PN-ABM-688
79809

Monoclonal Antibodies: Production and Application, pages 359–393
© 1989 Alan R. Liss, Inc.

C5-187

Monoclonal Antibodies as Catalysts and Templates for Organic Chemical Reactions

Bernard S. Green

Department of Pharmaceutical Chemistry, Faculty of Medicine—School of Pharmacy, The Hebrew University of Jerusalem, Jerusalem 91120, Israel

I. Background 361
A. History and Development 361
B. Approach and Rationale 367
II. Recent Results 369
A. Antibody-Promoted Hydrolysis 369
B. Antibody-Catalyzed Hydrolysis 373
C. Asymmetric Synthesis 377
D. Templates for Chemical Reactions 378
1. Restriction of Molecular Conformation 378
2. Intermolecular Interactions 380
III. Future Directions 381
A. Expanded Scope of Chemical Reactions 381
B. Correlation of Hapten Structure and Reaction Catalyzed 382
C. Metal Ion and Other Cofactors 383
D. Chemically Modified Antibodies 383
E. Combination Antibody-Chemical Systems 384
F. Site-Directed Mutagenesis 385
G. Anti-Anti-Enzyme Antibodies 385
IV. Biotechnological Implications 385
V. Medical Implications 386
VI. Summary 386

Chemists and biologists have long been preoccupied with imitating the properties of enzymes: rapid, highly efficient, stereospecific catalysis acting under mild conditions. An increasingly active area of research that has been termed "biomimetic chemistry" aims to use simple or, more often, complex,

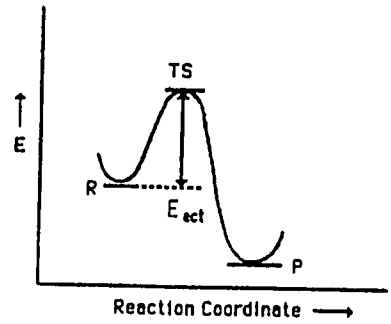

Fig. 1. Simplified schematic energy diagram of a chemical reaction, reactant (R) —> product (P).

difficult-to-synthesize chemical models which imitate some aspect of the enzyme-mediated process (see, for example, 1–14 and further refs. therein). In almost all of these systems, a major effort is expended in the careful design, synthesis, and demonstration of binding for a substrate molecule. The catalytic or chemical reaction properties are then built in, generally requiring even greater synthetic prowess.

Since extraordinarily selective binding is directly and simply achieved by raising antihapten antibodies, one might have expected that chemists would have explored this appealing approach; the details of antibody-hapten complementarity have been studied and have been relatively well-described for some time [15,16]. Indeed, analytical chemists and biologists routinely use sensitive immunoassay techniques, but little thought was apparently given to going a step further and considering the antibody-combining site for chemical reaction.

It has also been argued that enzymes achieve the major part of their catalytic power by utilizing the available (noncovalent) binding energy to lower the free-energy barrier for reaction (E_{act} in Fig. 1): "This process requires a large, complex molecule. Therefore it is probable that enzymic rate-enhancements are attainable only with artificial enzymes of similar complexity" [17]. Antibody molecules certainly have the required "similar complexity" and, indeed, have often been compared with enzymes: antibodies and enzymes are both composed of the same amino acid building blocks, and the combining sites of the former and active sites of the latter have approximately the same dimensions [18].

After a rather long period of induction, the idea of catalytic antibodies has clearly "arrived." This chapter seeks to review the history and the rationale of this idea, some of the approaches that are possible, and the work reported thus far. Only the surface of this field has been scratched and therefore a

review may appear premature. However, the results are clearly going to open up an important and exciting new direction in biotechnology, and it is therefore worthwhile to bring these ideas before audiences who seek new approaches and who may readily apply them.

I. BACKGROUND

A. History and Development

The history and development of the idea of, and experiments designed to demonstrate, antibody-induced chemistry provide an interesting and instructive science story. This field dates back to Linus Pauling's ideas about enzyme catalysis and his proposal that enzymes are complementary to the transition states of the reactions they catalyze [19,20]. Chemical reactions may be pictured in a simplified view (Fig. 1) as the transformation of a reactant R to product P via the transition state (TS), a species situated on an energy peak, having fleeting existence and no possibility of isolation. Pauling suggested that enzymes evolved so as to be complementary to the transition states of the reactions they catalyze, thereby selectively stabilizing the TS and lowering the energy of activation, E_{act}, for the reaction.

These ideas were largely ignored for many years, and until relatively recently, thinking about enzymes generally centered on how they fit the substrate and not the transition state. But Pauling's views received strong support when transition-state analogs, stable substances that mimicked the presumed transition-state structures of enzyme-catalyzed reactions, were found, as predicted, to bind to enzymes orders of magnitude more strongly than the natural enzyme substrates [21–23]. The study of transition-state analogs as selective enzyme inhibitors has become important in the design of drugs [24–29].

Pauling and coworkers had also worked intensively on the specificity of the interactions between an antibody molecule and an antigen or hapten, and this led them to consider the preparation of an antibody against the best inhibitor of catalase, assuming that this inhibitor was a transition-state analog, to see if the resulting antibody would have catalase-type activity [30]. (Related ideas of Pauling's led to experiments involving silica gel precipitation in the presence of dye molecules and the demonstration that the resulting "templated silica gel" was molecularly selective for the structure present during its formation [31].) No such experiments to induce chemical reactions with antibodies were reported at that time, but the research on antibody-hapten binding led to a clear view of the complementarity of this interaction and actually set the stage for successful experiments many years before they were first reported.

The first published attempt to raise specific antibodies in order to examine

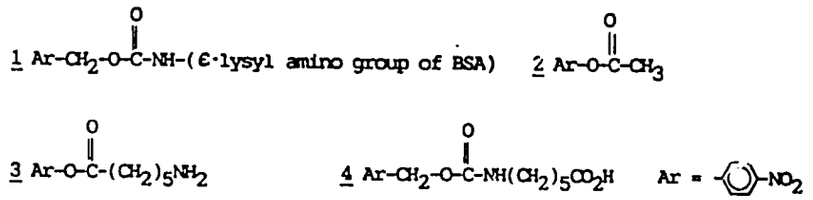

Fig. 2. Antibodies raised against the antigen *1* failed to enhance hydrolysis of analogous esters *2* and *3*.

the effects of antibody binding on a selective chemical reaction was made by Slobin, who raised antibodies against p-nitrocarbobenzoxy-BSA, *1* [32]. The resulting purified anti-p-nitrophenyl antibodies significantly *inhibited* the hydrolysis of p-nitrophenyl acetate (*2*) and of p-nitrophenyl-5-amino-hexanoate (*3*). When the antibody combining sites were blocked with the hapten 6-N-(p-nitrocarbobenzoxy) aminohexanoic acid, *4*, the hydrolysis of the esters was no longer inhibited (Fig. 2).

This example is important in emphasizing the importance of *avoiding* tight antibody binding to the substrate, thereby lowering its energy and increasing, rather than decreasing, the E_{act} of the reaction (Fig. 1). Other unpublished studies have been related to the author in which antibodies, directed against substrates, were also found to stabilize these substances toward chemical reaction.

The earliest succinct presentation of the rationale for antibody-induced catalysis was made by Jencks in 1969 [33]:

> If complementarity between the active site and the transition state contributes significantly to enzymatic catalysis, it should be possible to synthesize an enzyme by constructing such an active site. One way to do this is to prepare an antibody to a haptenic group which resembles the transition state of a given reaction. The combining site of such antibodies should be complementary to the transition state and should cause an acceleration by forcing bound substrates to resemble the transition state. .

Several years later a detailed and well-planned attempt to design a hapten and raise antibodies having catalytic properties was reported by Raso and Stollar [34]. These workers prepared antibodies against the reduced Schiff base (*5*) and a cyclic analog (*6*), both of which may be expected to mimic the Schiff base (*9*) [35,36]. This intermediate is formed by condensation of L-tyrosine (*7*) and pyridoxal phosphate (*8*) as the first step in pyridoxal

4

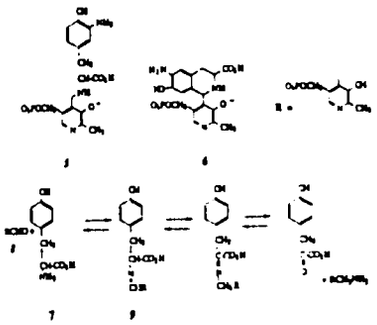

Fig. 3. Haptens *5* and *6* used to raise antibodies in order to enhance Schiff base (*9*) formation and transamination reaction of L-tyrosine (*7*) and pyridoxal phosphate, *8*.

phosphate-dependent enzymatic reactions such as transamination (illustrated in Fig. 3), decarboxylation, racemization, etc. Purified anti-*5* antibody was superior in binding the Schiff base intermediate (*9*) and was therefore examined in greater detail with regard to the rates of the condensation reaction and the overall transamination.

The authors disappointingly conclude that ". . . specific binding to antibody had no effect on the rate of Schiff base formation . . . ," and the transamination rate-enhancement, which was much less than that provided by enzymes, was considered to be of only theoretical interest [36]. However, this study may also be viewed with greater optimism. The antibodies *did* bind intermediate *9*, and Schiff base formation apparently *did* proceed within the antibody-combining sites (and the latter did *not* inhibit the condensation reaction, as would have been expected if the anti-*5* hapten antibodies were solely stabilizing reactant states). Most noteworthy is the fact that the pyridoxal phosphate-catalyzed transamination reaction was significantly enhanced by the specific antibody (Fig. 4) and this reaction apparently displayed true catalysis with turnover.

Of the attempted antibody-catalyzed reactions studied earlier using polyclonal antibodies, this reaction in particular merits closer attention using monoclonal antibodies; additional, different analogs of the transition state(s) for the reaction should also be studied.

Since antibody-bound substrates were invariably found to be less reactive than the unbound substrates [37] and since antibody binding protected labile substances from reaction it was authoritatively concluded that antibodies "are not capable of [catalyzing] the formation or breaking of covalent bonds" [18].

The first antibody-induced hydrolysis reaction to be reported was ob-

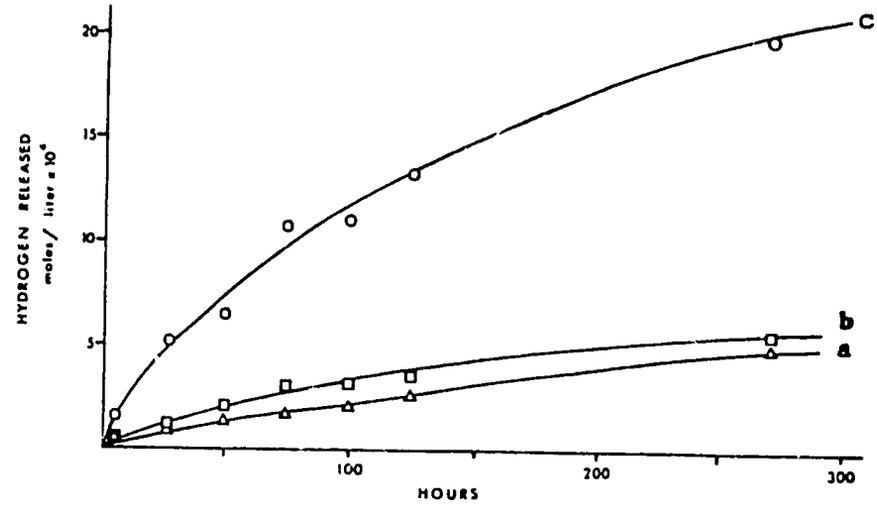

Fig. 4. Transamination of L-tyrosine (50×10^{-4} M) by pyridoxal phosphate 20×10^{-4}M at pH 5.5 a: No additive, triangles. b: With 15 mg/ml normal rabbit gamma globulin, squares. c: With 15 mg/ml (2×10^{-4}M combining sites) purified anti-5 antibody, circles. Product analysis indicated that the process proceeded according to reaction in Figure 3. All data taken from [36].

served accidentally. While developing a model for an improved immunoassay, in which the antibody, raised against 2,4-dinitrophenylated bovine serum albumin (DNP-BSA), was expected to stabilize a labile ester, fluorescein (N-2,4-dinitrophenyl)-5-aminohexanoate (*10*), towards hydrolysis, Burd and coworkers surprisingly found that the antibody apparently caused the ester to hydrolyze *more* rapidly [38]. These observations were probably ignored because the property of antibody-induced reactivity was a hindrance to the immunoassay objective. Since enhanced hydrolysis was also observed with avidin binding to a biotin ester, it may have appeared that the phenomenon was a chance result of protein binding. In addition, even though the results indicated that specific antibody binding caused hydrolysis (e.g., N-(2,4-dinitrophenyl)-3-aminopropionic acid (*11*), expected to be bound by the antibody, decreased hydrolysis) and that esterase or other enzyme impurities in the polyclonal antibody preparation were not responsible (antibody-induced reaction was not observed using fluorescein dibutyrate; and gamma-globulin from a rabbit not immunized with DNP-BSA had no effect on the hydrolysis of *10*), alternative explanations for the antibody-induced reaction were not ruled out. Also, there was no rationale given for the observations (Fig. 5).

Related studies which were also initially designed to produce immunoas-

6

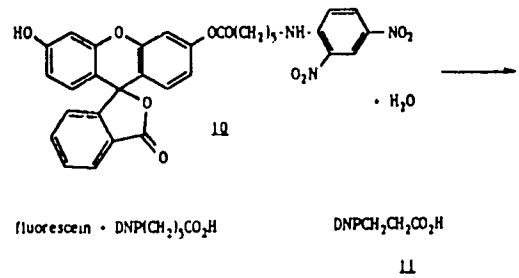

Fig. 5. Hydrolysis reaction promoted by a purified antibody preparation raised against 2,4-dinitrophenylated bovine serum albumin (DNP-BSA). The rate of this reaction is decreased in the presence of *11*.

says, based on antibody-binding stabilization of an ester whose hydrolysis would yield a fluorescent substance, afforded additional examples of unexplained promotion of hydrolysis by the antibody expected to inhibit reaction [39,40]; the molecular specificity possible was also demonstrated. Thus, antibody directed against 17-hydroxyprogesterone-7-carboxyethyl thioether, covalently linked to BSA (*13*-BSA) (in each of these cases the carboxylic acids were coupled to the protein via a carbodiimide reaction to give an amide linkage) promoted hydrolysis of the corresponding umbelliferone ester, *12*, but not those of *14* or *15*. Similarly, antibody directed against *16*-BSA enhanced the rate of hydrolysis of the umbelliferone ester of *16* but not of the ester *12* or that of *14*. The antibody-enhanced hydrolyses were also shown to be inhibited with high specificity by the presence of the corresponding steroidal acids.

Cross-reaction was also observed: the antibody directed against *16*-BSA hydrolyzed the umbelliferone esters of both *16* and the *15*-ester, which are closely similar in structure, at almost the same rates. However, *16* inhibited the hydrolysis of the umbelliferone esters of *16* or of *15* by this antibody to a much greater extent that did *15*, illustrating the sensitivity of the antibody-induced reaction to minute structural differences in inhibitors.

In this study, too, it was shown that immunoglobulin from a nonimmunized rabbit had no effect on the rate of hydrolysis of any of the four esters, and that the antibodies had no measurable effect on ethyl butyrate, a model substrate for esterase enzymes. Although no mechanisms were proposed for any of these reactions, it was suggested that selective antibody-induced reactions might have potential in diagnostic applications [39] (Fig. 6).

An explanation for all of these antibody-promoted reactions was proposed [41,42] when it was noted that each hapten had been linked via an amide bond to the protein carrier and that the ester bond which underwent hydrolysis could be expected, on the basis of the analogous structures of

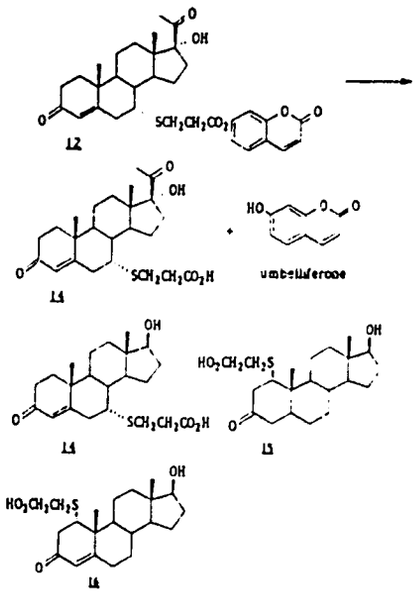

Fig. 6. Antibody-promoted hydrolysis of steroidal esters [39,40].

hapten and ester, to be bound within the antibody-combining site at the same position and in approximately the same orientation as the amide of the hapten-protein conjugate.

It was reasoned that the amide N-H group would be hydrogen-bonded within the antibody-combining site to one or more corresponding acceptor groups (e.g., carbonyl oxygen, phenolic or alcoholic oxygen, imidazole nitrogen, etc., represented by X in Fig. 7a). When these antibody functional groups, which originally stabilized the amide, are of the appropriate nature (—OH, —NH—, etc.) and orientation relative to the bound analogous ester, they may interact with the ester in a relatively destabilizing, electrostatic interaction. One may picture a more hydrophilic environment at the ether oxygen of the ester as a result of this interaction; an oriented water molecule within the otherwise hydrophobic antibody-combining site may become a relatively activated species and lead to states closer to the transition state, thereby lowering the activation energy, i.e., catalyzing the reaction (Fig. 7b). In addition, the functional group X— may serve as a nucleophilic catalyst (Fig. 7c) or, more likely, a general base catalyst (Fig. 7d). This rationale (see further discussion below) is meant to illustrate that antibody-induced chemical reactions are not only possible but may also be considered from a mechanistic viewpoint, implying the possible rational use of this phenomenon.

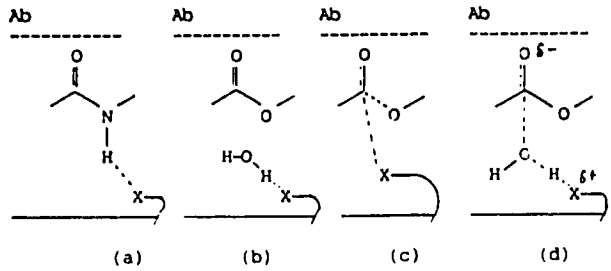

Fig. 7. Proposed rationale for the observed ester hydrolysis promoted by antibodies raised against the corresponding amide. **a:** Schematic view of antibody combining sites which are complementary to the amide group of the hapten-protein conjugate. X represents one or more hydrogen-bond acceptor groups of the antibody combining site. **b:** Ester replacing amide in the combining site and activated toward hydrolysis by a water molecule. **c:** Nucleophilic catalysis of ester hydrolysis within the combining site. **d:** General base catalyzed hydrolysis of the bound ester.

B. Approach and Rationale

Before discussing the results published to date using monoclonal antibodies, it may be useful to present the approach and rationale which has guided us and probably also others in this field. When one chooses to prepare a catalytic antibody for a given reaction, one may consider the scheme depicted in Figure 8.

This scheme illustrates the flow of the research and the multidisciplinary input which is implicitly or explicitly required. The cyclic rather than linear development of the research is also emphasized; the nature of the field at present, as discussed below, requires much more information than is available even from the combined contributions of all the disciplines indicated. Thus, each example of a successful or unsuccessful monoclonal antibody catalyst reflects back upon the rationale for the transition state analog and mechanism of the reaction. Some future developments which may be anticipated are also included.

The mechanism of any chemical reaction can be approached on the basis of the available accumulated literature data [43]. Effort may also be expended to better define the mechanism of a particular, required reaction [44]. The choice of groups for designing the TS analog may come from intuition and experience, such as the data accumulated by pharmaceutical chemists who have developed the concept of "bioisosteres," groups or molecules which have chemical and/or physical similarities to one another in their biological properties [45]. Additional sources of data which are important in designing substances which will mimic TS or intermediates come from studies in crystallography and solid-state chemistry [46]. Func-

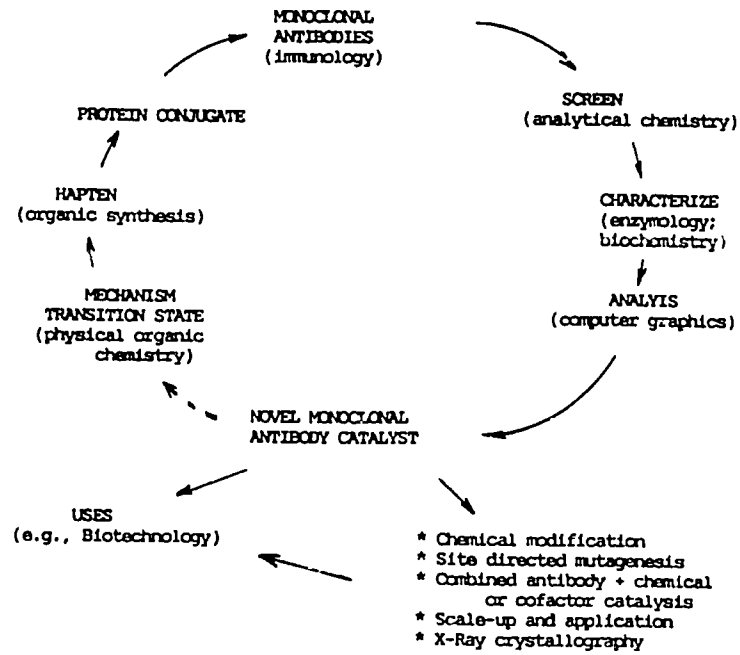

Fig. 8. Outline of program leading to catalytic monoclonal antibodies.

tional groups which replace one another in a structure or substances which form solid solutions may be expected to have closely similar attributes.

The availability of monoclonal antibodies, as a result of the development of hybridoma technology [47], has probably been the single most important factor in the recent rapid developments in the field of antibody-induced catalysis. A radically different situation was created by these developments, which make available unlimited quantities of molecularly homogeneous antibodies directed against essentially any desired chemical structure. An especially important aspect for our purposes is that one can also raise such antibodies against a weakly immunogenic structure, one that would possibly not have even been detected in a polyclonal antiserum sample.

The combination of monoclonal antibody production and the screening of clones for the desired chemical reaction (rather than for hapten binding as is routinely done) allows one to have an even better chance of a successful catalytic antibody even if the hapten chosen was not optimal as a transition-state analog. This idea, first suggested by I.R. Cohen [48], also provides a flexibility based on the multimolecular response of the immune system which may allow one to obtain combining site features that a single response or one restricted solely to the antibodies which are rigidly complementary to the hapten, would possibly not reveal.

One may successfully use an idealized reaction energy diagram having a single transition state, as in Figure 1, for some reactions such as rearrangements; and one may also be moderately successful for simple unimolecular displacement reactions or other reactions which have large driving force (ΔG exothermic). The stable analog which is synthesized in order to raise antihapten antibodies may also be a mimic of a high-energy metastable intermediate and not the true TS analog. Such species are more easily defined and characterized (and may be detected in some cases) and should be close to the TS in energy and structure; they can be expected to approximate more closely the TS than the substrate [49]. However, we feel that far broader success will result from what one may call the "reaction pathway" approach, which does not ignore the TS of the reaction, but which recognizes that more than one TS may be involved as well as several stable/metastable intermediates. This approach attempts to provide an optimal antibody-combining-site structure for guiding the reactant(s) along the reaction coordinate to product(s) by attempting to identify all the structures involved along the reactant(s) → products(s) pathway. This includes consideration of the access and approach geometry of "outside" reactants (e.g., water, oxygen, etc.); the desirable approach and binding of reactants to the antibody combining site; as well as features which enhance the stability of, say, a leaving group in order to maximize forward rate constants in all reaction equilibria. One should aim to elicit a combining-site structure which will best provide the desired approach geometry between reactants or reaction centers, as well as maximal stabilization of transition states and ready release of product(s). This may sometimes require more than one "pocket" on the antibody-combining site and sometimes a delicate balance of opposing forces.

In order to achieve this situation one should preferably synthesize several stable chemical substances which have been designed to produce the desired antibody-combining-site structure; this gives the immune system several "chances" to provide the best catalytic antibody. In addition to providing a better chance of detecting an antibody which is a good catalyst, the comparison of hapten structures (and surmised complementary antibody-combining-site structures) which produce more or better catalysts with those that provide fewer or poorer catalysts will provide an important insight into some of the factors that are important for catalysis as well as point the way for future, improved antibody-based catalysts.

II. RECENT RESULTS

A. Antibody-Promoted Hydrolysis

The first unambiguous and intentional demonstration 1) that an antibody molecule *can* induce a hydrolysis reaction; 2) that this reaction indeed takes

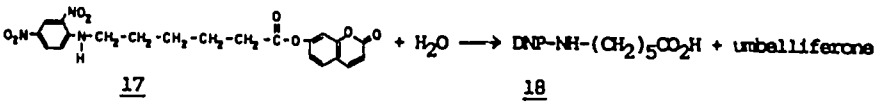

Fig. 9. Hydrolysis reaction promoted by monoclonal antibody SPE-21 raised against 2,4-dinitrophenylated BSA.

place within the antibody-combining site; and 3) that the reaction observed is selective for the substrate which is analogous to the epitope against which the antibody was raised was provided by a series of monoclonal anti-2,4-dinitrophenyl (DNP) antibodies [50].

The antibodies used were obtained from hybridomas produced by fusion of myeloma cells with spleen cells from mice immunized with 2,4-dinitrophenylated keyhole limpet hemocyanin (DNP-KLH) and purified by affinity chromatography; they had binding constants for N-(2,4-dinitrophenyl)-6-aminohexanoic acid (*18* in Fig. 9; cf. Fig. 16) of 1×10^9 liter/mol. One of the anti-DNP-secreting clones studied in detail (SPE-21), promoted, as might be expected from the results and rationale presented above, the hydrolysis of the ester 7-hydroxycoumarin N-(2,4-dinitrophenyl)-6-aminohexanoate (*17* in Fig. 9). The reaction, however, is stoichiometric rather than catalytic; the antibody enhances the rate of disappearance of *17* until 2 mol of ester have reacted per mole of antibody (Fig. 10). The combining sites strongly bind the product, acid *18*, and additional *17* is thereby prevented from entering the combining sites. When the SPE-21–*18* complex was dissociated by passage over an ion-exchange column, the antibody was again active in promoting the hydrolysis of *17*. This suggested that in a similar system where the product would not be so tightly bound, turnover and true catalysis would be displayed; this was indeed found to be the case, as is described below.

The hydrolytic activity of the SPE-21 monoclonal antibody was explained in the same way as before [41,42]. The use of monoclonal antibodies allowed one to rule out the effect of strong binding alone as the reason for hydrolysis. Other clones (e.g., SPE-25, SPE-7) also provided antibodies with equally strong DNP binding, but the former was devoid of rate-enhancement activity, while the latter was somewhat less effective than SPE-21. Several DNP-binding mouse myeloma proteins also promoted the hydrolysis of *17*; MOPC-315 was as active as SPE-21, while MOPC-460 was appreciably less effective.

In order to ascertain the nature of the purported group(s) (X in Fig. 7) which hydrogen bond to the amide antigen and are then believed to provide local hydrophilic environments for the ester group or act as nucleophilic or

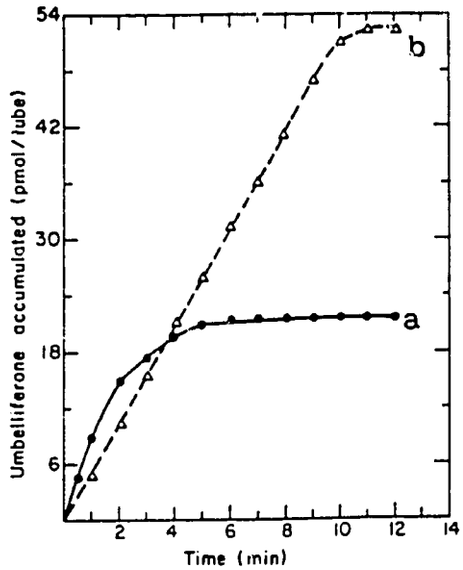

Fig. 10. Hydrolysis of *17* by a) monoclonal antibody SPE-21, resulting in a stoichiometic reaction (no turnover, solid circles); and b) hog liver esterase, resulting in a true catalytic reaction (open circles).

general base catalysts, several protein-modifying reagents were allowed to react with SPE-21 antibody. Cysteine and imidazole modification—using 5,5′-dithiobis-(2-nitrobenzoic acid) and ethoxyformic anhydride, respectively—had no effect on the hydrolytic activity of SPE-21. Tetranitromethane, which selectively nitrates tyrosine residues, significantly decreased the hydrolytic activity, suggesting that tyrosine plays a significant role in the mechanism of hydrolysis.

The availability of monoclonal antibodies from mouse tumours (myeloma proteins) [51] predates those available via hybridoma technology [47]. It is noteworthy that the binding specificity of several of these myeloma proteins, which were only recently shown to induce chemical reactions, were actually known for some time and structural data have also been available for some of these for several years [52]. Thus the murine myeloma proteins MOPC-167, TEPC-15, and MCPC-603 (discussed below), are all known to have a high binding affinity for phosphoryl choline esters.

The use of MOPC-315 and MOPC-460 to hydrolyze *17* was probably the first example of myeloma protein-induced chemical reactions [50]. More recently, the phosphorylcholine-binding murine myeloma protein TEPC-15

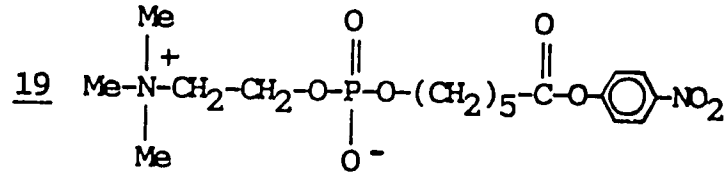

Fig. 11. Chemical structure of p-nitrophenyl 6-(phosphorylcholine) hexanoate, *19*.

was shown to accelerate the hydrolysis of p-nitrophenyl 6-(phosphorylcholine) hexanoate, *19* [53] (Fig. 11).

The mechanism of this reaction is not clear since the ester bond cleaved is apparently distant from the combining-site region, which is believed to be complementary to the phosphodiester group. From the pH profile of the reaction it was argued that *19* acylates a lysine amino group of the combining site. The reaction of *19* is also stoichiometric rather than catalytic; the kinetic constants reported for the hydrolytic acceleration are $K_m = 12.5\ \mu M$ at pH 8.0; $k_2/K_m = 2.6 \times 10^3\ M^{-1}\ s^{-1}$ at pH 9.9.

Tramontano, Janda, and Lerner reported the first example of selectively raised monoclonal antibodies which promoted a chemical reaction with more significant rate enhancement [54]. Antibodies were raised against the haptens *20* and *21*. The picolinic structure in *21* was designed to include a metal ion, but the work reported thus far has not involved any metal ions. It was found that several of the hybridoma-secreting antibodies which were selective for the phosphonate *21* enhance the hydrolysis of the ester *22*, and the most active (6D4) was studied in greater detail. The chemistry of the anti-*20* antibodies has not yet been reported, but it was found that both *23* and *24* inhibited the hydrolysis reaction by this antibody. The fact that *25* is not hydrolyzed by the antibody may seem surprising in view of the close similarity in size of the CF_3 and CH_3 groups; however, the strong electron-withdrawing effect of the former could drastically change the acidity of the trifluoroacetamide N-H group relative to that of the acetamide and the complementary functional groups within antibody combining site which accommodate the former are apparently inappropriate for the neutral acetamide. The reaction of *22* was stoichiometric, but, unlike the system described above (Fig. 9), the lack of turnover did not appear to be due to inhibition by carboxylic acid product *26*. Significant regeneration of antibody activity by hydroxylamine or alkaline conditions implied that a covalent acyl intermediate is formed. Reaction of 6D4 with protein modification

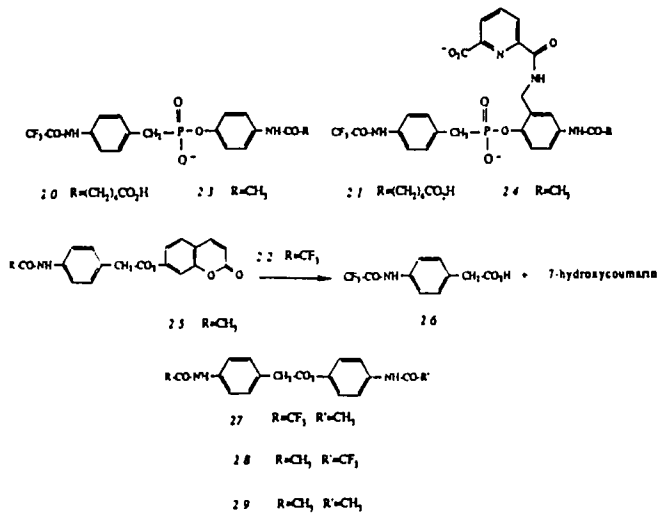

Fig. 12. Anti-*21* monoclonal antibody promotes the hydrolysis of *22* in a stoichiometric reaction and catalyzes the hydrolysis of *27* in an enzyme-like fashion.

reagents strongly suggested the presence of a histidine (quenching of all activity at a 10:1 diethyl pyrocarbonate: protein ratio) and a tyrosine (60% reduction of hydrolytic activity with a 100:1 tetranitromethane: protein ratio) in the antibody combining site and possibly also a lysine (25% reduced activity with a 5,000-fold excess of N-succinimidyl propionate) (Fig. 12).

B. Antibody-Catalyzed Hydrolysis

By judiciously changing the substrate with the same anti-*21* monoclonal antibody (hybridoma 6D4) Tramontano, Janada, and Lerner were able to demonstrate true catalysis, with enzyme-like turnover, using ester *27* [55]. Furthermore, the specificity of the antibody relative to an enzyme, hog liver esterase, was dramatic. The enzyme rapidly hydrolyzed the ester bonds of *27*, *28*, and *29* at approximately the same rate. However, the antibody, which was active with *27* showed no enhanced hydrolysis rates with *28* or *29*. The kinetic parameters for reaction of *27* catalyzed by antibody at pH 8 are $K_m = 1.9\ \mu M$, $V_{max} = 2.2 \times 10^{-9}\ Ms^{-1}$, $k_{cat} = 2.7 \times 10^{-2}\ s^{-1}$, and $k_{cat}/k_{uncat} = 960$.

Pollack, Jacobs, and Schultz carried out a conceptually simple and interesting study using the well-characterized myeloma protein MOPC-167, known to be specific for phosphorylcholine monoesters such as *30* (Fig. 13) [56]. On the basis of the known combining-site structure in related antibodies (that of MCPC-603 has been reported [57]), it was suggested that MOPC-167

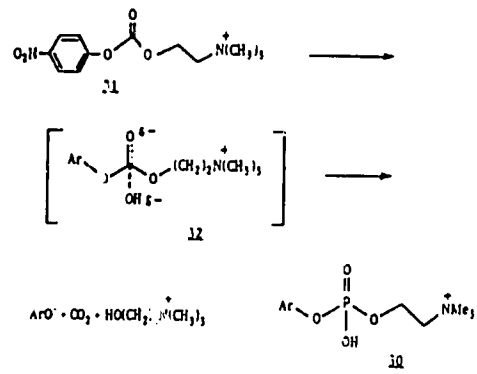

Fig. 13. Antibody MOPC-167 binds *30* and is an enzyme-like catalyst for the hydrolysis reaction of carbonate, *31*, whose expected transition-state, *32*, is closely represented by *30*.

antibody would stabilize the expected transition state *32* for the hydrolysis of the analogous carbonate *31*.

The kinetic constants for the MOPC-167-catalyzed reaction are K_m-208 μM at pH 7; k_{cat}/k_{uncat} = 770. The mechanism of the reaction could be ascribed to transition-state stabilization or to covalent catalysis by a nucleophilic group at the combining site followed by rapid hydrolysis of this intermediate in a second step. A tyrosine, known to be present within the combining site of phosphoryicholine-ester binding proteins [57], was implicated in this reaction, as in the reactions discussed before, by the complete elimination of catalytic activity on treatment of MOPC-167 with tetranitromethane.

Monoclonal antibodies were also specifically raised against related transition-state analogs designed to produce selective catalysts for 4-nitrophenyl carbonate hydrolysis [58]. The hapten 4-nitrophenyl phosphonate (*33*) linked to BSA was the immunogen (Fig. 14). One IgG which had high affinity for hapten *33* was found to catalyze efficiently and selectively the hydrolysis of methyl 4-nitrophenyl carbonate (*34*). The kinetic parameters for this reaction at pH 8.5 were k_{cat} = 1.4 min^{-1}, K_m = 660 μM. The substrate specificity was demonstrated by the absence of any appreciable catalysis using methyl 2-nitrophenyl carbonate (*35*). With a methyl substituted derivative (*36*), antibody-catalyzed hydrolysis was observed but with much less binding affinity (K_m = 7 mM) and an appreciably slower rate. The authors also report a catalytic monoclonal IgG raised against KLH-33 conjugate having k_{cat} = 29 min^{-1} and K_m = 350 μM for the hydrolysis of *34*; these values represent a rate acceleration of 16,000 over spontaneous background hydrolysis.

Recently, the combined elements of a tetrahedral phosphorus and an

16

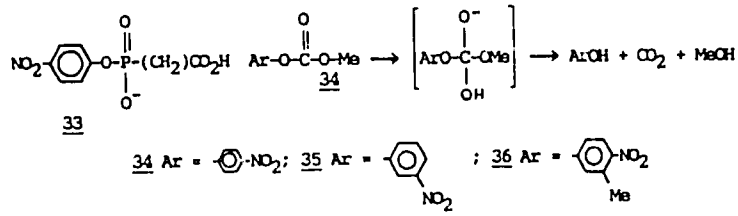

Fig. 14. Monoclonal catalytic antibodies raised against hapten *33* selectively catalyze hydrolysis of carbonate *34*.

amide were used in a hapten, *37*. The anti-*37*-BSA monoclonal antibodies were screened for both hapten binding in the usual way and also for the release of 4-nitrophenol using alkaline hydrolysis of ethyl 4-nitrophenyl carbonate (*38*) as substrate. Several of the clones secreted antibodies which were powerful catalysts for this hydrolytic reaction [59]. The results suggest that the simple idea of directly selecting antibodies on the basis of the chemical reaction which is being investigated [48] may greatly increase the chances of obtaining highly active catalytic antibodies for a wide variety of reactions. In addition, it is suggested that one may possibly uncover separate haptenic structural elements which contribute to a given antibody-induced reaction whose additive effects may lead to greatly improved catalytic antibodies. Finally, it is especially noteworthy that antibodies raised against a neutral hapten such as *37* can be good catalysts. The hydrolytic antihapten antibodies reported thus far were raised against ionized phosphonate groups [54,58], and it appeared that this electrostatic interaction was critical in giving rise to catalytic antibodies. Combining-site complementarity to this ionized group is apparently not an essential element in providing catalysts for hydrolysis (Fig. 15).

In work just completed [60] the anti-DNP monoclonal antibody SPE-21 was reexamined using carbonate substrates, *39–41* (Fig. 16). It will be recalled that this antibody was a stoichiometric catalyst for the hydrolysis of ester *17* (Fig. 9); it was suggested that the carboxylic acid product *18* was too tightly bound by the combining site to be released and allow turnover [50].

It was reasoned that if an analogous carbonate, *39*, reacted in the same way, an unstable carbonic acid, *42*, would be produced, which would lose carbon dioxide to give the DNP-aminoalcohol, *43*. The latter, which would be expected to have weak affinity for the antibody, would be readily released, and allow additional carbonate *39* to enter the combining site and react. This was indeed observed; SPE-21 induced true catalysis with *39*, displaying turnover and enzyme-like kinetics [60].

Furthermore, if the catalytic site of the antibody-combining site is indeed

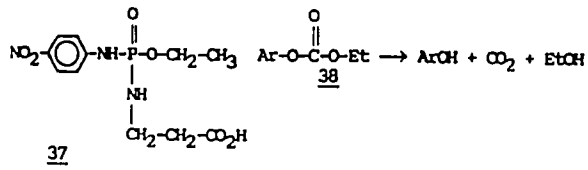

Fig. 15. Monoclonal antibodies elicited by *37* catalyze the hydrolysis of ethyl 4-nitrophenyl carbonate, *38*.

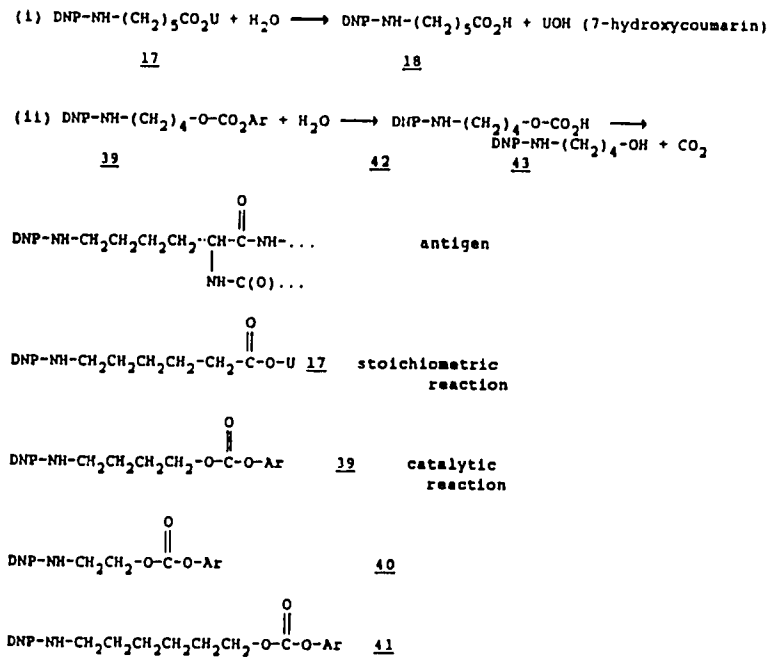

Fig. 16. Monoclonal antibody SPE-21, directed against DNP-BSA (antigen), showed enhanced but stoichiometric reactivity with *17* and catalytic activity with *39*. DNP = 2,4-dinitrophenyl; U = 7-coumaryl (umbelliferyl); Ar = 4-nitrophenyl.

at that region which is complementary to the amide of the antigen and assuming that the DNP group is the immunodominant subgroup in these molecules (as has been found with anti-DNP antibodies) [61], the antibody could be expected to display selectivity for the carbonate *39*, having an analogous structure to the antigen, and display less reactivity with carbonate substrates such as *40* or *41* having shorter or longer chain lengths, respectively. Indeed, comparison of SPE-21-catalyzed hydrolysis of the three carbonates—*39*, *40*, and *41*—with the corresponding spontaneous

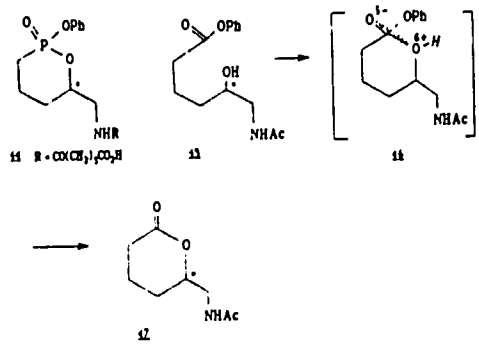

Fig. 17. High enantiomeric discrimination in the cyclization of *45* is observed using an antibody raised against optically active hapten *44*.

(background) hydrolysis in the presence of an irrelevant IgG molecule, showed that catalysis was appreciably greater with *39* than with either *40* or *41* [60].

C. Asymmetric Synthesis

The chirality of the antibody molecule should lead to enantiomeric ratios different from that observed in all cases where a chemical reaction gives a chiral product or in a reaction where one of the reactants is chiral [62]. Chiral discrimination between the enantiomers of N-benzoyl phenylglycine by polyclonal antibodies raised against diazotized N(p-aminobenzoyl)phenyl glycine was first reported by Landsteiner and van der Scheer in 1928 (!) [63] and was later shown to be general for tartrate [64] malate [65] and many other chiral substances. Enantiomeric discrimination is very high, as is reflected in binding ratios of 100:1 for the enantiomers. This is routine for such polyclonal antibodies [66] and demonstrates the powerful potential of antibodies for chiral recognition and separation.

The first asymmetric synthesis using an antibody-catalyzed reaction was reported recently [67]. Monoclonal antibodies were raised against the cyclic phosphonate ester hapten *44* (Fig. 17), which may mimic the transition-state *46* involved in the intramolecular cyclization of *45* to yield the lactone *47*. One of the antibodies studied was found to catalyze the reaction at pH 7.0 with kinetic parameters K_m = 76 μM, V_{max} 0.99. μM min^{-1}, k cat = 0.003 min^{-1}. From comparison of this rate to the rate of the spontaneous, uncatalyzed reaction, the acceleration by the antibody was found to be 167. The substrate *45* has a chiral center, indicated by an asterisk, and therefore one can inquire whether an antibody raised against a resolved, enantiomerically pure hapten would, as expected on the basis of high enantiomeric

recognition of antibodies, selectively react with one substrate enantiomer. The antibodies elicited above were raised against a resolved enantiomer of *44* and indeed demonstrated high enantiomeric preference for one of the substrate enantiomers. The isolated *47* had an enantiomeric excess ("optical yield") of 66%, but if one corrects for the spontaneous reaction which takes place alongside the antibody-catalyzed cyclization, it appears that the latter is nearly stereospecific.

Some features of the hybridoma technology merit special note in relation to chiral separations and synthesis. Generally, in order to perform an asymmetric synthesis, one of the initial starting materials has to be resolved. Alternatively, a naturally occurring chiral material may be used, but then one is severely restricted to those substances which are available. At first sight it would seem that use of a chiral hapten to induce enantiomer-selective antibodies would also demand resolution of the hapten, but this is not so: a *racemic* hapten may be used to produce monoclonal antibodies which are selective for *each* of the enantiomers! Monoclonal antibodies secreted by each clone will preferentially bind one enantiomer. By using appropriate selective screening procedures which detect each enantiomer, the clones displaying the highest selectivity for each enantiomer may be identified. Thus, both enantiomers may be obtained without having to resolve the hapten which gave rise to them. Furthermore, *both* of the resolved enantiomeric substances are not needed in order to obtain monoclonal antibodies selective for both enantiomers: by suitable screening with a racemic mixture and *one* of the enantiomeric substances it should also be possible to obtain monoclonal antibodies which are highly selective for both enantiomers. Of course, the same approach may also be used for diastereomers, geometrical isomers, etc.

Surprisingly, antibodies have apparently not been utilized thus far as a means for resolving enantiomers. A special advantage expected of antibodies relative to other proteins such as bovine serum albumin [68–70] or avidin [71], etc., for resolution or chemical reaction is that the absolute configuration of a related series of substances should be readily assigned by knowledge of the absolute configuration of one member of the series or of the hapten used to raise a monoclonal antibody. The use of antibodies to establish steric configurations of molecules was suggested many years ago by Landsteiner [64,72].

D. Templates for Chemical Reactions

1. Restriction of molecular conformation. The selection and preferential stabilization of one molecular conformation may be considered a kind of chemical reaction; in cases where the energy barrier between different conformations is sufficiently great, separately isolable substances are possible. Conditions which stabilize a conformation on the pathway to product in

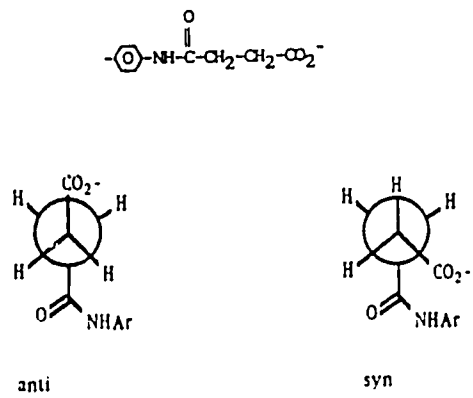

Fig. 18. Antibodies raised against the succinanilate group appear to stabilize the syn conformation.

a chemical reaction will enhance that reaction and the catalyzed cyclization reaction referred to earlier probably acts in part by this mechanism.

It was suggested many years ago that the polyclonal antibodies raised against the succinanilate group hold this and related substances in a preferred syn- rather than anti-gauche conformation about the ethane bond (Fig. 18) [73]. It would be necessary to estimate independently the ratio of these conformations in solution and when antibody-bound in order to assess the relative contributions of the antibody and the intramolecular hydrogen-bonding which also favors the syn conformation. Alternatively, the syn:anti ratio could be measured using molecules in which this hydrogen bonding is absent.

Early examples where antibody binding apparently favored selected molecular conformations are provided by studies of immunologically active peptides corresponding to sequences or protein and polypeptide antigens. A nonhelical tetradecapeptide fragment of sperm whale myoglobin (which *is* helical in the protein metmyoglobin) inhibited binding of metmyoglobin or apomyoglobin by antisera to metmyoglobin [74]. One explanation is that the helical conformation was stabilized by antibody interaction.

Fab fragments of antibodies were raised against the high molecular weight copolymer which is composed of the repeating sequence (L-tyr-L-Ala-L-Glu)$_n$ and possesses a helical structure. The antibodies were found to bind peptides such as (L-Tyr-L-Ala-L-Glu)$_{13}$, which have a very low helical content, and on the basis of induced circular dichroic spectra, these peptides adopted a more helical structure upon antibody binding [75]. These antibodies may thus induce or stabilize the helical conformations of peptides which are largely of random coil form in free solution.

A different kind of experiment suggesting that antibodies may induce significant and extensive conformational change upon binding was reported, based on an antigen containing p-aminobenzenearsonate (Ars) groups which are apparently not available for inducing antibody formation: the resulting antibodies did not bind Ars groups [76]. However, this antigen *could* be efficiently bound by anti-Ars antibodies elicited by other Ars-containing antigens These results are compatible with the view that in the original antigen the conformation was such that the Ars groups were buried and not able to be presented for immune recognition. Alternatively, those conformations in which Ars groups are accessible are nearly absent. The anti-Ars antibodies either cause conformational changes in the antigen or stabilize those conformations having accessible Ars groups.

The use of antipeptide antibodies which are directed against short sequences of a protein to bind intact proteins is an area of tremendous importance in biology and medicine. Antibody binding has been explained as being due to the antibody "trapping" of those protein determinant conformations which resemble those of the peptide used to raise the antibodies or, possibly, to the antibody-antigen binding causing a conformational change in the determinant as it is bound [75].

The use of monoclonal antibodies as agents to study the conformations of proteins and conformational changes induced by ligands or other conditions (heat, adsorption on solid supports, etc.) is becoming increasingly important and has been recently reviewed [78].

2. Intermolecular interactions. There have been many studies in which antibodies were raised against haptens or antigens which may be considered to be made up of separate, covalently linked units, e.g., A-B-C-D . . . , and then the relative antibody binding of different portions of this structure, e.g., A-B-C, A-B, B-C-D, etc., was determined. However, apparently no studies have been made of the *simultaneous* binding of separate groups—A-B *and* C-D, A-B *and* D, etc.—within an antibody combining site [79].

One of the earliest studied examples of a hapten comprising two determinant groups was the isophthalic acid derivative linked to glycine and leucine, *48* [80,81]. Anti-*48* antibodies were found that preferentially bound the hapten *48;* other antibody fractions bound the benzoyl leucine *49*, while still other fractions had greatest affinity for benzoyl glycine *50*. But the possible simultaneous binding of two groups, e.g., *51* and *52*, within the combining site was not investigated (Fig. 19).

One can imagine a variety of ways to exploit systems which bind two components for chemical reactions, spectroscopic changes, etc. A recent report indicates that preferential alignment of two molecules within an antibody-combining site may be achieved [82].

Antibodies were raised against one of the photodimeric products obtained

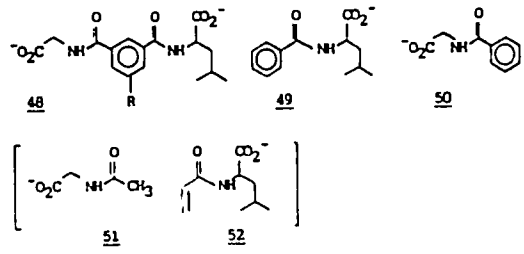

Fig. 19. The hapten *48* elicited antibody fractions displaying affinity for the separate components of *48* (*49* and *50*, respectively) as well as antibody fractions with affinity for the original hapten *48*. The question of whether antibodies of the latter type could simultaneously bind two separate molecules (e.g. *50* + *51*) has not yet been explored.

during the solution photoreaction of methyl 4-nitrocinnamate (*53*). The latter affords several photodimers (*54*, *55*,) which are believed to be formed by close approach of a photoexcited molecule and a ground-state molecule (Fig. 20). In the absence of additives, irradiation of solutions of *53* yields *55* as the major photodimeric product. On the basis of the close similarity in molecular volume, as deduced by x-ray crystallography, of pairs of monomers and the corresponding photodimer [83], it was felt that anti-*54* antibodies would stabilize the intermolecular, pre-photodimerization orientation pre-*54* or enhance the probability of excited state and ground-state interactions to produce this orientation, thereby preferentially producing photodimer *54*.

Anti-*54* antibodies both increased the rate of disappearance of monomer *53* and nearly reversed the ratio of products, causing *54* instead of *55* to be the major product formed [82]. Using *53*, this reaction can, of course, only be a stoichiometric and not a catalytic one. Future work will undoubtedly provide additional, improved examples of antibody-combining sites as catalytic templates.

A recent report in which a monoclonal antibody was raised against an immune complex of monoclonal antibody and human thyrotropin and found to be specific for the complex [84] suggests studies to inquire whether antibodies may enhance the attraction of weakly binding substances. A variety of applications may be considered for such systems.

III. FUTURE DIRECTIONS

One can foresee several directions for research that will undoubtedly guide researchers in the near future.

A. Expanded Scope of Chemical Reactions

There is no reason why the antibody approach should not be possible for the catalysis of virtually any chemical reaction. The kind of hapten required

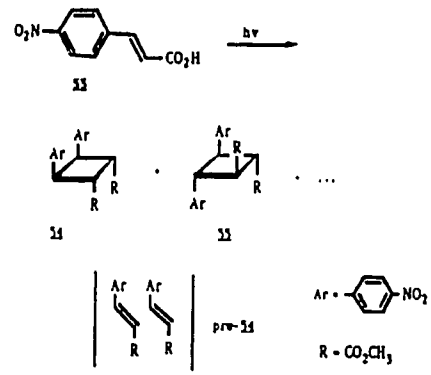

Fig. 20. Antibodies raised against a derivative of *54* enhance the formation of *54* during photoreaction of *53*.

to elicit antibodies capable of promoting, for example, decarboxylations, exchange reactions, nucleophilic substitution reactions, electrophilic substitution reactions on aromatic rings, etc., may be readily formulated on the basis of the mechanistic studies which have been carried out over the years on such reactions. It now remains only to design stable analogs of the transition states or intermediates involved, then synthesize them and test the resulting antibodies for reaction enhancement.

All such work will have value in corroborating the mechanistic pathway originally posited. Indeed, antibody catalysis provides a fascinating and novel way of testing the validity of reaction mechanisms and transition-state structures. New mechanisms for known reactions may also be uncovered by this approach, and a choice between two or more proposed mechanisms may also be based on an experimental test that was not feasible before. The use of a selectively raised antibody to act as a template and bring together two molecules for reaction [82] suggests more challenging organic synthesis using antibody-combining sites. Many important addition reactions involving two different substrates, A+B —> A-B, should be considered; photoaddition reactions, Diels-Alder and other orbital-symmetry allowed thermal addition reactions should be especially amenable to antibody-induced catalysis.

B. Correlation of Hapten Structure and Reaction Catalyzed

The antibody-catalyzed hydrolysis and lactonization reactions described thus far successfully used a tetrahedral phosphorus hapten or a carboxylic amide to produce catalytic antibodies. A deeper understanding, or at least improved correlation and predictability, of the reaction catalyzed and the substrate structure which best reacts when an antibody is raised against a given hapten is greatly needed. Research designed systematically to probe

the structural elements which give rise to catalytic antihapten antibodies for the hydrolysis of analogous esters, carbonates, carbamates, and amides, for example, may delineate the parameters for haptens that serve as transition-state analogs and/or reaction intermediates in hydrolysis reactions. It will be important to learn whether the separate hapten structural elements that elicit catalytic antibodies are additive, as has been suggested by current work (Fig. 15).

C. Metal Ion and Other Cofactors

Many enzymes utilize metal ions or coenzymes. The pyridoxal-phosphate tyrosine system (Figs. 3, 4) is an example which merits reinvestigation with monoclonal antibodies. The hapten *21* (Fig. 12) included a metal-ion binding site but this feature has not yet been utilized in chemical reactions. The effect of metal ions and cofactors on antibody-promoted reactions will certainly be investigated further.

Monoclonal antibodies have been prepared which display extraordinary selectivity for metal ions even though the metal ion is "insulated" from the antibody by an EDTA derivative [85]. Antibodies could also bind metal ions directly, and several suggestions for using antibodies as selective ion binding compounds plus reaction centers have been made [9,86,87].

The use of antibodies to activate a substrate for chemical reaction and act as a template for aligning molecules leads to the idea of antibodies binding a molecule related to the original hapten in a configuration closer to that of the hapten and different from that generally held by the molecule. E. Keinan suggested that transition-metal complexes may be useful in examining the transformation of binding energy into molecular deformation. The possibility of antibody-mediated chemical reaction of ligands at the metal atom may also be studied [87].

D. Chemically Modified Antibodies

The idea of transforming an antibody into an enzyme by chemical modification was probably first pursued by M. Wilchek and D. Givol in 1976 [88]. Intending to convert the DNP-specific myeloma protein MOPC-315 into a papain-like enzyme, these workers prepared the bromoacetyl derivatives of two different DNP derivatives to selectively modify the combining site. Reagent *56* had been shown to label Tyr 34 of the light chain selectively, while reagent *57* selectively labeled Lys 54 of the heavy chain [89,90]. They prepared reagents (*58* and *59*) analogous to *56* and *57* but having DNP groups which could be readily removed by mercaptoethanol. The idea was to treat MOPC-315 with *58*, which was expected to react like *56* with Tyr 34, and then remove the DNP with mercaptoethanol to release an —SH group on the

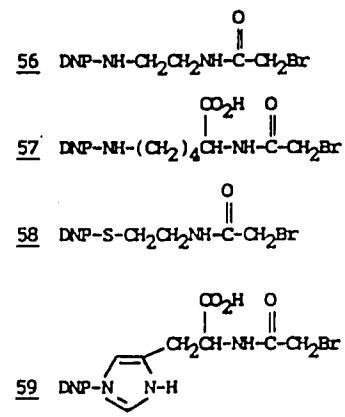

Fig. 21. A proposed example of chemical modification of an antibody (MOPC315) to produce a catalyst. The DNP-binding MOPC315 is affinity-labeled by the bromoacetyl reagents *56* and *57*. It was therefore suggested that affinity-labeling with analogous substances *58* and *59* (with chemical removal of the DNP groups after each reaction) would introduce thiol and imidazole groups whose presence may now confer papain-like activity with appropriate substrates.

protein. Subsequent reaction with *59* and removal of the DNP group was expected to provide the antibody with enzyme-like properties (Fig. 21).

Although not completed, this work illustrates the kind of chemical modification that may be introduced into an antibody-combining site. Many other chemical changes may be considered (e.g., conversion of an amino acid side chain to an aldehyde which is capable of catalyzing ester hydrolysis [12]).

E. Combination Antibody-Chemical Systems

A system in which the antibody provides molecular recognition and a covalently linked chemical catalyst or reagent induces the chemical change represents a different extension of the catalytic antibody approach. Support for one example of this approach is provided by the work of Whitesides and coworkers, who demonstrated rhodium catalyzed asymmetric hydrogenation in aqueous solution, where the chiral environment was provided by the protein avidin, which tightly binds biotin in a manner comparable to that of antibody and hapten [71]. The rhodium complex was covalently linked to the biotin. The use of a substrate-recognizing antibody instead of avidin in such a system, in which rhodium is covalently linked to the antibody near the combining site, might provide both efficient catalysis by the rhodium

(catalytic hydrogenation or other reactions) and afford predictable product stereochemistry.

F. Site-Directed Mutagenesis

The technology of specific amino acid replacement which is establishing structure-function relationships in enzymes, and allowing researchers to engineer proteins with new or different activities, specificities, or stability, may also be anticipated with antibodies [91].

G. Anti-Anti-Enzyme Antibodies

An intriguing idea which may also be considered is to raise antibodies against an enzyme active site and use the resulting anti-enzyme antibodies as an antigen to elicit antibodies (anti-idiotype antibodies). If the latter are complementary to the combining sites which were complementary to the enzyme active site, they may share the catalytic properties of the enzyme. In hormone receptors and other systems, related ideas have been successfully applied [92].

IV. BIOTECHNOLOGICAL IMPLICATIONS

The use of enzymes for organic chemical transformations is becoming increasingly attractive to the organic chemist and to the chemical industry [93,94]; enzyme-catalyzed reactions have long been routinely used by the pharmaceutical industry. The preparation of new, mutant enzymes by microbiological methods or by site-directed mutagenesis provides an element of control in order to obtain enzymes with desired properties. Chemical modification of enzymes may also lead to changes in substrate specificity as well as different reaction specificity [95]. However, the scope of known enzymes is limited and there still remain a host of important reactions for which no enzyme is known, or for which a chemical catalyst is insufficiently elective. For example, the selective hydrolysis of a polypeptide at any desired peptide bond represents the kind of problem (one of tremendous biological and medical importance) that has no solution at present. Catalytic antibodies and chemically modified antibodies may eventually find wide application and provide some advantages over both the enzyme and chemical catalyst approaches. Chemical catalysts are generally not specific and require drastic conditions, while enzymes are generally labile and demand careful handling.

The technology transfer from one system with a given antibody to another system using a different antibody should be especially facile: unlike enzymes, each of which has different lability and demands unique conditions of temperature, pH, etc., essentially all antibody molecules may be handled

under nearly identical conditions. Similarly, changeover from one enzyme to another often requires totally different linking or immobilizing conditions and a corresponding expenditure of time and effort, whereas the linking or immobilization of two different antibody molecules invariably proceeds with the identical recipe.

V. MEDICAL IMPLICATIONS

The immune system provides us with our most important defense against disease. It is tempting to speculate whether catalytic antibodies could be induced which would provide even wider protection or novel therapy approaches. The idea of selective antibodies which would not only *bind* to bacteria, viruses, cancer cells, etc., but would also *catalyze* their disruption is tantalizing. The future will certainly see attempts made in these directions.

Another possible direction for fruitful research lies in a totally new medical approach to the many diseases resulting from inborn errors of metabolism [96]. There is today limited therapy for patients with hereditary metabolic disorders characterized by insufficient activity of a particular enzyme. Enzyme replacement has been suggested and studied [97], but many difficult problems accompany this treatment, including the short lifetime of the administered enzyme, possible immune response, and access to the desired tissue or organ.

If the immune system of the patient is functioning adequately, one can imagine situations where immunization with a suitable transition-state analog conjugate would elicit a level of antibodies which would mimic the absent or decreased enzyme. Furthermore, the activity of the antibody providing the enzyme-like reactions could be readily controlled by administration of the TS analog, which would act as a powerful "enzyme" inhibitor. Support and therapy of individuals suffering from such diseases may some day result from this approach.

VI. SUMMARY

The motives for mimicking enzymes are twofold [6]: 1) to gain information and deeper understanding which will be relevant to biochemical catalysis, and 2) to extend the chemistry of living organisms in order to be able to invent new reactions—reactions that enzymes either cannot perform, or can perform only with difficulty.

It has taken a long time for the bridge of catalytic antibodies to be erected and join organic chemistry and immunology. This is certainly in part because of differences in language, approach, and goals [98]. Increasingly, many researchers in both disciplines are coming closer as a result of the unifying

nature of molecular biology. The importance of the basic questions being addressed as well as the tremendous potential for application in diverse areas ensures that the recent initial results will be greatly expanded in the future. Concurrent developments in biology, chemistry, and physical techniques may also have great impact on the use of catalytic antibodies. Chimeric antibodies, where the binding site (variable region or hypervariable region) is derived from a mouse and chosen at will, while the majority of the antibody molecule is human-derived, may allow wide human application. Bifunctional antibodies with two different catalytic reactions taking place in each antibody molecule may be considered. The accurate depiction of the details of antigen-antibody interaction from x-ray crystallography [99] and the prediction of structure [100] will greatly assist those planning experiments. The future of "dial-a-property" catalytic antibodies looks promising and exciting.

ACKNOWLEDGEMENTS

The author thanks Professor David Givol for helping to stimulate his initial interest in antibodies. I am grateful to my colleagues Professors Z. Eshhar, I.R. Cohen, D. Sulitzeanu, and Dr. R. Arad-Yellin for valuable discussions and comments. I thank Drs. F. Kohen, M. Werber, Professors M. Sela, and E. Katzir for helpful comments, suggestions, and references. I acknowledge the support of the Agency for International Development US-Israel CDR Program.

REFERENCES

1. Lehn JM: Supramolecular chemistry; receptors, catalysts and carriers. Science 227: 849–856, 1985.
2. Rebek J: Model studies in molecular recognition. Science 235:1478–1484, 1987.
3. Tabushi I: Cyclodextrin catalysis as a model for enzyme action. Accounts Chem Res 15:66–72, 1982.
4. Vogtle F, Sieger H, Muller WM: Complexation of uncharged molecules and anions by crown-type host molecules. Top Curr Chem 98:107–157, 1981.
5. Weber E, Vogtle F: Crown-type compounds—an introductory overview. Top Curr Chem 98:1–35, 1981.
6. Breslow R: Artificial enzymes and enzyme models. Adv Enzymol 58:1–60, 1986.
7. Green BS, Ashani Y, Chipman D (eds): "Chemical Approaches to Understanding Enzyme Catalysis. Biomimetic Chemistry and Transition-State Analogs." Amsterdam: Elsevier, 1981.
8. van Binst G (ed): "Design and Synthesis of Organic Molecules Based on Molecular Recognition" (Solvay Conference on Chemistry). B[illegible] Springer-Verlag, 1986.
9. Werber MM: Synthetic enzymes, past, present and future: Possible diagnostic use. Adv Clin Enzymol 5:123–130, 1987.

10. Schmidtchen FP: Molecular catalysis by polyammonium receptors. Top Curr Chem 132:101–133, 1986.
11. Cram DJ: Cavitands: Organic hosts with enforced cavities. Science 219:1177–1183, 1983.
12. Menger FM, Whitesell LG: A protease mimic with turnover capabilities. J Am Chem Soc 107:707, 708, 1985.
13. Rebek J: Model studies in molecular recognition. Science 235:1478–1484, 1987.
14. Moss RA, Alwis KW, Bizzigotti GO: *o*-Iodosobenzoate: Catalyst for the micellar cleavage of activated esters and phosphates. J Am Chem Soc 105:681, 682, 1983.
15. Landsteiner K: "The Specifity of Serological Reactions." Cambridge, Massachusetts: Harvard University Press, 1945.
16. Pressman D, Grossberg AL: "The Structural Basis of Antibody Specificity." New York: WA Bejamin, 1968.
17. Sinnott ML: Inimitable enzymes? Chem Britain 15:293–297, 1978.
18. Givol D: A structural basis for molecular recognition: the antibody case. In Cuatrecasas P, Greaves MF (eds): "Receptors and Recognition." London: Chapman and Hall, 1976, pp 3–42.
19. Pauling L: Molecular architecture and biological reactions. Chem Eng News 24:1375–1377, 1946.
20. Pauling L: Chemical achievement and hope for the future. Am Sci 36:51–58, 1948.
21. Wolfenden R: Transition state analogs for enzyme catalysis. Nature 223:704, 705, 1969.
22. Wolfenden R: Analog approaches to the structure of the transition state in enzyme reactions. Accounts Chem Res 5:10–18. 1972.
23. Lienhard GE: Enzymatic catalysis and transition-state theory. Science 180:149–154, 1973.
24. Lindquist RN: The design of enzyme inhibitors: Transition-state analogs. In Ariens EJ (ed): "Drug Design." New York: Academic Press, 1975, Vol 5, pp 24–80.
25. Wolfenden R: Reversible analog inhibitors and enzyme catalysis. Anna Rev Biophys Bioeng 5:271–306, 1976.
26. Wolfenden R: Transition-state affinity as a basis for the design of enzyme inhibitors. In Grandour RD, Schowen RL (eds): "Transition States of Biochemical Processes." New York: Plenum Press, 1978, p 555.
27. Coward JK: Transition-state theory and reaction mechanism in drug action and drug design. In Grandour RD, Schowen RL (eds): "Transition States of Biochemical Processes." New York: Plenum Press, 1978, p 579.
28. Lienhard GN: Transition-state analogues. In Sandler M (ed): "Enzyme Inhibitors as Drugs." London: MacMillan Press, 1980, p 43.
29. Andrews PR, Winkler DA: The design and medicinal applications of transition state analogues. In Jolles G, Wooldridge KRH (eds): "Drug Design: Fact or Fantasy?" London: Academic Press, 1984, pp 145–174.
30. Pauling L: personal communication, 1988.
31. Dickey FH: Specific adsorption. J Phys Chem 59:695–707, 1955.
32. Slobin LI: Preparation and some properties of antibodies with specifity towards p-nitrophenyl esters. Biochemistry 5:2836–2844, 1966.
33. Jencks WP: "Catalysis in Chemistry and Enzymology." New York: McGraw-Hill, 1969, p 288.
34. Raso V, Stollar BD: Antibodies specific for conformationally distinct coenzyme-substrate transition state analogs. A fluorescence, nuclear magnetic resonance, circular dichrosim, and antibody study of N-(5-phosphopyridoxyl)-3′-amino-L-tyrosine. J Am Chem Soc 95:1621–1628, 1973.

30

35. Raso V, Stollar BD: The antibody-enzyme analogy: Characterization of antibodies to phosphopyridoxyltyrosine derivatives. Biochemistry 14:584–591, 1975.
36. Raso V, Stollar BD: The antibody-enzyme analogy. Comparison of enzymes and antibodies specific for phosphopyridoxyltyrosine. Biochemistry 14:591–599, 1975.
37. Wilchek M, Givol D, Weinstein Y: Anti-DNP protects DNP ligands against chemical reduction. Isr J Med Sci 13(10):1050 (1977).
38. Burd JF, Carrico RJ, Fetter MC, Buckler RT, Johnson RD: Specific protein binding reactions monitored by enzymatic hydrolysis of ligand-fluorescent dye conjugates. Anal Biochem 77:56–67, 1977.
39. Kohen F, Hollander Z, Burd JF, Boguslaski RC: A steroid immunoassay based on antibody-enhanced hydrolysis of a steroid-umbelliferone conjugate. FEBS Lett 100:137–140, 1979.
40. Kohen F, Kim JB, Barnard G, Lindner HR: Antibody-enhanced hydrolysis of steroid esters. Biochim Biophys Acta 629:328–337, 1980.
41. Green BS: Seminar, Weizmann Institute of Science, Jan. 1978.
42. Green BS: Antibody molecules as enzyme-like catalysts for organic reactions. Biochemical approaches to organic synthesis. Satellite Symposium of 13th European Biochemical Societies, FEBS, Jerusalem:20, 21 Aug. 1980, Abstracts.
43. Knipe AC, Watts WE (eds): "Organic Reaction Mechanisms. 1985. ' Chichester: John Wiley, 1987.
44. Weissberger A (ed): "Techniques of Chemistry." Lewis ES (ed): Part 1, Hammes GG (ed): Part 2: "Investigation of Rates and Mechanisms of Reactions," Vol 6, 3rd Ed. New York: John Wiley, 1974.
45. Lipinski CA: Bioisosterism in drug design. In Bailey DM (ed): "Annual Reports in Medicinal Chemistry," Vol. 21. Orlando: Academic Press, 1986, pp 283–291.
46. Desiraju GRP: "Organic Solid State Chemistry." Amsterdam: Elsevier, 1987.
47. Kohler G, Milstein C: Continuous cultures of fused cells secreting antibody of predetermined specificity. Science 256:495, 1975.
48. Cohen IR: Personal communication, Jan. 1985.
49. Hammond GS: A correlation of reaction rates. J Am Chem Soc 77:334–338, 1955.
50. Kohen F, Kim JB, Lindner HR, Eshhar Z, Green BS: Monoclonal immunoglobin G augments hydrolysis of an ester of the homologous hapten. An esterase-like activity of the antibody combining site? FEBS Lett 111:427–431, 1980.
51. Potter M: Antigen-binding myeloma proteins of mice. Adv Immunol 25:141–211, 1977.
52. Davies DR, Padlan EA, Segal DM: Three-dimensional structure of immunoglobulins. Annu Rev Biochem 44:639–667, 1975.
53. Rao G, Philipp M: TEPC-15, a phosphorylcholine-binding antibody, accelerates the hydrolysis of a specific nitrophenyl ester. Fed Proc 46:Abstract 1634, 1987.
54. Tramontano A, Janda KD, Lerner RA: Chemical reactivity at an antibody binding site elicited by mechanistic design of a synthetic antigen. Proc Natl Acad Sci USA 83:6736–6740, 1986.
55. Tramontano A, Janda KD, Lerner RA: Catalytic antibodies. Science 234:1566–1570, 1986.
56. Pollack SJ, Jacobs JW, Schultz PG: Selective chemical catalysis by an antibody. Science 234:1570–1573, 1986.
57. Padlan EA: Structural basis of the specificity of antibody–antigen reactions and structural mechanisms for the diversification of antigen-binding specificities. Q Rev Biophys 10:35–61, 1977.
58. Jacobs J, Schultz PG, Sugasawara R, Powell M: Catalytic antibodies. J Am Chem Soc 109:2174–2176, 1987.

59. Balan A, Cohen IR, Green BS, Elias D, to be published.
60. Amer H, Green BS, Eshhar Z: SPE-21 monoclonal antibody selectively catalyzes the hydrolysis of a carbonate substrate. Abstracts. Israel Chemical Society 53rd Annual Meeting, Beersheva, 9, 10 Feb 1988; paper in preparation.
61. Haselkorn D, Friedman S, Givol D, Pecht I: Kinetic mapping of the antibody combining site by chemical relaxation spectrometry. Biochemistry 13:2210–2222, 1974.
62. Pressman D, Bryden JH, Pauling L: The serological properties of simple substances. X. A hapten inhibition experiment substantiating the intrinsic molecular assymmetry of antibodies. J Am Chem Soc 67:1219–1222, 1945.
63. Landsteiner K, van der Scheer J: Serological differentiation of steric isomers. J Exp Med 48:315–320, 1928.
64. Landsteiner K, van der Scheer J: Serological differentiation of steric isomers (antigens containing tartaric acids). J Exp Med 50:407–417, 1929.
65. Landsteiner K, van der Scheer J: Note on the serological differentiation of steric isomers. Proc Soc Exp Biol Med 29:1261–1263, 1932.
66. Karush F: The interaction of purified antibody with optically isomeric haptens. J Am Chem Soc 78:5519–5526, 1956.
67. Napper AD, Benkovic SJ, Tramontano A, Lerner RA: A stereospecific cyclization catalyzed by an antibody. Science 237:1041–1043, 1987.
68. Dean PDG, Johnson WS, Middle FA (eds): "Affinity Chromatography. A Practical Approach." Oxford: IRL Press, 1985, pp 108–114.
69. Chaiken IM, Wilchek M, Parikh I (eds): "Affinity Chromatography and Biological Recognition." New York: Academic Press, 1983, p 209.
70. Sugimoto T, Kokubo T, Matsumura Y, Miyazaki J, Tanimoto S, Okano M: Asymmetric reduction of alkyl aryl ketones in the binding domain of bovine serum albumin. Bioorg Chem 10:104–113, 1981.
71. Wilson ME, Whitesides GM: Conversion of a protein to a homogeneous asymmetric hydrogenation catalyst by site-specific modification with a diphosphorhodium(I) moiety. J Am Chem Soc 100:306–307, 1978.
72. Landsteiner K: Ueber einige neure Ergebnisse der Serologie. Naturwissenschaften 18:653–659, 1930.
73. Pressman D, Grossberg AL: "The Structural Basis of Antibody Specificity." New York: WA Benjamin, 1968, pp 112–116.
74. Crumpton MJ, Small PA: Conformation of immunologically-active fragments of sperm whale myoglobin in aqueous solution. J Mol Biol 26:143–146, 1967.
75. Schechter B, Conway-Jacobs A, Sela M: Conformational changes in a synthetic antigen induced by specific antibodies. Eur J Biochem 20:321–324, 1971.
76. Conway-Jacobs A, Schechter B, Sela M: Extrinsic Cotton effect and immunological properties of the p-azobenzenearsonate hapten attached to a helical amino acid copolymer. Biochemistry 9:4870–4875, 1970.
77. Lerner RA: Antibodies of predetermined specificity in biology and medicine. Adv Immunol 36:1–44, 1984.
78. Katchalski-Katzir E, Kenett D: Use of monoclonal antibodies in the study of the conformation and conformational alterations in proteins. Chem Soc Jpn 61:133–139, 1988.
79. Absolom DR, van Oss CJ: The nature of the antigen-antibody bond and the factors affecting its association and dissassociation. In Atassi MZ (ed): "Critical Reviews in Immunology." Boca Raton, Florida: CRC Press, 1986, pp 1–46.
80. Landsteiner K, van der Scheer J: On cross reactions of immune sera to azoproteins. II.

Antigens with azocomponents containing two determinant groups. J Exp Med 67: 709–723, 1938.
81. Kreiter VP, Pressman D: Antibodies to a hapten with two determinant groups. Immunochemistry 1: 151–163, 1964.
82. Balan A, Doctor BP, Green BS, Torten M, Ziffer H: Antibody combining sites as templates for selective organic chemical reactions. JCS Chem Comm 106–108, 1988.
83. Nakanishi H, Jones W, Thomas JM, Hursthouse MB, Motevalli M: Static and dynamic single-crystal X-ray diffraction studies of some solid-state photodimerization reactions. J Phys Chem 85:3636–3642, 1981.
84. Benkirane MM, Dominique B, Cordeil M, Delori P, Delaage MA: Immunization with immune complexes: Characterization of monoclonal antibodies against a TSH-antibody complex. Mol Immunol 24:1309–1315, 1987.
85. Reardon DT, Meares CF, Goodwin DA, McTigue, David GS, Stone MR, Leung JP, Bartholomew RM, Frincke JM: Antibodies against metal chelates. Nature 316:265–268, 1985.
86. May SW, Green BS: Work in progress.
87. Eren D, Green BS, Keinan E: Work in progress.
88. Wilchek M: Personal communication, 1988.
89. Strausbauch PH, Weinstein Y, Wilchek M, Shaltiel S, Givol D: A homologous series of affinity labeling reagents and their use in the study of antibody binding sites. Biochemistry 10:4342–4348, 1971.
90. Haimovich J, Eisen HN, Hurwitz E, Givol D: Localization of affinity-labeled residues on the heavy and light chain of two myeloma proteins with anti-hapten activity. Biochemistry 11:2389–2398, 1972.
91. Knowles JR: Tinkering with enzymes: What are we learning? Science 236:1252, 1987.
92. Strosberg AD, Courand PO, Schreiber A: Immunological studies of hormone receptors: A two-way approach. Immunol Today 75–79, 1981.
93. Jones JB, Sih CI, Perlman D (eds): "Applications of Biochemical Systems in Organic Chemistry," Parts 1 and 2. In Weissburger A (ed): "Techniques of Chemistry," Vol 10. New York: J. Wiley and Sons, 1976.
94. Porter R, Clark S (eds): "Enzymes in Organic Synthesis" (Ciba Foundation Symposium 111). London: Pitman, 1985.
95. Kaiser ET, Radziejewski C: The design of new enzyme active sites for the catalysis of specific chemical reactions. In Porter R, Clark S (eds): "Enzymes in Organic Synthesis" (Ciba Foundation Symposium 111). London: Pitman, 1985, pp 219–230.
96. Stanbury JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS (eds): "The Metabolic Basis of Inherited Disease," Fifth Edition. New York: McGraw-Hill, 1983.
97. Brady RO: Enzyme Replacement Therapy. In Boyer PD (ed): "The Enzymes," 3rd ed. New York: Academic Press, 1983, Vol 16, pp 679–691.
98. Stevenson R: A gulf between chemists and biologists. Chem Britain 23:302, 1987.
99. Amit AG, Mariuzza RA, Phillips SEV, Poljak RJ: Three-dimensional structure of an antigen-antibody complex at 2.8 A resolution. Science 233:747–753, 1986.
100. Chothia C, Lesk AM, Levitt M, Amit AG, Mariuzza RA, Phillips SEV, and Poljak RJ: The predicted structure of immunoglobulin D1.3 and its comparison with the crystal structure. Science 233:755–758, 1986.

NOTE ADDED IN PROOF

In the period since submission of this manuscript, several important papers in the field of antibody-catalyzed chemical reactions have been published, demonstrating the rapidly expanding nature of this research area.

The "interplay between chemistry and biology" as a theme in the design of enzyme-like catalysts has been clearly presented with regard to several antibody-catalyzed organic reactions and other approaches (Schultz PG: The interplay between chemistry and biology in the design of enzymatic catalysts. Science 240:426–433, 1988).

Monoclonal antibodies have been raised against the hapten *20* (Fig. 12). Out of 20 monoclonal antibodies which displayed high affinity for *20* and *23*, five were found to hydrolyze ester *27*. One of these, when tested for esterolytic activity using a variety of substrates, showed even higher activity with *29*, and when the R′ of *29* was replaced by a $-(CH_2)_3CO_2H$ group, the observed rate enhancement relative to uncatalyzed hydrolysis (k_{cat}/k_{uncat}) was 6.25×10^6 (Tramontano A, Ammann AA, Lerner RA: Antibody catalysis approaching the activity of enzymes. J. Am Chem Soc 110:2282–2286, 1988). This demonstration that catalytic antibodies can achieve enzyme-like rate enhancements should provide added incentive for utilizing such systems in biotechnological and other applications.

Monaryl antibodies raised against aryl phosphonamidates were found to catalyze the amidation of corresponding aryl esters in a bimolecular reaction (Janda KD, Lerner RA, Tramontano A: Antibody catalysis of bimolecular amide formation. J Am Chem Soc 110:4835–4837, 1988).

An especially interesting Claisen rearrangement, the transformation of chorismate to prephenate, has also been achieved using a monoclonal antibody raised against a potent inhibitor (designed as a transition-state analog) of the enzyme, chorismate dismutase, which catalyzes this reaction (Jackson DY, Jacobs JW, Sugasawara R, Reich SH, Bartlett PA, Schultz PG: An antibody-catalyzed Claisen rearrangement. J Am Chem Soc 110:4841–4842, 1988). The enzyme catalyzes the rearrangement by a factor of 3×10^6 while one of the anti-hapten monoclonal antibodies displayed a rate enhancement of 1×10^4 under the same reaction conditions. In addition to demonstrating a new antibody-catalyzed reaction, the process may prove important for a better understanding of the enzymatic reaction.

Each of these reactions increases our confidence that catalytic antibodies may be found for a wide range of chemical reactions. These results also raise many interesting questions regarding the detailed mechanisms of the processes whose answers will, along with future work, certainly lead to a much deeper insight into the nature of biocatalysts as well as the ability to

eventually design efficient antibody-based catalysts for essentially any chemical reaction.

The continuing advances in immunology and molecular biology provide novel substances and techniques for achieving molecular compositions that were not previously available and thus contribute directly to new possibilities with catalytic antibodies. For example, the expression of the variable fragment, Fv, of an antibody (McPC603) has recently been achieved (Skerra A, Plückthun A: Assembly of a functional immunoglobulin Fv fragment in *Escherichia coli*. Science 240:1038–1041, 1988). This approach provides relatively simple (MW 26,000) heterodimers which will be of great use in further experiments such as amino acid modification or site-directed mutagenesis.