Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 373–377, January 1993
Biochemistry

catELISA: A facile general route to catalytic antibodies

(enzyme mimic/biocatalyst/direct screening/immunoassay/ester hydrolysis)

DAN S. TAWFIK*†, BERNARD S. GREEN†‡, RACHEL CHAP*, MICHAEL SELA*, AND ZELIG ESHHAR*

*Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel; and †Department of Pharmaceutical Chemistry, The Hebrew University, School of Pharmacy–Faculty of Medicine, P.O. Box 12065, Jerusalem 91120, Israel

Contributed by Michael Sela, September 21, 1992

ABSTRACT **The low abundance and activity of catalytic antibodies are major obstacles to their selection from the virtually unlimited repertoire of antibody binding sites. The requirement for new screening methodologies is further emphasized by the availability of combinatorial libraries, in which a functional polypeptide has to be selected out of millions of possibilities. We present a simple and sensitive screening approach (termed catELISA) based on immobilized substrates and immunodetection of the end product of the catalyzed reaction. The feasibility of catELISA is demonstrated here by the generation of potent ester-hydrolyzing antibodies by direct screening of hybridoma supernatants. We show that this approach is not only facile but general: it is not limited by type of reaction, substrate, or catalyst (enzymes, catalytic antibodies, chemical catalysts). catELISA opens a route to catalytic antibodies that replaces existing lengthy and arduous methods, thus allowing us to expand their number and improve their quality and to address questions that would otherwise be difficult to answer.**

The ability of catalytic antibodies to be tailor-made to a predesigned substrate, combined with rate enhancement and turnover, has led to the most intriguing enzyme mimics described thus far (1–3). Catalytic antibodies are elicited against a hapten, typically a stable synthetic analog of the transition state (TS) of the catalyzed reaction. The repertoire resulting after immunization is immortalized as hybridomas (4), which are then screened to select those clones producing monoclonal antibodies that bind the hapten. The direct screening of culture supernatants of these hybridomas for antibody catalysis was heretofore not possible due to relatively high background reaction, the generally low catalytic efficiency of antibodies, and contaminating enzymes that catalyze the same reaction (5). Therefore, to detect catalytic activity, large quantities (usually from ascites fluid) of purified monoclonal antibodies are needed. Only a few, and occasionally none, of the dozens of clones that bind a hapten are catalytic; it is therefore widely recognized that these inefficient and labor-intensive procedures must be replaced by rapid and direct screening procedures (2, 3, 6–8, 27). Novel, nonhybridoma, methodologies, such as combinatorial variable-region cloning in phage (9, 10), were also used to generate antibodies. Yet, as noted (11), future applications of these methodologies for obtaining catalytic antibodies depend upon appropriate screening.

Analysis of the unique problems involved in direct screening of hybridoma supernatants for antibody-mediated catalysis (5) led us to *catELISA*, an assay involving a substrate-protein conjugate immobilized on microtiter plates. Antibody-catalyzed conversion of any "solid-phase" substrate to a product is then detected by ordinary ELISA, using binding anti-product antibodies (Fig. 1). Employing catELISA, we were able to rapidly screen thousands of hybridoma clones elicited against both a phosphonate TS analog and an amide substrate to detect catalytic cleavage of the corresponding p-nitrobenzyl ester.

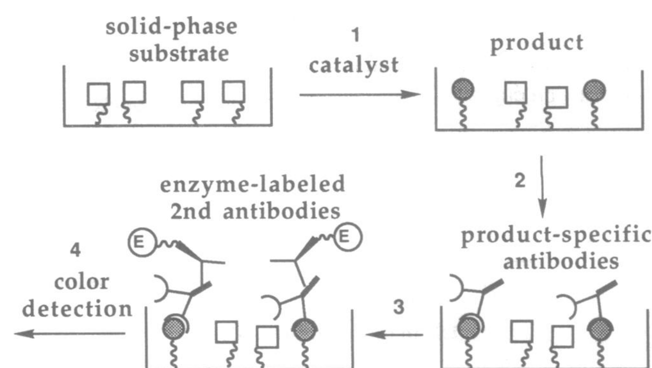

FIG. 1. Schematic presentation of catELISA.

MATERIALS AND METHODS

Preparation of Substrates and Haptens. All of the synthesized substances were purified to homogeneity (judged by thin-layer chromatography and NMR) by crystallization or silica column chromatography. Structures were confirmed by NMR and mass spectrum; satisfactory elemental analyses were obtained for all crystalline compounds. Esters **1a**, **1b**, and **1e** and amide **2** (Fig. 2) were prepared by the following procedures: (*i*) allowing the corresponding alcohol or amine to react with glutaric anhydride in the presence of a base (**1b**, ethanol, sodium ethoxide, reflux; **1a** and **1e**, p-nitrobenzyl alcohol or o-nitrobenzyl alcohol, 1,8-diazabicyclo[5.4.0]-undecane (DBU); **2**, p-nitrobenzylamine; Et_3N); (*ii*) coupling of t-butyl glycinate by using the acid chloride prepared with thionyl chloride; and (*iii*) removal of the t-butyl ester in the presence of trifluoroacetic acid. The methyl ester **1c** was prepared by esterification of N-glutaryl-O-benzylglycine with diazomethane, and the 2-fluoroethyl ester **1d** was prepared by esterification with 2-fluoroethanol in the presence of thionyl chloride; removal of the benzyl esters by catalytic hydrogenation gave the products. Imide **5** was prepared by heating glutaric anhydride and glycine benzyl ester in the presence of acetic anhydride; the benzyl ester was then removed by catalytic hydrogenation. Substrates were conjugated to BSA or KLH via their N-hydroxysuccinimide esters.

For the preparation of the protein conjugates of carboxylic acid **3** (product), the corresponding p-iodophenyl ester **1f** was synthesized: t-butyl glycinate was added to glutaric anhy-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: catELISA, catalytic assay using ELISA; BSA, bovine serum albumin; KLH, keyhole limpet hemocyanin; TS, transition state.

‡To whom reprint requests should be addressed.

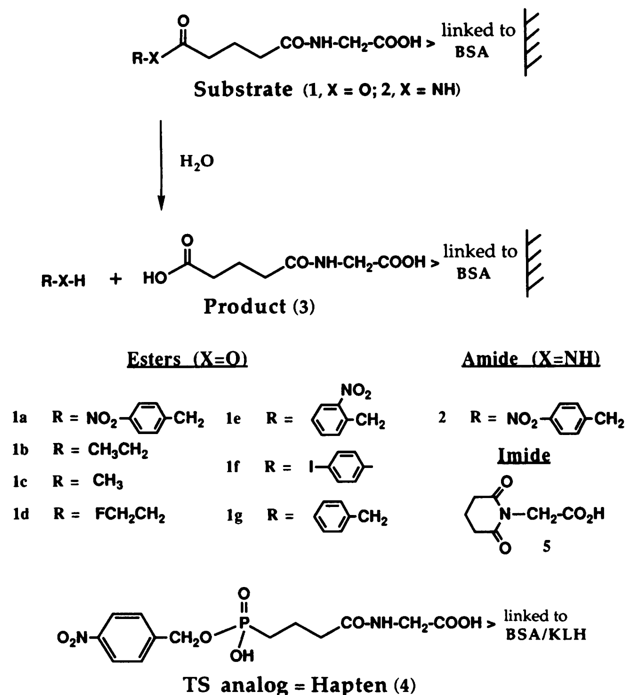

FIG. 2. Structures of substrates, **1a–g**, **2**, and **5**, the product of their hydrolysis, acid **3**, and the hapten, **4**. All of these substances were linked to a carrier protein [bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH)] via the carboxyl group; the protein conjugates appear in the text as, for example, **1a**-BSA.

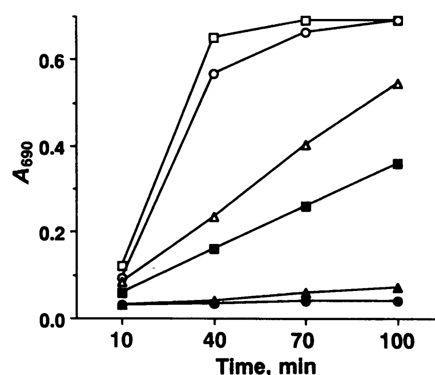

FIG. 3. Base- and enzyme-catalyzed hydrolysis of ethyl ester **1b**-BSA was followed by catELISA. Conjugate **1b**-BSA, coated on microtiter plates (0.5 μg/ml) was treated for increasing time periods with lipase (□, 1.4 mg/ml; ○, 0.35 mg/ml; △, 0.07 mg/ml) diluted in PBS; 0.1 M Na_2CO_3, pH 10.9 (■); TBS, pH 8.25 (●); or p-nitrobenzyl ester-hydrolyzing antibody D2.3, 50 nM (▲).

dride, and the resulting mono acid was then coupled to p-iodophenol by using N,N'-dicyclohexylcarbodiimide. After removal of the t-butyl ester the resulting **1f** was coupled to protein (via the N-hydroxysuccinimide ester) and then hydrolyzed (0.1 M sodium carbonate, 3 hr) to give the carboxylic acid **3**-BSA and **3**-KLH. The synthesis and conjugation of phosphonate **4** are described elsewhere (12).

catELISA. Anti-product antibodies were prepared by immunizing rabbits with the KLH conjugate of carboxylic product **3** [hapten density = 17 per carrier molecule (100 kDa); 100 μg per rabbit, emulsified in complete Freund's adjuvant (CFA), followed by a boost, 14 days later, in incomplete Freund's adjuvant (IFA)]. The sera taken after 24 days exhibited high binding affinity to the product (**3**-BSA, hapten density = 10, titer = 1:5000) and minimal crossreactivity (<10% at 1:5000 dilution) with the various substrate conjugates (**1a–d**, **2**, and **5**-BSA). For the catELISA, microtiter plates (Nunc, Maxisorb) were coated with **1a**-BSA (hapten density = 15, 1.0 μg/ml, 1 hr) and then blocked with BSA (1 mg/ml, 0.5 hr). The hybridoma supernatants (50 μl) were incubated for 3 hr and the plates were then washed with phosphate-buffered saline (PBS; 10 mM sodium phosphate/ 0.14 M NaCl, pH 7.4) + 0.04% Tween 20. The rabbit anti-product **3** serum was then added (1:5000 dilution in PBS + 0.04% Tween 20, 1 hr). After washings and incubation with peroxidase-linked mouse anti-rabbit immunoglobulin antibodies (Jackson ImmunoResearch, diluted 1:5000 in PBS) the substrate, 2,2'-azinobis(3-ethyl benzthiazolinesulfonic acid) (ATBS), was added and the absorbance at 690 nm was measured. Base (0.1 M sodium carbonate, pH 10.9) or lipase (porcine pancreatic, crude extract, Sigma L-3126, 50 μg/ml) was used as a positive standard for hydrolysis.

The conditions for screening supernatants for hydrolysis of the various substrates were optimized so that the background signal in the absence of a catalyst is minimized and the positive signal observed after complete cleavage with sodium carbonate or lipase is maximal. Thus, different concentrations of conjugates were used for coating the plates for each of the substrates (0.1–5 μg/ml). Optimal results were obtained with substrate-BSA conjugates having hapten densities of 10–20.

Hybridomas and Monoclonal Antibodies. BALB/c mice were immunized (foot pad injection) with **4**-KLH (hapten density = 16, 50 μg per mouse in complete Freund's adjuvant). After 14 days, a boost was administered (50 μg per mouse in incomplete Freund's adjuvant) and two more injections (i.p., 50 μg per mouse) were given 4 and 3 days before fusion (45 days from immunization) with NSO cells. A short immunization protocol was used with **2**-KLH (hapten density = 16); three BALB/c and three C57BL/6 mice were immunized (50 μg per mouse in complete Freund's adjuvant). After 17 days a boost in incomplete Freund's adjuvant was given, and 3 days later the draining lymph node cells were fused with NSO cells. Two weeks after fusions, the supernatants of the resulting hybrid clones were screened by catELISA. Positive clones were propagated as ascites in BALB/c mice. The antibodies were purified by staphylococcal protein A (Pharmacia) affinity chromatography and dialyzed against Tris-buffered saline [TBS; 50 mM tris(hydroxymethyl)aminomethane/0.14 M NaCl], pH 8.25 or 9.0. Homogeneity of the purified antibodies was judged by SDS/PAGE, which yielded only heavy and light chains under reducing conditions, with Coomassie blue staining. For details concerning fusions, growth of hybridoma cell lines, immunoassays, and production of monoclonal antibodies in ascites fluid, see refs. 13 and 14.

Kinetic Analyses. The catalytic activity of the protein A-purified antibodies was determined by measuring the appearance of p-nitrobenzyl alcohol and the disappearance of substrate **1a** in homogeneous solutions, using HPLC for detection and quantification. The substrate, in dimethyl sulfoxide, was added to the antibody in TBS, pH 8.25 or 9.0 (final organic solvent <1%). Aliquots of the reaction mixtures were quenched (acetonitrile + 0.1% trifluoroacetic acid) and analyzed by HPLC (RP-8, 100 × 5 mm column; 27:73, water/acetonitrile + 0.1% trifluoroacetic acid; flow 1.0 ml/ min; detection by absorbance at 277 nm; retention times for substrate **1a**, 6.9 min, and for the product, p-nitrobenzyl alcohol, 4.1 min). The concentration of the antibodies (Ab_o) was determined by active site titration with **4** (as **4**-N-benzylamide). The uncatalyzed rate of hydrolysis (k_{uncat}) of ester **1a**, determined by initial rate analysis and extrapolated to zero buffer concentration, is 2.8×10^{-5} min^{-1} at pH 8.25. The activities of the catalytic antibodies with the o-nitrobenzyl and benzyl esters (**1e** and **1g**, respectively; 1 mM) were studied by HPLC; no rate enhancement was observed in the presence of the purified antibodies (0.3–5 μM).

RESULTS AND DISCUSSION

The system presented here was designed to study a variety of antibody-catalyzed hydrolytic reactions. Cleavage of immobilized esters **1a–g**, amides (e.g., **2**), or imide **5** leaves the carboxylic acid product **3** bound to the solid phase (Fig. 2). Anti-product **3** polyclonal antibodies which do not cross-react with any of the substrate conjugates were used for detection.

To establish the feasibility of the catELISA system we studied the enzyme- or base-catalyzed hydrolysis of esters **1a–d** and imide **5**. The BSA conjugates of these substrates, coated on microtiter plates, were treated with mild base (sodium carbonate, 0.1 M, pH 10.9) or pancreatic lipase for various time periods. Formation of the resulting acid product **3** was then determined by conventional ELISA using the rabbit anti-**3** antibodies followed by peroxidase-linked, anti-rabbit immunoglobulin antibodies (Fig. 3). No signal is observed with amides—e.g., **2**—which represent substrates that are stable to base or to lipase-catalyzed hydrolysis. As expected, imide **5** is hydrolyzed in the presence of sodium carbonate ($t_{1/2}$ = 45 min compared with 90 min for the methyl ester, **1c**) yet not by the lipase. The rates of hydrolysis of esters **1a–d** in the presence of various dilutions of lipase reach a maximum at relatively low enzyme concentration (Fig. 3). This is ascribed to the very small amount of substrate that is coated on the solid phase (ca. 10–50 pmol per well). The kinetics of the enzyme- and base-catalyzed reactions shows that as little as 5% of substrate conversion to product can be measured. The activity of less than 0.01 unit of lipase can be readily detected even with ester substrates such as **1a–d**, which are far from optimal structures for this enzyme. These and additional experiments indicated that catELISA is sensitive and selective and can be applied for the direct screening of catalytic antibodies in hybridoma supernatants.

To generate catalytic antibodies we used a phosphonate hapten primarily because of the demonstrated ability of phosphonoesters to mimic the tetrahedral, negatively charged, TS/intermediate of ester hydrolysis and to elicit ester-hydrolyzing antibodies (1). Spleen cells from a mouse immunized with the *p*-nitrobenzyl phosphonoester conjugate, **4**-KLH, were fused with myeloma cells, and the resulting 1570 hybrid clones (970 of which were hapten-binding clones) were assayed by catELISA for their ability to induce hydrolysis of ester **1a** and amide **2**. Representative data from the screening of the initial hybridoma supernatants are given in Fig. 4; as can be seen, although dozens of clones bind the phosphonate hapten, only two scored positive in the catELISA. Altogether, 9 clones that catalyze the cleavage of ester **1a** were identified.

Control experiments provided conclusive evidence that the catELISA signal observed in the presence of the hybridoma supernatants is indeed due to antibody-catalyzed hydrolysis of the *p*-nitrobenzyl ester substrate, **1a**. None of the selected clones gave a signal in catELISA when esters other than the *p*-nitrobenzyl ester (**1b–d**) were used as substrates (e.g., antibody D2.3 on **1b**, Fig. 3). The affinity pattern of the catalytic site (Fig. 5) is as expected for an antibody elicited against hapten **4**—i.e., hapten **4** > "short" hapten > amide substrate > *p*-nitrobenzyl alcohol (product). Indeed, the same order of affinity was observed when the inhibition of binding of these antibodies to the hapten-BSA conjugate was measured by ordinary competitive inhibition immunoassay (CIEIA; for a similar analysis, see ref. 13). Product inhibition, pH-activity profiles, substrate specificity, and other properties that characterize the overall quality of these antibodies as catalysts were all conveniently determined by catELISA prior to the purification of the antibodies (data not shown).

Purified antibodies from ascites fluid were assayed by HPLC for their ability to catalyze the hydrolysis of the free, unconjugated *p*-nitrobenzyl ester **1a** (Fig. 6). The results confirmed the catELISA selection. The identification of clones exhibiting relatively low rate accelerations (e.g., an-

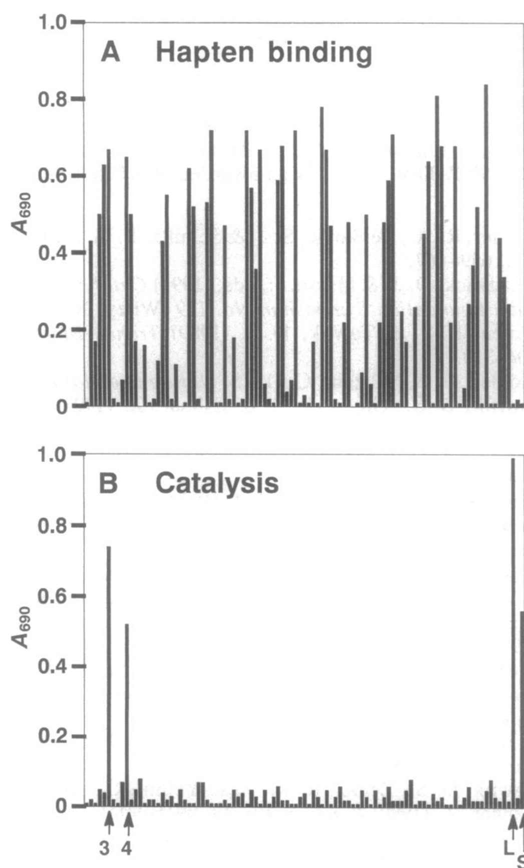

FIG. 4. Results of screening of one microculture plate (96 hybrid clones, each represented by a bar) for hapten binding and for catalytic activity. Hapten binding (*A*) was determined by ELISA using 50 μl of hybridoma supernatants in microtiter plates coated with **4**-BSA (1 μg/ml); catELISA (*B*) was performed with the *p*-nitrobenzyl ester substrate **1a**-BSA. The last three bars are controls: L, lipase (A_{690} = 1.0); TBS, pH 8.3; and S, 0.1 M sodium carbonate. Two catalytic clones, D2.3 (3) and D2.4 (4) were identified from this plate.

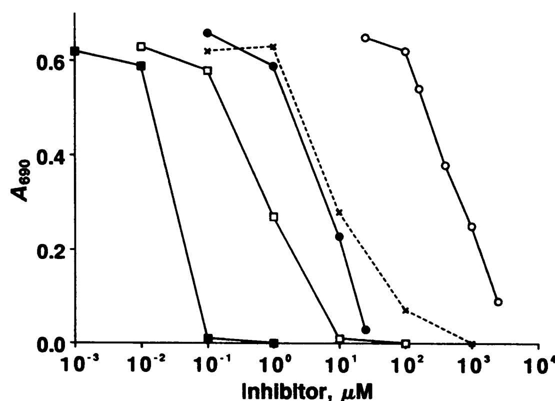

FIG. 5. Inhibition pattern of esterolytic activity of D2.3 determined by catELISA. Antibody D2.3 (protein A-purified preparation, 20 nM in TBS, pH 8.25) was incubated on microtiter plates coated with **1a**-BSA in the presence of various dilutions of the following: ■, phosphonate hapten **4** (as **4**-*N*-benzylamide); □, a "short" hapten (mono-*p*-nitrobenzyl methyl phosphonate); ●, amide substrate **2** (**2**-*N*-methylamide); and ○, *p*-nitrobenzyl alcohol. ×, Inhibition of the signal was also observed when the added rabbit sera (applied after incubation with the catalytic antibody) contained product **3** (**3**-*N*-methylamide).

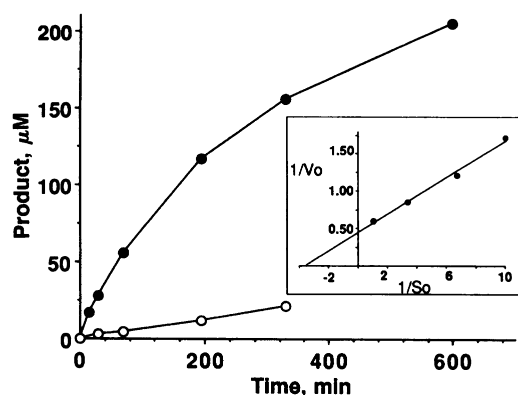

FIG. 6. Hydrolysis of the unconjugated ester substrate **1a** (0.3 mM) by monoclonal antibody D2.3 (0.3 μM) (●) was determined by HPLC; activity is inhibited in the presence of hapten **4** (4-*N*-benzylamide, 1.0 μM) (○). (*Inset*) Kinetic parameters were determined by a Lineweaver–Burk analysis performed with **1a** at pH 8.25 (Ab_o = 0.3 μM; $1/S_o$ is given in mM^{-1} and $1/v_o$ in min·μM^{-1}).

tibody clone D2.1: $k_{cat}/k_{uncat} \approx 700$, at pH 9.0) underlines the sensitivity and selectivity of the catELISA; neither "background" hydrolysis of the ester substrate nor the presence of enzymes in the hybridoma supernatants interfered. catELISA indicated that solid-phase esters **1a–d** are stable in the presence of most esterases that hydrolyze the soluble, unconjugated esters (e.g., porcine liver esterase, acetylcholinesterase). Using a solid-phase substrate affords two additional distinct advantages: the catalytic antibody has increased affinity for the substrate that includes the protein carrier structure (i.e., lower K_m values), and the relatively low substrate concentration ensures that pseudo-first-order conditions are maintained ($K_m >> S_o$). We have previously shown that these two factors directly increase the ability to detect antibody-mediated catalysis (5).

Another antibody, D2.3, exemplifies a truly efficient, enzyme-like catalyst (Fig. 6; $k_{cat} = 7.35$ min^{-1}, $K_m = 0.28$ mM, at pH 8.25), exhibiting not only significant rate enhancement ($k_{cat}/k_{uncat} = 2.6 \times 10^5$) with an unactivated ester substrate but also multiple turnovers (>1000). Turnover—i.e., the ability of a single binding site to repetitively process substrate molecules—is often found to be limited by severe product inhibition in catalytic antibodies (for examples, see refs. 15–20). In spite of the presence of the nitrophenyl group (15), the *p*-nitrobenzyl alcohol product binds to antibody D2.3 with low enough affinity (K_d = 52 μM) to allow efficient turnover (Fig. 6). D2.3 is also characterized by its notable substrate specificity; it recognizes not only the *p*-nitrobenzyl moiety (i.e., no catalysis is observed with the benzyl or *o*-nitrobenzyl esters, **1g** and **1e**) but also the acyl part of the substrate: the k_{cat}/K_m value with *p*-nitrobenzyl acetate was estimated to be less than 10 $sec^{-1} \cdot M^{-1}$, compared with 440 with *N*-(*O*-*p*-nitrobenzylglutaryl)glycine (**1a**). Direct screening, therefore, not only can save the effort of handling hundreds of hapten-binding noncatalytic clones, but also it should increase the variety and quality of the selected catalytic antibodies.

Because phosphonates were suggested as haptens for generating amide or peptide bond-hydrolyzing antibodies (21, 22), we screened hybrid clones elicited against **4**-KLH for hydrolysis of the amide substrate, **2** (2430 clones, comprising the 1570 clones referred to above and an additional 860 clones from a second fusion). None of the hybridoma clones afforded antibodies which cleave this substrate. Catalyzing the hydrolysis of an amide bond is far more demanding than catalyzing the hydrolysis of an ester bond and probably requires the use of a better mimic of the TS than a phosphonoester structure. Formation of the TS or intermediate involved in amide hydrolysis is significantly different from that of ester hydrolysis and includes, in the rate-determining step, protonation of the amino leaving group. The design and synthesis of new TS analogs is therefore still a key element in this field.

catELISA may open new routes in a rapidly evolving field and clarify some fundamental issues, such as the following: Can a ground state substrate induce the formation of antibodies that catalyze its cleavage (23)? Is the repertoire of antibodies elicited against a single hapten sufficiently diverse so that making haptens that better mimic the transition state is unnecessary? (See refs. 24 and 25 and other references in ref. 2). We have screened a repertoire of hybridoma clones elicited against a substrate, *p*-nitrobenzylamide, **2**-KLH. None of the 890 clones (out of which 220 bind this hapten) were found to catalyze the hydrolysis of the amide itself (**2**-BSA) or the corresponding ester, **1a**. This result suggests that ground state molecules probably do not efficiently select for B-cell clones which make antibodies having catalytic properties and that immunization with TS analogs is indeed demanded.

The paradigm of Jencks (26), ". . . it should be possible to synthesize an enzyme by . . . [preparing] an antibody to a haptenic group which resembles the transition state of a given reaction . . ." is proving to be very fruitful for an increasing variety of model reactions (1–3). The use of improved transition state analogs and novel antibody production technologies combined with direct screening methodologies such as that presented here promises to afford useful antibody-based catalysts for more demanding chemical reactions and challenging biomedical applications.

We thank Dr. George Lowell for his interest and help. We are grateful to Prof. Israel Shechter for his helpful comments. This research was supported by U.S. Army Medical Research Grant DAMD17-90-Z-0010. D.S.T. is an awardee of a Clore Scholarship.

1. Lerner, R. A., Benkovic, S. J. & Schultz, P. G. (1991) *Science* **252**, 659–667.
2. Chadwick, D. J. & Marsh, J., eds. (1991) *Catalytic Antibodies, Ciba Foundation Symposium No. 159* (Wiley, New York).
3. Green, B. S. & Tawfik, D. S. (1989) *Trends Biotechnol.* **7**, 304–310.
4. Köhler, G. & Milstein, C. (1975) *Nature (London)* **256**, 495.
5. Tawfik, D. S., Green, B. S. & Eshhar, Z. (1992) *Anal. Biochem.* **202**, 35–39.
6. Schultz, P. G. (1989) *Angew. Chem. Int. Ed. Engl.* **28**, 1283–1444.
7. Lerner, R. A. & Benkovic, S. J. (1988) *Bioessays* **9**, 107–112.
8. Schultz, P. G., Lerner, R. A. & Benkovic, S. J. (1990) *Chem. Eng. News* **68** (22), 26–40.
9. Winter, G. & Milstein, C. (1991) *Nature (London)* **349**, 293–299.
10. Huse, W. D., Sastry, L., Iverson, S., Kang, A. S., Alting-Mees, M., Burton, D. R., Benkovic, S. J. & Lerner, R. A. (1989) *Science* **246**, 1275–1281.
11. Iverson, S. A., Sastry, L., Huse, W. D., Sorge, J. A., Benkovic, S. J. & Lerner, R. A. (1989) *Cold Spring Harbor Symp. Quant. Biol.* **54**, 273–281.
12. Tawfik, D. S., Eshhar, Z. & Green, B. S. (1992) *Phosphorus and Sulfur*, in press.
13. Tawfik, D. S., Zemel, R. R., Arad-Yellin, R., Green, B. S. & Eshhar, Z. (1990) *Biochemistry* **29**, 9916–9921.
14. Eshhar, Z. (1985) in *Hybridomas in Biotechnology and Medicine*, ed. Springer, T. (Plenum, New York), pp. 1–41.
15. Benkovic, S. J., Adams, J. A., Borders, C. L., Jr., Janda, K. D. & Lerner, R. A. (1990) *Science* **250**, 1135–1139.
16. Martin, M. T., Napper, A. D., Schultz, P. G. & Rees, A. R. (1991) *Biochemistry* **30**, 9757–9761.
17. Janda, K. D., Ashley, J. A., Jones, T. M., McLeod, D. A., Schloeder, D. M., Weinhouse, M. I., Lerner, R. A., Gibbs, R. A., Benkovic, P. A., Hillhorst, R., & Benkovic, S. J. (1991) *J. Am. Chem. Soc.* **113**, 291–297.
18. Iverson, B. L., Cameron, K. E., Jahangiri, G. K. & Pasternak, D. S. (1990) *J. Am. Chem. Soc.* **112**, 5320–5323.

19. Cochran, A. G. & Schultz, P. G. (1990) *Science* **249,** 781–783.
20. Jacobsen, J. R., Prudent, J. R., Kochersperger, L., Yonkovich, S. & Schultz, P. G. (1992) *Science* **256,** 365–367.
21. Lerner, R. A. (1984) *Adv. Immunol.* **36,** 1–44.
22. Pollack, S. J., Hsuin, P. & Schultz, P. G. (1989) *J. Am. Chem. Soc.* **111,** 5960–5961.
23. Paul, S., Volle, D. J., Beach, C. M., Johnson, D. R., Powell, M. J. & Massey, R. J. (1989) *Science* **244,** 1158–1162.
24. Blackburn, G. M., Kingsbury, G., Jayaweera, S. & Burton, D. R. (1991) in *Catalytic Antibodies, Ciba Foundation Symposium No. 159*, eds. Chadwick, D. J. & Marsh, J. (Wiley, New York), pp. 211–226.
25. Paul, S., Johnson, D. R. & Massey, R. J. (1991) in *Catalytic Antibodies, Ciba Foundation Symposium No. 159*, eds. Chadwick, D. J. & Marsh, J. (Wiley, New York) pp. 156–173.
26. Jencks, W. P. (1969) *Catalysis in Chemistry and Enzymology* (McGraw–Hill, New York), p. 288.
27. Green, B. S. (1989) *Adv. Biotechnol. Processes* **7,** 359–393.