Antibody Catalysis of Carbon–Carbon Bond Formation and Cleavage

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Nearly a century ago, the lock-and-key description of enzyme action was formulated by Emil Fischer.[1] When modified to account for the possibility of ligand and protein conformational changes, this simple metaphor still conveys the basic principle of catalysis: that each enzyme must possess an "active site" tailored for recognition and stabilization of the rate-limiting transition state of the reaction it promotes. This metaphor can also be applied to the field of catalytic antibodies,[2] in which rationally designed haptens unlock the catalytic potential of the humoral immune system.

Preparation of catalytic antibodies exploits the immune system's sophisticated ability to produce selective binding pockets for almost any molecule of interest. To induce a binding site with a topology and stereoelectronic environment suitable for catalysis, a stable molecule which mimics the structure of the short-lived transition state of the target reaction is employed as a hapten. This approach to tailored catalyst molecules is very general, since transition-state analogs for many reaction types can be designed using an understanding of chemical mechanism and the basic principles of physical organic chemistry. In the last six years, well over 50 reactions have been accelerated by antibodies.[2] These include simple hydrolytic reactions, pericyclic processes lacking biological counterparts, and transformations that are otherwise chemically disfavored. In addition to transition-state analog design, two less widely-appreciated factors are also critical to the success of a catalytic antibody experiment: the use of monoclonal antibodies and the application of effective screening methods. Given the many ways in which an asymmetric molecule can be bound by a protein, only a small fraction of the many different antibodies that recognize an individual hapten may possess the desired catalytic properties. Rapid assay methods substantially increase the probability of finding an efficient catalyst by facilitating the preliminary evaluation of large numbers of possible candidates, while monoclonal antibody technology permits the reproducible study of individual immunoglobulins in pure form.

The field of catalytic antibodies has been extensively reviewed in recent years.[2] Rather than provide another broad overview of this subject, the present Account will focus on a few transformations involving the formation and cleavage of carbon–carbon bonds. Although the availability of a large number of well-characterized transition-state analogs for hydrolytic reactions has made them the primary targets of antibody catalysis to date, reactions in which carbon atoms are connected or disconnected may be of greater intrinsic interest to the synthetic organic chemist. These transformations are crucial to the survival of all living organisms. They are also central to *in vitro* synthetic strategies leading to the construction of useful molecules of all kinds, from relatively simple commodity chemicals to novel polymeric materials and densely-functionalized natural products. Because the regio- and stereoselectivity of C–C bond forming reactions is often difficult to control using conventional methodologies, the creation of tailored antibody catalysts may yield useful new tools for asymmetric synthesis. For example, the aldol reaction remains one of the most powerful methods for forming C–C bonds; antibodies with significant aldolase activity would greatly extend current chemoenzymatic approaches[4] to the synthesis of sugars and other compounds via this reaction.

Aside from their practical value, reactions in which C–C bonds are made or broken are also mechanistically interesting and can be used to illuminate some of the basic strategies which enzymes exploit to reduce free energies of activation. Enzymes are thought to achieve their remarkable catalytic effects through a combination of substrate destabilization, proximity effects, and chemical catalysis mediated by appropriately positioned nucleophiles, general acids and bases, metal ions, and other diverse cofactors.[5] However, the contribution of individual factors to the overall efficiency of an enzyme-catalyzed reaction has been difficult to measure experimentally because of the inherent complexity of these systems. Catalytic antibody technology now makes it possible to examine specific mechanistic issues in the absence of competing effects by using tailor-made active sites and model reactions that are particularly sensitive to one factor or another. The knowledge gained in the course of these studies has important implications for the (re)design of transition-state analogs. This knowledge is also essential for the development of general

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rules that will guide and improve future efforts to capitalize on the immune system's "hidden" capacity for catalysis.

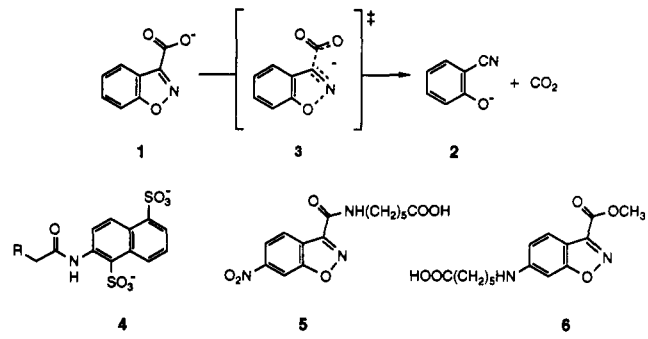
Decarboxylations and Substrate Destabilization

Carboxylation and decarboxylation reactions are crucial components of aerobic and anaerobic metabolism, as exemplified by the fixation of carbon dioxide in photosynthesis and the decarboxylation of α- and β-ketoacids in the Krebs cycle. Most enzymes that promote these reactions require metal ions or a cofactor such as thiamine pyrophosphate to effect catalysis.[6] However, substrate destabilization (through desolvation of the negatively charged carboxylate moiety bound at the active site) is also thought to contribute to the overall catalytic efficiency of these agents.[6,7] Lienhard showed some years ago that the thiamine-catalyzed decarboxylation of α-ketoacids could be greatly enhanced by transferring the reaction from water to nonaqueous solvents.[7] More recently, crystallographic studies of histidine decarboxylase from *Lactobacillus 30a* have established that the carboxylate binding site is predominantly hydrophobic.[8]

To probe the importance of medium effects and desolvation in catalysis, we have examined a mechanistically simple decarboxylation reaction.[9] The unimolecular conversion of 3-carboxybenzisoxazoles (1) into salicylonitriles (2) is a concerted, intermediateless process that proceeds through a charge-delocalized transition state (3)[10] (see Scheme I). This reaction is remarkably sensitive to solvent microenvironment; its reaction rate increases as much as 10^8-fold upon transfer of the reactant from aqueous solution to aprotic dipolar solvents. This dramatic range of reactivity has been attributed mainly to two factors: destabilization of the ground-state carboxylate upon removal of hydrogen-bonding interactions with water, and concomitant stabilization of the charge-delocalized transition state in organic solvents through dispersion interactions.[10] The rate-retarding effect of ion-pair formation in solvents of low dielectric constant has also been noted.[10,11]

We employed the napthalene disulfonate derivative 4 to generate a number of monoclonal antibodies capable of catalyzing the decarboxylation of 5-nitro-3-carboxybenzisoxazole.[9] The best of these, 21D8, accelerates this reaction 19 000-fold over the rate in aqueous buffer. The successful hapten (4) combined a large aromatic moiety and anionic substituents in order to elicit an appropriately-sized apolar pocket containing complementary positively-charged residues that might facilitate anion binding. In related experiments with haptens 5 and 6, which contain a neutral amide and ester in place of the carboxylate group, no catalysts

Scheme I



1 3 2

4 5 6

were obtained.[9] In the latter cases, it is possible that the available binding energy was insufficient to bury the substrate carboxylate in a hydrophobic pocket.

Like a typical enzyme, 21D8 displays saturation kinetics and multiple turnovers. The cleavage of the carbon–carbon bond is fully rate-determining, as judged by measuring the carbon kinetic isotope effect (k^{12}/k^{13}) on the rate of the antibody-catalyzed decarboxylation.[12] Moreover, similar carbon kinetic isotope effects for the reaction in water, in dioxane, and in the active site of 21D8 indicate that transition-state structure does not change significantly when the solvent microenvironment is altered,[12] even though the rate of reaction varies by a factor of $>10^4$.

Decarboxylation of 3-carboxybenzisoxazoles is not subject to general acid–base catalysis or to stereochemical constraints.[10] Hence, the large rate acceleration provided by the antibody can be ascribed almost entirely to medium effects. This conclusion is supported by experiments using fluorescent hapten derivatives as reporter groups. These studies showed that the antibody binding pocket is apolar and virtually inaccessible to water when occupied by a ligand.[9] Analysis of the thermodynamic activation parameters also indicates that antibody catalysis of the decarboxylation, like the solvent-associated rate acceleration, is due to a more favorable enthalpy of activation ($\Delta\Delta H^{\ddagger} = -8.1$ kcal/mol). Unlike reactions in organic solvents, the antibody's entropy of activation is significantly less favorable than that of the spontaneous reaction in water ($-T\Delta\Delta S^{\ddagger} = +2.7$ kcal/mol).[9] These findings suggest that the antibody provides a rigid microenvironment for the reaction. Unlike free solvent, this environment cannot relax as the charge-delocalized transition state is approached.

Because the antibody's discrete and chemically well-defined structure is amenable to genetic alteration through site-directed mutagenesis, the specific factors influencing its ability to desolvate the substrate and accelerate the chemical reaction (e.g., dipolarity, electrostatics, and hydrogen bonding) can now be evaluated in a systematic fashion. It may also be possible to increase the potency of the catalyst through protein engineering. Multiparametric analysis of the rates at which 3-carboxybenzisoxazoles decarboxylate in 20 solvents having widely different properties indicates that solvent hydrogen bond acidity is likely to be the dominant rate-retarding factor.[13] If the rate enhance-

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(13) Grate, J. W.; McGill, R. A.; Hilvert, D. *J. Am. Chem. Soc.*, in press.

ment achieved by 21D8 is limited by interactions between the carboxylate and a hydrogen bond donor at the active site, repositioning or removing this residue to prevent the deleterious interactions might augment activity without compromising substrate binding.

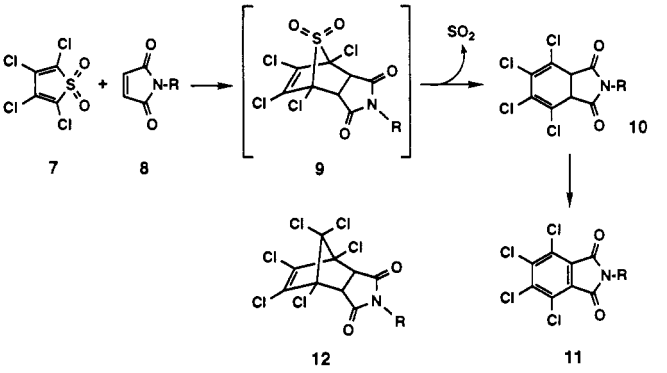
Many important chemical transformations, including aldol condensations, S_N2 substitutions, E2 eliminations, and even some hydrolytic reactions, can be greatly accelerated by altering their solvent microenvironment. They should therefore be particularly susceptible to antibody catalysis. Furthermore, desolvation of charged groups represents only one of several mechanisms by which substrate destabilization can be achieved. Geometric strain or distortion and electrostatic repulsion are also effective, potentially yielding very large rate accelerations, provided that the induced destabilization can be fully relieved at the transition state.[5] Cochran and Schultz have already exploited geometric strain to generate mimics of the enzyme ferrochelatase:[14] antibodies raised against an *N*-methylated porphyrin, which has a nonplanar structure corresponding to a distorted substrate conformation, substantially accelerated the metallation of mesoporphyrins by Zn^{2+}. Amide hydrolysis might be enhanced in a similar fashion if binding energy can be harnessed so as to force the amino group out of conjugation with the scissile carbonyl.[15] The catalytic advantage to be derived from substrate destabilization is limited only by the amount of binding energy available to force the substrate molecule into the destabilizing environment. Thus, this strategy is likely to be increasingly exploited in the development of a wide variety of catalytic antibodies.

Diels–Alder Reactions and Catalysis by Approximation

Many chemical reactions are limited by high entropic barriers. For example, bimolecular versions of the synthetically useful Diels–Alder reaction, in which an olefin adds to a conjugated diene to give a cyclohexene product, typically have an unfavorable entropy of activation ($\Delta S^{\ddagger}$) in the range −30 to −40 cal K^{-1} mol^{-1}. One of the simplest ways in which an enzyme can speed such a process is to act as an "entropy trap", utilizing binding energy to bring the two substrate molecules together in the correct orientation for reaction.[5] This is sometimes referred to as catalysis by approximation.

Despite the importance of the Diels–Alder reaction to organic synthesis,[16] enzymes that catalyze such cycloadditions have not yet been found in Nature. For this reason, recent demonstrations[17,18] that antibodies can promote these reactions are an exciting advance. Here, suitable hapten design is dictated by the boatlike structure of the uncharged, highly-ordered Diels–Alder transition state. This species resembles the cyclohexene product more than it does the individual starting materials. However, the product itself is generally a poor choice of hapten,[19] given the likelihood of severe

Scheme II



product inhibition in the induced binding pockets, and novel strategies must be developed to facilitate catalyst turnover.

One way to minimize product inhibition draws on chemical or conformational change to drive product release. We used this approach to catalyze the Diels–Alder reaction between tetrachlorothiophene dioxide (**7**) and *N*-alkylmaleimides (**8**)[17] (see Scheme II), a reaction that occurs in two steps, with the initially formed tricyclic adduct **9** undergoing facile chelotropic elimination of sulfur dioxide to give the dihydrophthalimide **10**. The latter is subsequently oxidized to **11** under the reaction conditions. The transition states for both the cycloaddition and the cycloreversion resemble the high-energy intermediate **9**. The stable hexachloronorbornene derivative **12** mimics key geometric features of this species, including the boat conformation of the cyclohexene ring. It was used to generate antibodies that catalyze the reaction between **7** and **8** efficiently, with substantial rate accelerations and multiple turnovers.

The effective molarity for one of these antibodies, 1E9, was determined to be in excess of 10^2 M from comparison of the pseudo-first-order rate constant for the reaction at the antibody active site (k_{cat}) with the second-order rate constant for the uncatalyzed process in free solution (k_{un}).[17] This value indicates the concentration of substrate that would be needed in the uncatalyzed reaction to achieve the same rate as seen in the enzyme ternary complex and thus provides a measure of catalytic efficiency. Page and Jencks have estimated that the maximum kinetic advantage to be derived from converting a bimolecular to a pseudo-unimolecular reaction is on the order of 10^8 M for 1 M

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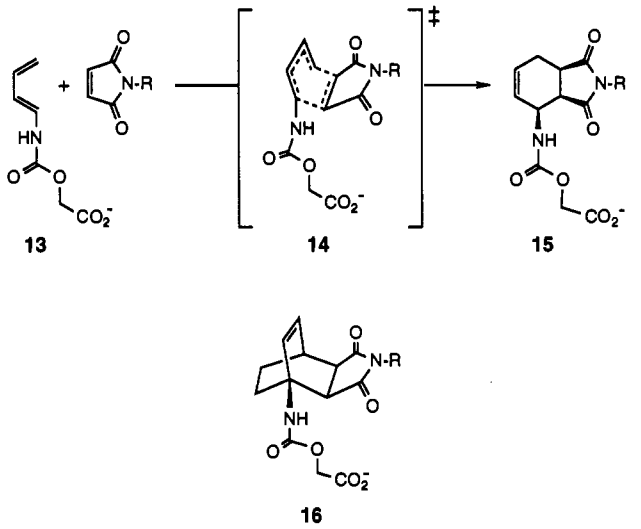
Scheme III



13 14 15 16

standard states.[20] While 1E9 does not approach this level of efficiency, its effective molarity is still 5 orders of magnitude larger than the solubility limit of tetrachlorothiophene dioxide in the reaction medium.

Structural work on 1E9 is at an early stage, but sequencing experiments[21] have shown that this immunoglobulin has high homology with the progesterone-binding antibody DB3.[22] Crystallographic studies of DB3 have revealed a very hydrophobic binding pocket that almost completely envelops its steroid ligand.[22] A similar active site geometry can be inferred for 1E9. Kinetic experiments have shown that binding interactions to nonreacting, hydrophobic portions of the maleimide substrate substantially increase catalytic efficiency. Thus, *N*-alkylmaleimides with long alkyl side chains are better substrates than maleimides with short side chains (i.e., R = butyl ≈ propyl > ethyl > methyl ≫ H).[23] Kinetic studies[23] have also established that the spontaneous elimination of SO_2 from **9** is rapid and that the final product of the reaction is bound only weakly by the antibody, presumably because of poor shape complementarity. The dissociation constant for *N*-ethylphthalimide was determined to be 0.5 mM, only 40 times smaller than that of *N*-ethylmaleimide (K_m = 18 mM). The hapten (K_i = 8 nM), on the other hand, binds nearly 5 orders of magnitude more avidly than the phthalimide and 6 orders of magnitude more tightly than either reactant. Together, these results provide strong validation for the principles used to design the hapten for these experiments.

Braisted and Schultz adopted a similar strategy to catalyze the Diels–Alder reaction between the acyclic diene **13** and *N*-phenylmaleimide[18] (see Scheme III). They synthesized the bicyclooctene hapten **16** as a mimic of transition-state structure **14**. The analog contains an ethano bridge (rather than the CCl_2 unit in **12**) to lock the cyclohexene ring into a boat conformation. In this case, the product's tendency to undergo an energetically favorable conformational change, rather than a second chemical reaction, was expected

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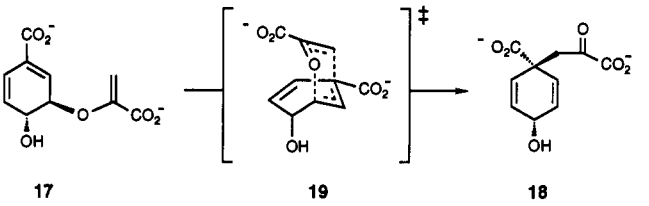
Scheme IV



17 19 18

to reduce its affinity for the active site. Antibody 39,-A11 was raised against **16** and accelerates the target reaction with multiple turnovers. As expected, product binds only 75–100 times more tightly than either substrate, but the catalyst is rather inefficient as judged by its low effective molarity (0.35 M). These results indicate poor use of antibody binding energy. It is possible that the active site generated by the [2,2,2]-bicyclooctene is simply too large to completely freeze out the degrees of freedom available to a substrate diene lacking a structural equivalent of the ethano bridge. More extensive screening of the immunological response to haptens like **16** will probably be necessary to find catalysts with high activity.

Virtually all bimolecular reactions will be amenable to antibody catalysis through proximity effects. Moreover, the use of antigens resembling high-energy product conformers or unstable reaction intermediates is likely to be a general solution to the problem of product inhibition in these reactions. Extension of these ideas to new systems consequently has the potential to provide versatile catalysts for a variety of practical applications. Given the precise control over ligand binding that antibodies exercise, for example, antibodies reversing normally observed Diels–Alder endo/exo ratios or favoring rare product regioisomers should be accessible.

Sigmatropic Rearrangements and in Vivo Applications

Recent progress in producing immunoglobulins in microorganisms such as *Escherichia coli* and yeast opens the door to many exciting applications of catalytic antibodies *in vivo*. For instance, it may be possible to engineer new properties such as pesticide resistance or unusual biosynthetic capabilities into living organisms by intracellular synthesis of these agents. Alternatively, given their biocompatibility and selectivity, tailored antibody catalysts might some day be used to complement human metabolic disorders.

The rearrangement of (–)-chorismate **17** into prephenate **18** (see Scheme IV), which is a key step in the biosynthesis of the aromatic amino acids tyrosine and phenylalanine in plants and lower organisms (Figure 1),[24] is an excellent model system for evaluating the feasibility of antibody catalysis *in vivo*. This reaction, formally a Claisen rearrangement, is a rare example of a pericyclic process in primary metabolism. The enzyme chorismate mutase accelerates the reaction by more than 10^6-fold and is essential for normal cell growth on minimal medium. Strains of yeast or *E. coli* engineered to lack an active version of this enzyme are auxotrophic for tyrosine and phenylalanine.

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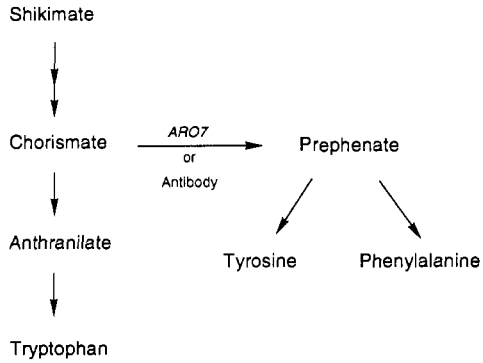
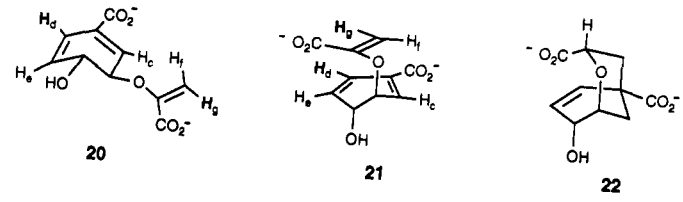


Figure 1. Shikimate pathway for the biosynthesis of aromatic amino acids in yeast. *ARO7* encodes the enzyme chorismate mutase, which promotes the rearrangement of chorismate into prephenate.

While the uncatalyzed rearrangement of chorismate has been well studied, the detailed mechanism of the mutase reaction is still unknown. Like the Diels–Alder reaction discussed in the previous section, this sigmatropic rearrangement should be sensitive to proximity effects. Stereochemical studies have established that both the enzyme-catalyzed and spontaneous reactions occur via a compact, chairlike transition state (**19**).[25] In aqueous solution, however, the substrate adopts an extended conformation (e.g., **20**) and must undergo a conformational change to position the enolpyruvate side chain properly for reaction.[26] Given this, it has been proposed[27,28] that the enzyme may increase the probability of reaction by selectively binding and stabilizing the reactive pseudo-diaxial conformer **21**. The very favorable entropy of activation for the enzyme-catalyzed rearrangement ($\Delta S^{\ddagger} \approx 0$ cal K^{-1} mol^{-1}) compared to that of the spontaneous reaction ($\Delta S^{\ddagger} = -12.9$ cal K^{-1} mol^{-1}) is consistent with this suggestion.[28] Considerable dipolar character in the transition state has also been inferred from isotope effect studies,[29] with C–O bond cleavage preceding C–C bond formation. Thus, electrostatic and/or hydrogen-bonding interactions may also contribute to the enzyme's overall catalytic efficiency.[30]

The oxabicyclic dicarboxylic acid **22** has been used in two different laboratories to raise antibodies with chorismate mutase activity.[31,32] This compound resembles the conformationally restricted transition-state structure **19** and is currently the most potent inhibitor of chorismate mutase available.[33] Antibody 11F1-2E9 was elicited with this compound by Schultz and coworkers[31] and shown to effect a remarkable 10^4-fold acceleration of the rearrangement (k_{cat}/k_{un}). The catalytic efficiency is only 10^2 times smaller than that



achieved by the natural *E. coli* enzyme under identical conditions. As seen with chorismate mutase itself, the antibody-catalyzed reaction is characterized by a favorable $\Delta S^{\ddagger}$ (−1.2 cal K^{-1} mol^{-1}) relative to the uncatalyzed reaction,[34] illustrating the importance of entropic effects.

A second chorismate mutase antibody (1F7), developed in our laboratory,[32] is roughly 10^2 times less active than 11F1-2E11. Desite its relatively sluggish activity, 1F7 is highly enantioselective, showing a >90:1 preference for rearrangement of the natural (−)-isomer of chorismate, a property that was successfully exploited in the kinetic resolution of racemic substrate.[35] In contrast to 11F1-2E9, 1F7 achieves its catalytic effects entirely by lowering the enthalpy of activation.[32] The entropy of activation is actually 10 cal K^{-1} mol^{-1} *less* favorable than for the spontaneous thermal rearrangement. While detailed interpretations of activation parameters obtained for reactions carried out in organized media are notably unreliable, the latter observation raises interesting questions regarding the conformation chorismate adopts at the active site of 1F7. To address this issue, we have carried out an NMR study using transferred nuclear Overhauser effects (TRNOE's).[36] Clear evidence was obtained for magnetization transfer between H_g and H_d and between H_f and H_c when chorismate was bound at the antibody active site. These results are diagnostic for the pseudodiaxial conformer **21** and indicate that the antibody pocket is able to preorganize its substrate to an appreciable extent, as dictated by the structure of the templating hapten. The detection of smaller, nonspecific TRNOE's in these experiments suggests, however, that 1F7 may not completely reduce the rotational degrees of freedom available to the enolpyruvyl group. The ability of the substrate to bind in catalytically-unproductive modes may account in part for the antibody's relatively low efficiency compared to 11F1-2E9 and natural chorismate mutase.

The isolation of two different antibodies with chorismate mutase activity underscores the importance of screening the immune response to a given hapten as widely as possible. Given the enormous diversity of the immunological repertoire, the probability of finding an effective catalyst will generally rise with the number of antibodies screened. In the chorismate mutase experiments, only about 50 hapten binders were examined, representing a tiny fraction of the estimated 10^7–10^8 antibody receptors potentially available for ligand recognition prior to somatic diversification.[3] By assaying larger numbers of hapten binders, catalysts even more active than 11F-2E11 might conceivably be found. It might also be possible to find antibodies that

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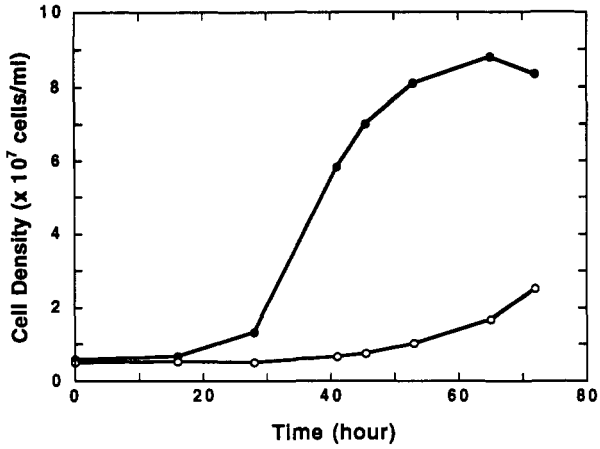

Figure 2. Growth curves[40] for a chorismate mutase-deficient yeast strain (351m), harboring (●) or lacking (○) a plasmid encoding the Fab fragment of the chorismate mutase antibody 1F7. The cells were grown under selective conditions at 30 °C in liquid medium lacking phenylalanine.

promote the stereospecific rearrangement of (+)-chorismate, since the transition-state analog **22** used in these experiments was racemic.

The distinctive kinetic and thermodynamic properties of the two chorismate mutase antibodies discussed here also illustrate the ability of the immune system to solve a specific catalytic task in a variety of interesting ways. This mechanistic versatility has also been noted in the context of hydrolytic antibodies, some of which exploit nucleophilic catalysis while others do not.[37] The precise structural basis for the differences between 1F7 and 11F1-2E9, and the features that differentiate them from the natural enzyme, will eventually be resolved through X-ray crystallographic studies. In this regard, recent successes growing small crystals of the Fab′ fragment of 1F7 that diffract to about 3.0-Å resolution represent a promising development.[38] The structure of the antibody–hapten complex is currently being solved by molecular replacement methods and will undoubtedly yield valuable insights into structure–function relationships in this catalyst.

In complementary molecular biological studies, the genes encoding the heavy and light chains of 1F7 have been cloned, sequenced, and engineered for intracellular expression in *Saccharomyces cerevisiae* as an Fab fragment.[39] Under optimal conditions, the antibody can be produced at levels corresponding to ca. 0.1% of total cellular protein in a variety of yeast strains lacking natural chorismate mutase. The yeast-derived Fab not only folds and assembles correctly within the cell, but its binding and catalytic properties *in vitro* are indistinguishable from those of the parent immunoglobulin.[39] More importantly, the Fab fragment has been shown to confer a substantial growth advantage to a permissive chorismate mutase-deficient host strain under auxotrophic conditions (Figure 2).[40] In control experiments, an antibody with esterolytic activity was unable to complement the host's chorismate mutase deficiency. These experiments demonstrate that antibodies can function *in vivo* to catalyze an essential metabolic transformation and thereby reconstitute a defective biosynthetic pathway. The ability to select for a phenotype altered in a predictable fashion also provides the means for increasing the activity of the first-generation catalyst. 1F7 is 4 orders of magnitude less potent than natural chorismate mutase. Through extensive random mutagenesis of the antibody-encoding genes and genetic selection it should be possible to exploit the host cell's requirement for antibody activity to identify 1F7 variants with increased potency. Experiments along these lines are currently underway in our laboratory.

Next Steps

As the above examples illustrate, catalytic antibody technology provides the means to create a wide variety of enzyme-like catalysts possessing tailored activities and specificities. Even reactions lacking physiological counterparts can be catalyzed. If the full promise of this technology is to be realized, however, a number of practical problems will have to be solved. The principal challenge for the future will be the production of truly efficient catalysts. Although large rate accelerations have been observed in some cases, most catalytic antibodies exhibit relatively low activities. Even the best cases fall several orders of magnitude short of the rate enhancements achieved by analogous naturally-occurring enzymes.

This low efficiency is not surprising given that stable haptens can never mimic transition structures perfectly. Antibodies raised against imperfect transition-state analogs cannot be expected to reduce free energies of activation to the same extent that authentic enzymes do. The challenge of effective hapten design is made even greater by the fact that many energetically demanding reactions require induction of multiple catalytic groups. Simple strategies exploiting charge complementarity to elicit general acids, general bases, and nucleophiles at the antibody combining site (the "bait-and-switch" approach) have been developed to address this problem,[41] but arrays of residues as sophisticated as the catalytic triad in serine proteases may be difficult to generate with a single hapten. In addition, for most reactions, transition-state analog design has not been optimized. Even for those reactions for which excellent haptens are available, such as ester hydrolysis, a correlation between hapten structure and the probability of inducing an active antibody has not been established in a statistically rigorous fashion. Complete, careful characterization of existing catalytic antibody active sites may help identify the important structural features of individual haptens that can improve the odds of generating highly active catalysts. Incorporation of metal ions[42] and other catalytic

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cofactors[43] into the antibody pocket may also extend the properties of these agents.

Another means of obtaining more efficient antibodies requires providing better access to the full diversity inherent in the immune repertoire. Over the last six years, the majority of the catalytic antibodies that have been reported were prepared using standard hybridoma techniques.[44] Given the difficulty and expense of maintaining large numbers of hybridomas, only a fraction of the immune response to any hapten has been assayed for catalysis. As the most active catalysts may also be the rarest, far larger numbers of antibodies will have to be screened to ensure that the best molecules are not missed. In fact, large combinatorial libraries of antibody fragments can now be generated readily and rapidly using the polymerase chain reaction (PCR).[45] When displayed on the surface of the filamentous phage virion, the desired active sites can be selected on the basis of antigen affinity.[46] If coupled with sensitive colorimetric or biological assays, this new technology is likely to be an enormously valuable tool in the search for highly active antibodies. By increasing the number of hapten binders screened for catalytic activity, it will also be possible to assess the efficacy of individual hapten molecules in a statistically rigorous way.

Finally, since catalytic antibodies with modest activity are now readily available, general strategies for augmenting their chemical efficiency directly must be developed. For that task, modern genetic techniques hold the greatest promise. Natural enzymes have been brought to peak efficiency through millions of years of evolution. It may be possible to adopt an evolutionary approach in the laboratory by subjecting first-generation antibody catalysts to extensive random mutagenesis and identifying improved variants by classical genetic selection. Strong selection assays can be devised for a broad range of transformations, including any metabolic reaction (e.g., reactions leading to the synthesis of an essential nutrient, drug, or hormone) and reactions that destroy pesticides or other toxins. As already mentioned, the viability of this approach is currently being tested with the chorismate mutase antibody 1F7. Characterization of the genetic changes that accumulate as the antibody's activity increases will provide fundamental insights into the evolution of molecular function and the relationship between structure and activity. Site-directed mutagenesis, on the other hand, is likely to become an increasingly important tool for probing and improving the properties of these molecules as detailed structural information becomes available for individual antibody combining sites.

Progress in the field of catalytic antibodies has been rapid over the past six years and is likely to continue apace. By learning more about fundamental structure–function relationships in these molecules and by optimizing our strategies for preparing them, we expect to be able to create immunoglobulins that rival the efficiency of naturally-occurring enzymes.

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