

Miniature Organic Models of Enzymes

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Received August 19, 1986 (Revised Manuscript Received January 5, 1987)

Introduction

Enzymes are proteins with catalytic activity that exhibit high specificity and large rate accelerations. Although enzymes are large and complex molecules, their power to catalyze reactions can be attributed mainly to binding and catalysis. Binding not only is responsible largely for the specificity of the reaction but by stereochemistry also brings the substrate in close proximity to and in the correct orientation to the active site. Other factors, such as the microscopic environment of the catalytic site and the stabilization of the transition state by hydrogen bonding, contribute to enzymatic activity, but binding (seen particularly in transition-state analogues¹) and catalysis are the two essential features of all enzymes.

Enzyme modeling is the science of synthetically mimicking the exact nature of the binding subsite in terms of shape, size, and microscopic environment as well as mimicking the active site in terms of identity of groups, stereochemistry, interatomic distances of various groups, and the mechanism of action of the enzyme. Extensive investigations² of chymotrypsin have revealed that the binding subsite of chymotrypsin is hydrophobic in nature, 10–12 Å deep and 3.5–4 Å by 5.5–6.5 Å in cross section,³ which gives a snug fit to an aromatic ring which is 6 Å wide and 3.5 Å thick (the aromatic ring is also hydrophobic in nature).⁴ The catalytic subsite of chymotrypsin has been shown to contain only three amino acids: (1) serine-195, (2) histidine-57, and (3) aspartate-102.² However, it is the *functional groups* that these amino acids carry that is most important.⁵ They are (1) a hydroxyl group (of serine-195), (2) an imidazole group (of histidine-57), and (3) a carboxylate ion (of aspartate-102). Interatomic distances⁶ are 2.8 Å between the Ser-195 O^γ and the His-57 N^{ε2} and 2.65 Å between the His-57 N^{δ1} and the Asp-102 O^{δ1}.

The "proton-transfer relay" system proposed for the mechanism of action of chymotrypsin (Figure 1)⁷ consists of two proton transfers, one initiated by carboxylate ion and the other initiated by imidazole. These increase the nucleophilicity of the hydroxyl oxygen atom of serine toward the carbonyl function of the amide or ester substrate bound in the hydrophobic pocket of the enzyme to give an acyl-enzyme intermediate.

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Deacylation occurs via the same two proton transfers, increasing the nucleophilicity of the hydroxyl group of water, which attacks the carbonyl group of the acyl-enzyme ester.² This mechanism is controversial,⁸ and the participation of carboxylate ion has been questioned.⁹ However, the experiments done in this laboratory with acyl-enzyme models (discussed later) and in another laboratory with mutated enzyme¹⁰ lend credence to the participation of carboxylate ion.

Thus, a miniature model of chymotrypsin should essentially contain a hydrophobic pocket to act as a binding subsite, attached to a hydroxyl group, an imidazole group, and a carboxylate ion (the catalytic subsite) placed at the right distances and correct stereochemistry to participate in a "proton-transfer relay system".

Binding

Binding of the substrate to the enzyme is an essential feature of any enzymatic reaction. Although covalent enzyme-substrate bonds are formed during some reactions, usually binding of the substrate to the active site of the enzyme involves noncovalent forces such as hydrophobic, van der Waals, or London dispersion forces, hydrogen bonding, and electrostatic interactions.¹¹ Cumulative effects of such forces produce tight binding if the binding subsite is complementary to the structure of the substrate or if conformational changes occur in the enzyme during the binding process.¹¹

The formation of an enzyme-substrate complex is an equilibrium process, and the magnitude of the equilibrium constant and energy of binding depend on the extent of interaction between the substrate and the enzyme. Thus, for a certain molecule to be a tight binding substrate for an enzyme there should be a maximum interaction between the guest and the host in terms of both structure and microscopic environment. The binding energy thus obtained is one of the important forces behind enzymatic activity. The terminology used to describe the effects of binding in enzymatic catalysis has been compiled by Jencks.¹²

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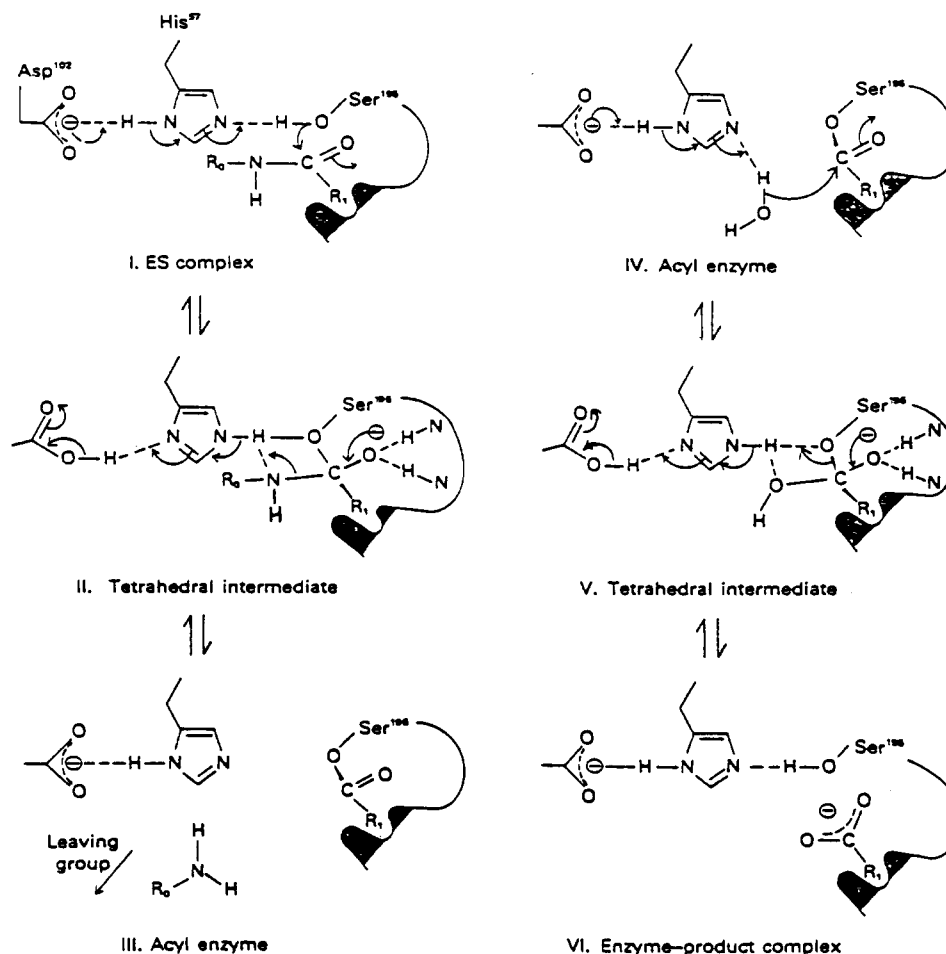


Figure 1. A mechanism of serine protease hydrolysis of peptides or amides. In this representation, the proton shuttle is concerted.

The binding subsite of chymotrypsin is essentially hydrophobic in nature and is capable of having maximum interaction with an aromatic ring to orient the oxygen atom of Ser-195 for a nucleophilic attack on the carbonyl carbon atom of the ester or amide substrate. Thus, the ideal molecule on which to base a model of chymotrypsin should have a cavity that (a) provides maximum hydrophobic interaction with a substrate to form complexes, (b) fits the aromatic ring of the substrate, and (c) orients the carbonyl carbon of the bound substrate toward an oxygen atom (of serine in the real enzyme) for nucleophilic attack.

Cyclodextrins consisting of 6, 7, or 8 units of α -1,4-linked D-glucopyranoses turned out to be the molecules of choice of this venture.¹³ Cyclodextrins have doughnut shapes with *secondary* hydroxyl groups at the C-2 and C-3 atoms of glucose units arranged in the more open end and *primary* hydroxyl groups at the C-6 atoms of the glucose unit located at the other end. The interior of the cavity, consisting of a ring of C-H groups, a ring of glycosidic oxygen atoms, and another ring of C-H groups, is hydrophobic in nature, similar to the binding subsite of chymotrypsin. The inner diameter of the cavities are approximately 4.5 Å in α -cyclodextrin, 7.0 Å in β -cyclodextrin, and 8.5 Å in γ -cyclodextrin. α - and β -cyclodextrins would give a snug fit for an aromatic ring. Formation of inclusion complexes with various compounds (binding) is one of the most important characteristics of cyclodextrins.¹³

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We discovered that the aromatic rings did bind to cyclodextrins with dissociation constants varying from 10^{-2} to 10^{-3} M, depending on the substituent on the ring,¹⁴ which are within the range of some enzymatic dissociation constants. Hydrophobic substituents on the phenyl ring led to a tighter binding, indicating that the binding was essentially due to hydrophobic interactions.¹⁴ Initial investigations on the effect of cyclodextrin on the hydrolysis of *p*-nitrophenyl acetate showed that it followed saturation kinetics similar to enzymes and enabled the data to be treated by a variant of Michaelis-Menten kinetics.¹⁴ This indicated that the accelerations in the presence of cyclodextrin were due to the formation of Michaelis-Menten type complexes. The investigations of the *stereochemistry of binding* by the effect of cyclodextrins on a series of substituted phenyl acetates produced chaos, in what we consider to be the world's worst Hammett plot, as compared to the normal Hammett plot produced by the effect of the monomeric methylglucoside (Figure 2).¹⁴ However, it can be seen that hydrolytic acceleration by cyclodextrin on any meta-substituted phenyl esters is larger than that on the corresponding para-substituted phenyl esters. This pattern indicates that electronic effects are *not* important. This evidence and the models of binding of *m*-*tert*-butylphenyl acetate and *p*-*tert*-butylphenyl acetate by the cyclodextrin (Figure 3)¹⁴ emphasize the fact that the *stereochemistry of binding* by cyclodextrin is important, as Emil Fisher predicted in

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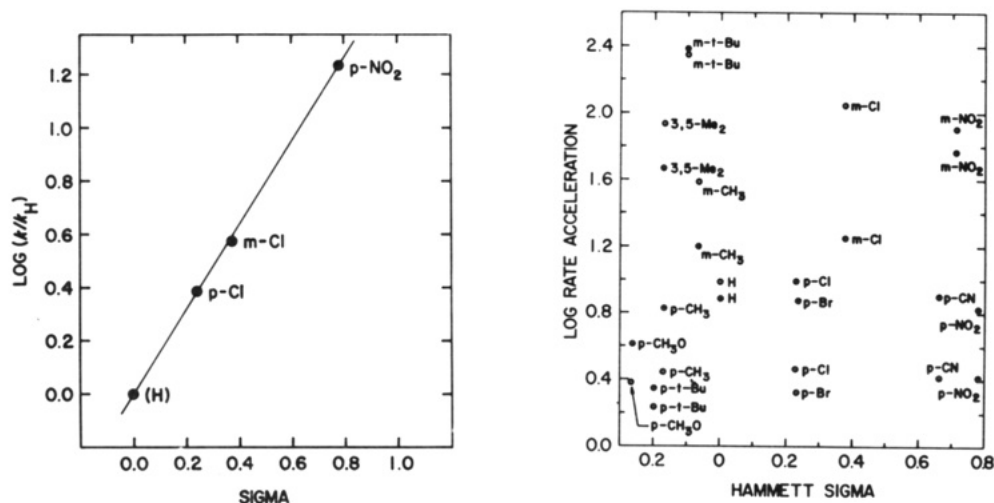


Figure 2. Hammett plots of acceleration of rate of hydrolysis of substituted phenyl esters due to (left) methylglucoside and (right) (●) β -cyclodextrin, (○) α -cyclodextrin.

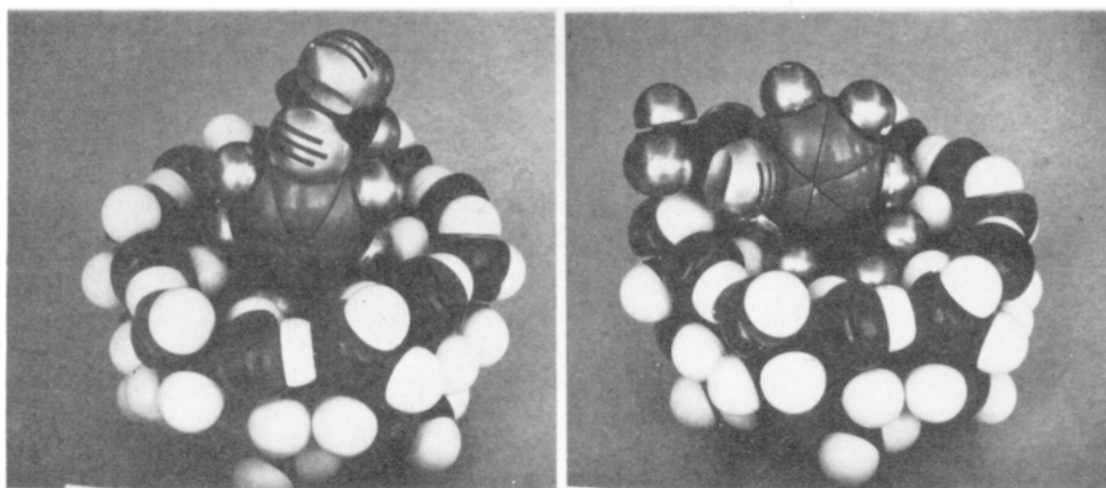


Figure 3. CPK models of α -cyclodextrin complexes: *p*-*tert*-butylphenyl acetate complex with *tert*-butyl group inserted into the cavity from the secondary hydroxyl side (left); *m*-*tert*-butylphenyl acetate complex similarly constructed (right).

his "lock and key" theory of enzymatic action in 1894.¹⁵ As seen in the models, the binding of meta-substituted phenyl acetates to cyclodextrin orients the carbonyl carbon atom of the ester substrate toward the oxygen atoms of the *secondary* hydroxyl groups of the cyclodextrin for nucleophilic attack, whereas the complexes of para-substituted phenyl esters orient the carbonyl carbon atom far from the *secondary* (or *primary*) hydroxyl groups. Thus, cyclodextrins are perfect candidates for mimicking the binding part of chymotrypsin in models.

Catalysis

The importance of intramolecularity in enzymatic catalysis has been well established. There are several reviews including a recent elegant piece by Menger which details the reasons for the large rate accelerations in intramolecular reactions.¹⁶ This Account primarily deals with how these large intramolecular accelerations can be utilized in enzyme models. One of the first steps in building an enzyme model is a complete understanding of the mechanism of action of the enzyme.

The mechanism of action of chymotrypsin involving the three active site functionalities, the hydroxyl group

of Ser-195, the imidazole group of His-57, and carboxylate ion of Asp-102, is shown in Figure 1. An important feature of this mechanism is that there are two distinct phases in the reaction: (1) the acyl transfer from the substrate to the hydroxyl group of the enzyme (acylation) to form an acyl-enzyme and (2) the deacylation of the acyl-enzyme (ester) by water. The common feature in both these reactions is that the negative charge from the carboxylate ion is transferred to the oxygen atom of the carbonyl function and concurrently the proton is transferred from the hydroxyl group (of serine in acylation, the first reaction, or of water in deacylation, the second reaction) via the imidazole group to the carboxylate ion (structural evidence is not as clear on the point as it is on the imidazole proton transfer) during the formation of the tetrahedral intermediate.⁴ Thus, this mechanism is known as the "charge relay" or, more recently, "proton-transfer relay". Thus, both the imidazole group and the carboxylate ion act as general base catalysts and not as nucleophilic catalysts.

Models of the acyl-enzyme were first synthesized to test these mechanistic features, excluding the contributions from the binding part of the enzymatic catalysis. The model of the acyl-enzyme consisted of a norborane backbone with an imidazole group in the

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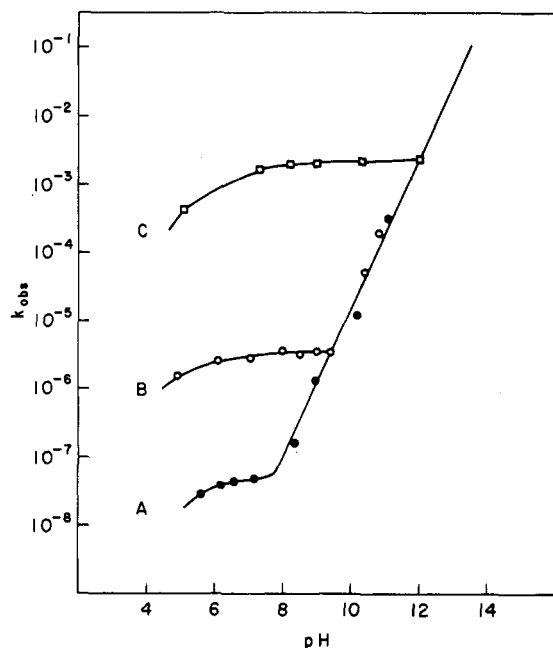


Figure 4. Hydrolysis of acyl-enzyme models: A, hydrolysis of 1; B, hydrolysis of 1 + 0.5 M benzoate ion; C, hydrolysis of 2.

endo 5-position and a cinnamoyl ester group in the endo 2-position (1).¹⁷ The positions were chosen so that the imidazole group would not act as a nucleophilic catalyst but allow a water molecule between the imidazole group and the carbonyl carbon atom of the ester function and thus act as a general basic catalyst. The deacylation of this model of acyl-enzyme ester showed (by the D₂O effect) that the imidazole group acts as general basic catalyst and not a nucleophilic catalyst.¹⁸ Computer-aided structures (using the CHEMGRAF program) of this model showed that the distances between the carbonyl carbon atom of the model of acyl-enzyme (ester function) and the two nitrogen atoms of the imidazole group were 2.43 and 2.80 Å. However, if a water molecule is inserted between the imidazole group and the ester group, the shortest distance between the nitrogen atom of the imidazole group and the hydrogen atom of the water molecule is 1.60 Å and the distance between the carbonyl carbon atom of the ester group and the oxygen atom of the water molecule is 1.37 Å. These distances support the experimental evidence that the imidazole acts as a general basic catalyst (and not as a nucleophilic catalyst). However, the most interesting feature of this model is that the rate of deacylation increased tremendously in the presence of benzoate ion. It was also observed that the rate of deacylation (in the presence of benzoate ion) is further increased by the addition of dioxane, which was used to simulate the apolar nature of the active site of chymotrypsin. The ratio of the rates in the presence of 0.5 M benzoate ion to that in the absence of benzoate ion resulted in a 2500-fold acceleration at a dioxane mole fraction of 0.42.¹⁹ Such an increase in the rate of deacylation in the presence of the apolar environment and the third component (carboxylate ion) of the active site of chymotrypsin indicated

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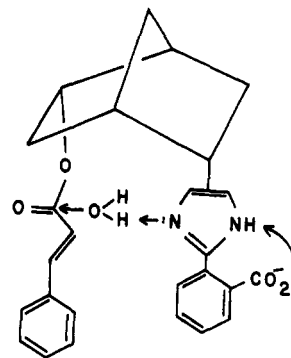


Figure 5. Complete model of acylchymotrypsin showing all three components of the catalytic triad (the oxy ester group, the imidazole group, and the carboxylate ion).

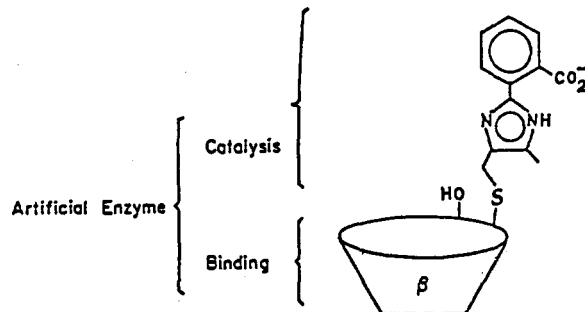


Figure 6. Miniature organic model of chymotrypsin.

that this is a good model for the charge relay system.¹⁹ However, this carboxylate ion is an intermolecular catalyst whereas chymotrypsin employs an intramolecular carboxylate ion. The advantage of intramolecular catalysis over intermolecular catalysis has been well established. Thus, it was imperative to build a model of chymotrypsin with an intramolecular carboxylate ion. This was achieved by synthesizing *endo,endo*-5-[2-(2-carboxyphenyl)-4(5)-imidazolyl]bicyclo[2.2.1]hept-2-yl *trans*-cinnamate (2).²⁰ This model has a rate of hydrolysis 10⁵–10⁶ (154 000) faster than norbornyl cinnamate ester and is only 18-fold slower than deacylation (although 1/20 the molecular weight) of real *trans*-cinnamoylchymotrypsin (Figure 4).²⁰ A mechanism, similar to the mechanism of deacylation of acylchymotrypsin, can be proposed to explain this acceleration (Figure 5).²⁰ It was suggested that if the same differential solvating system, as exists in real chymotrypsin, can be mimicked in the active site of the artificial acylchymotrypsin, then one could expect the artificial acylchymotrypsin to deacylate at the same rate as the real acylchymotrypsin.²⁰

Thus, a model of the catalytic subsite of chymotrypsin to which a binding subsite could be attached was then synthesized.

Synergism of Binding and Catalytic Groups To Produce an Enzyme Model

Having thus established the systems to mimic the binding subsite (cyclodextrin) and the catalytic subsite (*o*-imidazolylbenzoic acid) of chymotrypsin, it was logical to combine the two essential features of enzyme action to produce a miniature organic enzyme model. Cramer had demonstrated that inserting an imidazole group on the primary side of cyclodextrin led to a fair

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Table I.
Hydrolysis of Esters by Chymotrypsins (Real and Artificial)

enzyme	substrate	pH ^c	$k_{\text{cat}} \times 10^{-2}$, ^d s ⁻¹	$K_m \times 10^{-5}$, ^d M	k_{cat}/K_m , ^e M ⁻¹ s ⁻¹
chymotrypsin ^a	<i>p</i> -nitrophenyl acetate	8.0	1.1	4.0	275
artificial chymotrypsin ^b	<i>m</i> - <i>tert</i> -butylphenyl acetate	10.7	2.8	13.3	210

^aThe concentration of the stock solution was determined to be 1.0×10^{-3} M (83% purity) by active site titration.²⁸ ^bThe concentration of the stock solution was determined to be 3×10^{-3} M (96% purity) by UV absorbance. ^cThe pH selected was the predetermined optimum pH for both the real and artificial chymotrypsins. The use of identical pH's for the two systems would result in a low rate for real chymotrypsin at pH 10.7 (because of binding-lysine ionization) and a low rate for artificial chymotrypsin at pH 7.9 (because of cyclodextrin hydroxyl groups). ^dThe error limit in k_{cat} is $\pm 5\%$; the error limit in K_m is $\pm 10\%$. ^e $(k_{\text{cat}}/K_m)_{\text{real}}/(k_{\text{cat}}/K_m)_{\text{art.}} = 1.3$.

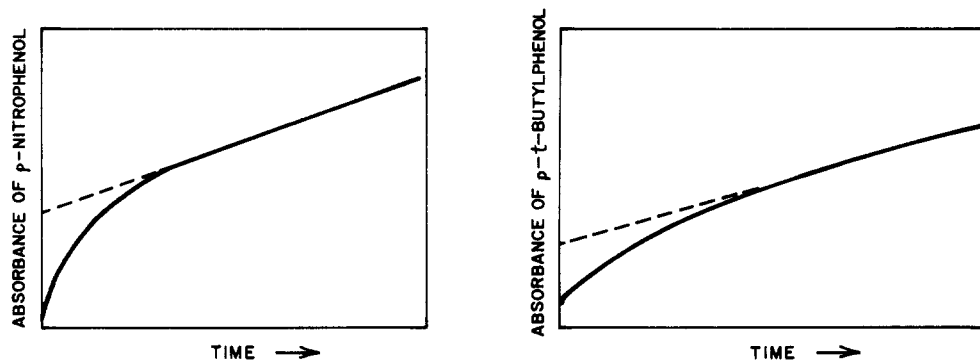


Figure 7. Real-chymotrypsin-catalyzed hydrolysis of *p*-nitrophenyl trimethylacetate (left); artificial-chymotrypsin-catalyzed hydrolysis of *m*-*tert*-butylphenyl trimethylacetate (right).

mimic of chymotrypsin.²¹ Breslow had demonstrated that modified cyclodextrins (although slower than the enzyme itself) could mimic ribonuclease (but produce a bell-shaped pH rate profile for the hydrolysis of cyclic phosphates).²² Subsequently, functionalized cyclodextrins have been used as models of transaminase,²³ carbonic anhydrase,²⁴ and a thiamine-dependent enzyme.²⁵

In our miniature organic model of chymotrypsin (Figure 6)²⁶ the catalytic subsite that had been so successfully designed had to be placed on the secondary side of cyclodextrin since it was established that the bound substrate would have its carbonyl function at the secondary side of cyclodextrin.

The synthesis was achieved by the reaction of β -cyclodextrin 2,3-epoxide (obtained from the reaction of β -cyclodextrin 2-tosylate by the reaction of ammonium bicarbonate) with *o*-[4(5)-mercaptomethyl-4(5)-methylimidazol-2-yl]benzoic acid (obtained by hydrolysis of the corresponding thioacetate in ammonium bicarbonate).²⁶ It was possible to obtain the pure product (96% purity by UV analysis) by anion-exchange chromatography. Artificial enzymes based on α - and γ -cyclodextrins have now been prepared by similar schemes.

These enzyme models were examined for their catalytic activity in ester hydrolysis. Since *m*-*tert*-butylphenyl acetate is known to bind well to the β -cyclodextrin cavity, it was decided to examine the rate of hydrolysis of this ester in the presence of β artificial

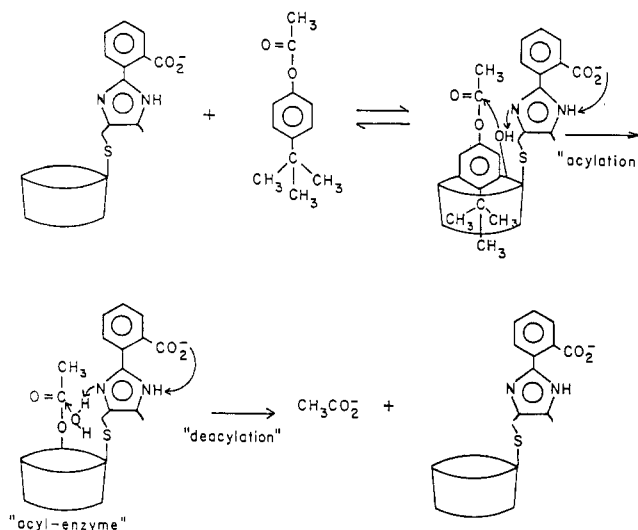


Figure 8. The mechanism of action of artificial chymotrypsin.

enzyme. The hydrolysis of more than 10 mol of substrate by 1 mol of artificial enzyme (determined spectrophotometrically) indicates that there is turnover.²⁷ The results given in Table I for β artificial enzyme obtained from Lineweaver-Burk plots indicate that both the artificial and real enzymes are comparable in their catalytic activity (both in rate and in binding constants).²⁶ The second-order constants (k_{cat}/K_m), the most important enzymatic rate constants, indicate that the artificial enzyme is as efficient as the real enzyme in its catalytic activity. The solvent isotope effect ($k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}} = 3$) shows that the hydrolysis of *m*-*tert*-butylphenyl acetate is catalyzed by a general basic mechanism rather than a nucleophilic attack by imidazole. Similar arguments have been put forward to

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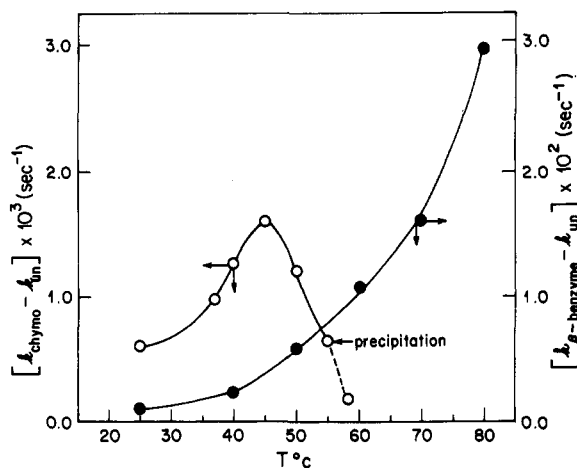


Figure 9. Thermal stabilities of real and artificial chymotrypsins.

explain the general basic character of real chymotrypsin catalysis. Interestingly, the three artificial enzymes have different specificities. Whereas α and β artificial enzymes are better than γ artificial enzyme in phenyl ester hydrolysis, γ artificial enzyme hydrolyzes tryptophan ethyl ester faster than the α and β artificial enzymes. In the hydrolysis of *p*-*tert*-butylphenyl trimethylacetate by the artificial enzyme, a two-phase reaction similar to the hydrolysis of *p*-nitrophenyl trimethylacetate by real chymotrypsin was observed (Figure 7).^{29,30} This is an indication that the artificial enzyme, like real chymotrypsin, involves a pre-steady-state, acylation (the curved portion), and a steady-state, deacylation and turnover (the straight portion). From the results obtained so far the mechanism of action of the miniature organic model of chymotrypsin can be compared to the mechanism of action of real chymotrypsin (Figure 8).²⁹ The mechanism assumes that 1:1 complexes seen in simpler reactions of cyclodextrin are still operative.

Indeed, there are differences between this miniature organic model of chymotrypsin and real chymotrypsin, both theoretically as well as in practice. The pH maxima for chymotrypsin is 7.9, dependent on the ionization of imidazole and on the conformation determined by a lysine ionization, whereas the pH maxima for model is beyond 10, determined by both the ionization of imidazole and the ionization of the *secondary* hydroxyl groups of the cyclodextrin.

One of the most important differences between the real and artificial enzymes is their stability. Chymotrypsin can undergo two kinds of inactivation: reversible inactivation and irreversible inactivation. Chymotrypsin, like other enzymes, depends on its conformation for its catalytic activity, which is dictated by hydrogen bonding, van der Waals interactions, configurational entropy, hindered rotation, permanent dipole interactions, electrostatic effects, electronic situations, and interaction of the protein with water. Forces, like temperature, that disturb these interactions tend to change the conformation of the enzyme and thus inactivate the enzyme. However, the conformation of the artificial enzyme (the cyclodextrin part) is not affected by such forces and is thus active at elevated temperatures. Figure 9 shows that real chymotrypsin has a

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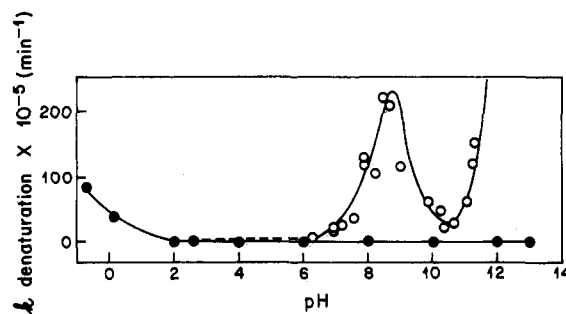


Figure 10. pH stabilities of real and artificial chymotrypsins.

temperature maxima around 45 °C, and after 55 °C the protein begins to precipitate and is rendered inactive whereas the activity of the artificial enzyme keeps increasing to at least 80 °C.

Irreversible inactivation of chymotrypsin is brought about by disruption of the protein by cleavage of peptide linkages or by blockage of the three groups of the catalytic subsite. Since chymotrypsin consists of 245 amino acids, several of which (tyrosine, tryptophan) are natural substrates for chymotrypsin, it undergoes self-proteolysis (cannibalistic denaturation) at its active pH range. The peptide linkages are also cleaved by hydroxide ion at high pH range to inactivate the enzyme (Figure 10). However, the artificial enzyme is made up of glucose units with α -1,4-glycosidic linkages. These are very stable under high pH conditions and do not undergo cannibalistic denaturation (since the artificial enzyme is not a substrate for itself). Only at very low pH can glycosidic bonds be broken to inactivate the artificial enzyme (Figure 10). However, the conditions (acidity and temperature) required for such cleavage are too strenuous to hinder practical use of the artificial enzyme. Thus, one of the greatest limitations of natural enzymes, i.e., instability, can be overcome by artificial enzymes.

Despite these differences, we have been able to copy the enzyme (MW 24 800) in abbreviated form (MW 1365), in terms of its two essential features, binding and catalysis, and synthesize a good catalyst. This is the ultimate proof of the mechanism of chymotrypsin catalysis.

Conclusions

The study of an enzyme from an organic chemist's point of view has a 2-fold function: (1) to understand the enzyme and be able to control it and (2) to build a system which can function as well as the enzyme or modify the system to fulfill his needs.

Although we have used cyclodextrins to synthesize artificial chymotrypsin, one could choose from a wide variety of synthetic hosts which are known to bind specific substrates. We have used a catalytic subsite that accelerates hydrolysis. However, one could use various systems to accelerate different reactions as desired. One that has been successfully used is a polymer with hydrophobic groups attached to it: poly(ethylenimine).³¹

Thus, this paper is a description of the pathway that biochemically oriented physical-organic chemists can follow to synthesize an artificial enzyme. If a chemist can synthesize a system with a binding subsite to bind

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the desired substrate adjacent to a catalytic subsite and thereby accelerate the desired reaction, then he or she can essentially achieve what nature has achieved through natural enzymes.

Authors are grateful to Dr. R. C. Gadwood of the Upjohn Co. for the help with the CHEMGRAF computer structures. This work

was financially aided by the National Institute of Health (Grant GM-20853), the National Science Foundation (Grant CHE-802697), Merck Sharp and Dohme Co., Hoffman-La Roche Co., Dow Chemical Co. Fdn., and Monsanto Chemical Co. It is now being supported by the Office of Naval Research. This support is gratefully acknowledged. Finally, the inspiration for these advances can be attributed to Dr. F. H. Westheimer.

Carbon-13 Shielding Tensors: Experimental and Theoretical Determination

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Received October 9, 1986 (Revised Manuscript Received January 27, 1987)

I. Introduction

With the formulation of rules for substituent effects¹⁻³ in the mid-1960s, ¹³C NMR spectroscopy became one of the premier methods for structural and conformational analysis in organic chemistry.⁴ It is not at all uncommon for practicing chemists to monitor chemical processes using both ¹H and ¹³C NMR spectral methods, as even subtle structural changes correlate nicely with variations in the isotropic chemical shift. The high sensitivity of such liquid data upon chemical structure has been very well documented during the past two decades, and extensive cataloging of trends and spectral features is now available for essentially all classes of organic chemicals.

The complete chemical shielding interaction, however, is given by a tensor which depends upon the electronic structure of the molecule and can be used to describe how the NMR resonance frequency changes with the molecular orientation in the external magnetic field. In nonviscous liquids only the average of the principal values of the tensor, i.e., the isotropic liquid shift, is observed due to the averaging from the rapid rotational motion.

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David M. Grant has worked in the area of nuclear magnetic resonance since his Ph.D. in 1957. After completing a postdoctoral year at the University of Illinois where he was closely associated with both H. S. Gutowsky and M. Karplus, he has been on the faculty at the University of Utah. His present interest in ¹³C shielding tensors culminates almost three decades of interest in ¹³C NMR methods as they relate to molecular structure and dynamics.

Josef Michl was born in 1939 in Prague, Czechoslovakia, where he obtained his M.S. degree from Charles University with V. Horák and P. Zuman and his Ph.D. degree from the Czechoslovak Academy of Sciences with R. Zahradník. After postdoctoral years with R. S. Becker at the University of Houston and M. J. S. Dewar at the University of Texas and a brief stay with A. C. Albrecht at Cornell University, he was a staff scientist at the Academy of Sciences in Prague. He left Czechoslovakia in 1968 and spent postdoctoral years with J. Linderberg at Aarhus University, Denmark, and with F. E. Harris at the University of Utah, where he subsequently joined the faculty. In 1986, he moved from Utah to the University of Texas at Austin, where he holds the Marvin K. Collie-Welch Regents Chair in Chemistry. Since 1984, he has been the editor of *Chemical Reviews*. His primary interest is physical organic chemistry.

These tensorial shifts with three principal values have the potential to reveal up to 3 times the information of isotropic shifts, and it is possible from these principal values to obtain "three-dimensional" information on molecular structure and the associated electronic features. Section II outlines in more detail the nature and importance of shielding tensors.

Except for limited, early pioneering work,⁵⁻⁷ the measurement of ¹³C shielding tensors for more than a few molecules has been left until recently. Due to the previous lack of a comprehensive body of experimental data as well as to earlier inadequacies of theoretical methods for systematizing the structural variations, correlations between measured shielding tensorial components and chemical structure are only now emerging. This relative lack of research in the area of ¹³C shielding tensors, in spite of their clear advantages, results in part from the difficulty of solid-state NMR techniques compared with standard isotropic liquid work.

The measurement of ¹³C shielding tensors involves the analysis of single-crystal rotation data or line shape analysis of powder samples where small crystallites orient in all possible directions. Use is made of the cross-polarization method to enhance the sensitivity with high-power ¹H decoupling to eliminate the ¹H-¹³C dipolar interactions. A detailed description of the experimental techniques is given in section III.

The principal tensorial shielding values can be obtained from spectral patterns obtained on powdered solids, providing tensorial patterns from nonequivalent nuclei are not so severely overlapping as to obscure break points (cusps and shoulders) and other spectral

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