Artificial Enzymes

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Summary. Simple chemical catalysts have been designed to achieve some desirable features of enzymes. These novel catalysts are not proteins, but they may incorporate the typical enzyme catalytic groups and they achieve selectivity in their reactions by use of geometric control, as do enzymes. Catalysts that carry out geometrically controlled chlorinations of aromatic rings and steroids have been constructed. Other catalysts achieve the selective synthesis of amino acids, and still others imitate ribonuclease in detailed mechanism and hydrolyze RNA. Optimization of geometries has led to a rate acceleration of over 10^8 in one instance.

Organic chemistry is only in part a "natural science." Much of the field is concerned with creating and understanding chemical substances and reactions other than those existing in nature. Thus the role of synthesis greatly exceeds that in other sciences. Most of the compounds and reactions examined are created by the chemists themselves. Chemistry is in this sense akin to electrical engineering, with its similar concern for understanding the behavior of humanly fabricated systems.

The inspiration for the design of new substances can come from considering natural compounds. For example, synthetic medicinals with structures related to those of important metabolites have been created. The motivation for such work is in part the desire to understand the properties of the natural substances by putting them into a general context, but the principal interest is in the creation of substances with novel properties. Chemistry is also concerned with reactions, that is, chemical transformations. The chemical reactions occurring in the natural world are almost exclusively the reactions of life. Thus it is reasonable that chemists have been interested in imitating and generalizing the biochemical reactions of nature. Since most such reactions are catalyzed by enzymes, efforts have been made to produce artificial enzymes.

Natural enzymes are proteins of considerable size, made up of hundreds of amino acids. They catalyze biochemical reactions by binding one or two small molecules into an active site, with catalytic groups of the enzyme held nearby so as to interact effectively with the bound substrate molecules. The result is reactions of great speed—velocities 10 billion times the speed of the uncatalyzed reaction are not uncommon. At least as remarkable is the typical selectivity of enzyme-catalyzed processes. Enzymes can select and bind one particular molecule among the many that are present in solution in a cell. Then the enzyme can selectively catalyze a reaction in one section of the molecule, which might not be the one most prone to simple chemical attack. This catalyzed reaction is normally under strong geometric control by the enzyme, so that products will be formed with particular spatial arrangements, including chirality ("handedness").

For many practical purposes, learning how to imitate the selectivity of enzyme-catalyzed reactions is more important than learning how to achieve their velocity. Simple chemical processes do not generally produce high yields of a single desired product that is free of impurities from side reactions. The achievement of good selectivity has been a major aim of work on artificial enzymes.

Selective Chlorinations

The cyclodextrins have excited much interest as possible components of artificial enzymes (*1*). They are doughnut-shaped molecules made up of glucose units, with an interior cavity whose size and shape is determined by the number of glucose units that make up the ring. In α-cyclodextrin (cyclohexaamylose, made up of six glucose units) the almost cylindrical cavity is approximately 7 angstroms deep and 5 angstroms in diameter. In β-cyclodextrin (cycloheptaamylose, seven glucose units) the depth is the same but the diameter is 7 angstroms, and in γ-cyclodextrin (eight glucose units) it is again 7 angstroms deep, but 9 angstroms in diameter.

Cyclodextrins are soluble in water, because of the many hydroxyl groups of glucose that rim the cavity. However, since the cavities themselves are hydrophobic (poorly solvated by water), the cyclodextrins have the ability to extract small organic molecules out of water solution and bind them into the cavities. This is similar to the ability of enzymes to bind substrates into interior cavities. Cyclodextrin binding is also selective for molecules that have the correct shape and hydrophobic character.

In free solution, the chlorination of anisole (methoxybenzene) normally yields a mixture of two products, *ortho*-chloroanisole (**1**) and *para*-chloroanisole (**2**) (Fig. 1). When α-cyclodextrin is present, the anisole binds into the cavity. Molecular models suggested that in the complex it should be difficult to chlorinate the anisole so as to form *ortho*-chloroanisole, since the ortho position is buried inside the cavity. This was the experimental finding: no *ortho*-chloroanisole was formed (*2*). At the same time, *para*-chloroanisole (**2**) was produced by a new chemical pathway, catalyzed by a hydroxyl group of α-cyclodextrin. By this path (Fig. 1), a chlorine atom was delivered to the bound anisole in the complex. The chlorination system catalyzed by α-cyclodextrin thus shows many enzyme-like properties. The cyclodextrin selectively binds particular molecules. It then selectively produces a single product by a catalyzed reaction within the complex. Finally, the product *para*-chloroanisole is released and a new anisole substrate molecule binds to start the cycle again.

Anisole can also be chlorinated by the enzyme chlorinase. However, with this enzyme, a mixture of products **1** and **2** is formed. In this instance, the α-cylodextrin system shows a more typical enzyme-like selectivity than does the enzyme itself. As this shows, there are many chemical reactions of interest for which no enzyme exists, or an enzyme exists that catalyzes the reaction only poorly. In such cases artificial enzymes can play an important role.

A more challenging problem is the development of highly selective reactions of large organic species such as

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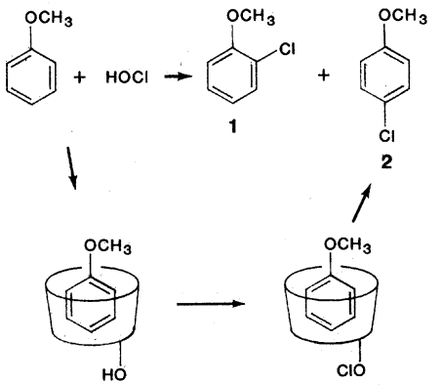

Fig. 1. The random chlorination of anisole in solution is converted to a selective process by a cyclodextrin catalyst, which binds the anisole in a cavity and delivers chlorine under geometric control.

steroid molecules. Reaction of a large hydrocarbon structure (for example those in Fig. 2) in simple chemical processes would normally be selective only for reactions involving one of the reactive groups of the molecule, and attack on the unreactive C–H groups is usually random, leading to hopeless mixtures. By contrast, enzymes are known that can selectively attack unreactive C–H bonds because of the geometry of the enzyme-substrate complex. Since steroids have great practical importance as medicinal compounds, we have tried to learn how to imitate this selective enzyme-catalyzed chemistry.

The first problem was to show that simple geometrical control could indeed be achieved, leading to selective functionalization. A template molecule carrying an iodine atom was attached to a steroid (**3**) (Fig. 2). When chlorination was performed, a chlorine became attached to the iodine atom and thus held so that it could reach and attack only one C–H group, the one shown. The 46 other hydrogens of the steroid were not attacked. This led, in a few simple steps, to the formation of a chlorinated steroid (**4**) in which the chlorine was selectively placed on a carbon (C-17) at the opposite end of the molecule from the carbon to which the template had been attached (C-3). We have described such reactions as "remote functionalizations" (*3*).

This process is catalyzed by the template species. It is faster than chlorination of a steroid without the template group, and it shows geometrically directed selectivity. Furthermore, the ester linkage that attaches the template to the steroid can be hydrolyzed so that the catalyst group can be reused. The direct chemical linkage between the substrate and the catalytic group in this instance contrasts with the simple binding in the anisole system described above or with that in enzymes.

Different selectivities can be achieved by changing the geometry of the templates. Thus with the shorter template in compound **5**, the chlorination has been directed to the hydrogen on a carbon (C-9) in the middle of the molecule. This template was also highly selective for the hydrogen shown among the 47 in the steroid; in particular, it afforded none of compound **4**. The C-9 halogenation is useful in the production of corticosteroids, and we have used it in the synthesis of cortisone from a more available steroid (*4*). Still another template (in **6**) has let us perform a selective chlorination at C-14, useful for the preparation of cardiac-active steroids. However, close geometric control is needed, as predicted from molecular models or computer modeling. Templates with incorrect geometries yield mixtures of products, directing the attack to several positions.

The imitation of the geometric control used to achieve selective enzymatic reactions of steroids has led to useful selective chemical processes. Even more utility, as well as a better imitation of enzymes, will come when such template control can be achieved with simple binding interactions, not chemical linkages, between substrates and catalysts.

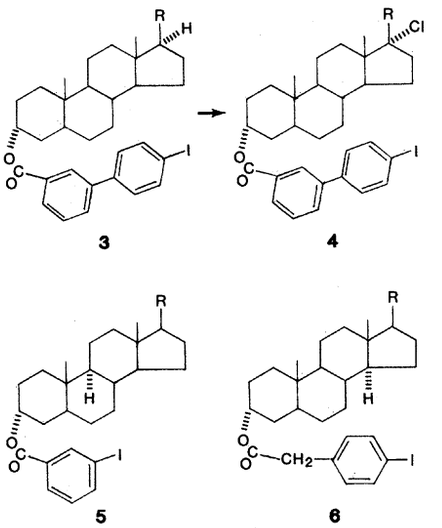

Fig. 2. The steroid, 3-α-cholestanol, can be selectively catalytically chlorinated at any one of the three hydrogens shown by different templates whose geometry determines the point of selective attack.

Mimics of Transaminase

Pyridoxal phosphate and the related pyridoxamine phosphate are the coenzymes for many enzymatic transformations involving amino acids. Of these, the reactions of most general importance are the transaminations by which amino acids are synthesized from keto acids. The transaminase enzymes that catalyze this process perform two reactions. In the first, an α-keto acid reacts with enzyme-bound pyridoxamine phosphate to form an imine structure (**7**) (Fig. 3). This imine then undergoes isomerization to another imine, which hydrolyzes to form the amino acid and a new coenzyme, pyridoxal phosphate (the actual enzymatic reaction is a bit more complicated than simple hydrolysis). The process then goes over the same path in the reverse direction (all the steps of Fig. 3), but with a different amino acid. The result is to regenerate the pyridoxamine form of the coenzyme, with the formation of a new keto acid. Since the total reversible path converts keto acid 1 and amino acid 2 to amino acid 1 and keto acid 2, with overall preservation of the structure of the coenzyme, the coenzyme is a catalyst (along with the enzyme).

Many elegant studies (*5*) have demonstrated that transamination (and other biochemical reactions involving these coenzymes) can be catalyzed in simple chemical systems by pyridoxal, pyridoxamine, or related compounds in the absence of enzymes. These nonenzymatic reactions of the coenzymes with amino or keto acids are slower than the enzyme-catalyzed processes, and they are also much less selective. We have designed more complicated catalysts to try to achieve better rates and selectivity.

The selectivity of the enzyme-catalyzed processes results in part from the ability of an enzyme to bind particular substrates, and not others. Furthermore, within the selectively formed complexes there are additional catalytic groups of the enzyme able to reach particular atoms, and these impose additional selectivity. Because these groups are in general not symmetrically arranged, they can produce optically active amino acids by adding a proton to one face of an intermediate rather than to the other one. If all of these types of selectivity could be achieved within artificial enzyme-like systems, it might be possible to use them in chemical syntheses of important amino acid derivatives.

In one approach to this objective, we have constructed an artificial transaminase enzyme that combines the pyridoxamine-pyridoxal coenzyme system with a cyclodextrin-binding group (*6*). The compound was constructed to show selectivity for amino acid derivatives carrying aromatic side chains that would

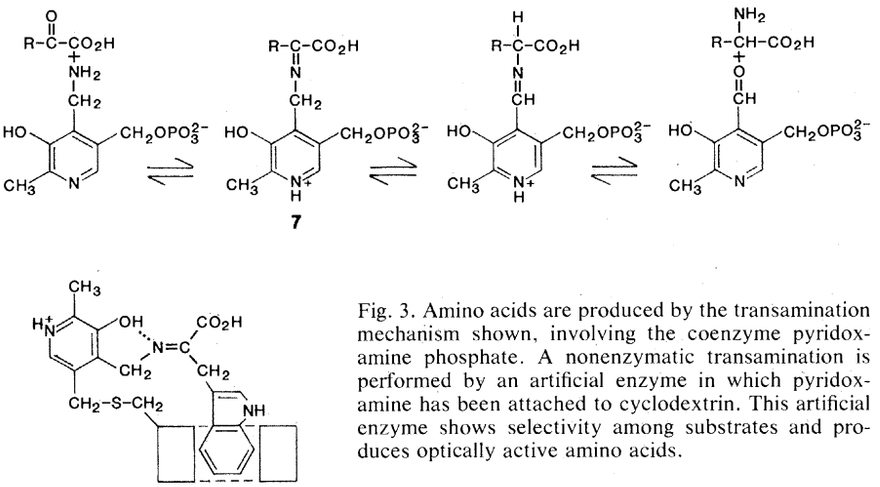

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Fig. 3. Amino acids are produced by the transamination mechanism shown, involving the coenzyme pyridoxamine phosphate. A nonenzymatic transamination is performed by an artificial enzyme in which pyridoxamine has been attached to cyclodextrin. This artificial enzyme shows selectivity among substrates and produces optically active amino acids.

bind into the cyclodextrin cavity. The desired selectivity was achieved. When the pyridoxamine catalyst carrying a cyclodextrin ring was allowed to react with a mixture of simple pyruvic acid and indolepyruvic acid (Fig. 3), the indole derivative was preferentially bound to the catalyst and selectively converted to the amino acid tryptophan. There was a 200-fold preference for the formation of tryptophan in competition with the formation of alanine from pyruvic acid, although with simple pyridoxamine carrying no cyclodextrin-binding group the competition led to essentially equal reactivities of the two compounds. Thus, a random reaction with respect to two substrates of the simple coenzyme has been converted to a selective reaction by the addition of a binding group.

An additional feature is that the cyclodextrin system is optically active, being composed of glucose units. When an amino acid is produced by our pyridoxamine-cyclodextrin system it is being formed in an asymmetric environment and can, in principle, be created in an optically selective fashion. We found that the reaction of phenylpyruvic acid to form phenylalanine did indeed show an optical preference, forming three times as much of the natural L-enantiomer as of the D-enantiomer (*6*). With simple pyridoxamine alone, no such optical selectivity is seen, since it is a completely achiral molecule. These systems are catalytic; in the presence of a second amino acid they can perform the entire transamination cycle repeatedly, turning one keto acid into an amino acid while catalyzing the reverse process for a second amino acid.

The enzymatic reaction also differs from the simple chemistry of the coenzymes themselves in the presence of additional catalytic groups in the enzyme. We have been studying mimics of this situation by adding catalytic groups to pyridoxamine and pyridoxal that can catalyze the removals and additions of protons required in the overall mechanism for transamination and other related processes (*7*). We find that we can attach chains carrying basic groups at the same position in pyridoxamine to which we attached the cyclodextrin. Some of these compounds are much more effective than simple pyridoxamine as catalysts for transamination. The most effective catalysts are those with a flexible chain that permits the basic group to reach both the carbon from which a proton must be removed and the other carbon to which a proton must be delivered in the reactions of Fig. 3. This is consistent with the finding for the enzyme that the same basic group is removing the proton from one carbon and carrying it to the other. Work is still under way to produce a catalyst that combines the pyridoxamine-pyridoxal coenzyme with a binding group to create substrate selectivity and an additional catalytic group to perform the proton transfers. Such a species could be a very selective and effective artificial transaminase.

Mimics of Ribonuclease

Ribonuclease, which catalyzes the hydrolytic cleavage of RNA, is one of the better understood enzymes (*8*). The cleavage occurs in two steps (Fig. 4) that are closely related in mechanism. In the first step, a hydroxyl group of RNA itself attacks the phosphorus atom, converting the original phosphate diester of RNA into a cyclic phosphate diester group. This results in a break in the RNA chain. In the second step the enzyme catalyzes an attack on this cyclic phosphate diester by water, producing a phosphate monoester on C-3 of the ribose sugar unit or RNA, while regenerating the hydroxyl group at C-2 of that ribose unit. Thus in the overall hydrolytic cleavage of the RNA chain, the first step involves the cleavage and the second step involves the hydrolysis of an intermediate product.

The enzyme catalyzes both steps by closely related mechanisms. In the first reaction, a basic catalytic group of the enzyme removes the proton from the attacking hydroxyl group, and an acidic catalytic group of the enzyme puts a proton onto the leaving oxygen atom. In the second step, the proton is put back on the C-2 oxygen of ribose, which is now the leaving group, while a basic catalytic group of the enzyme removes the proton from an attacking water molecule. Thus in both steps, a basic and an acidic catalyst are needed, the base assisting the removal of a proton from an attacking oxygen atom while the acid group is putting a proton onto the departing oxygen atom.

In ribonuclease, these two catalytic groups are both imidazole rings of histidine-12 and histidine-119. Catalysis occurs when one of them is present as the free imidazole ring, capable of acting as a base catalyst, and the other one is present as the protonated imidazolium ring, able to act as an acid catalyst. Thus the enzyme shows a rate maximum near *p*H 6. At a lower *p*H both rings are protonated and the needed basic catalytic group is absent, whereas at high *p*H, both rings are unprotonated and the acidic catalyst is no longer available. There is an additional important functional group in the enzyme, the ammonium group of lysine-41. This binds the phosphate anion of RNA at which the catalyzed reaction is to occur, and the hydrogen bond established makes the phosphate more reactive and thus serves a direct catalytic function.

We have produced two types of mimics of ribonuclease. In the first series, the principal catalytic groups of ribonuclease were mounted on a cyclodextrin framework so as to cause binding of the substrate and to bring about catalyzed reactions in the complex. By the use of rigid reagents it is possible to activate pairs of hydroxyl groups in cyclodextrin selectively (*9*). Using such selectively activated cyclodextrins, we have prepared cyclodextrin derivatives carrying two imidazole rings to see if they could mimic the catalytic reaction by which one of them acts as a base and the other one, in its protonated form, acts as an acid group to catalyze phosphate cleavages (*10, 11*).

A cyclodextrin bisimidazole (compound **8**) has two of the correct catalytic groups to imitate ribonuclease, but the binding site is not optimized for the binding of RNA itself. We have therefore used these cyclodextrin-based systems to examine other substrates whose geometry is optimal for binding and reactions with our catalyst. Artificial enzymes would be expected to have different selectivities from natural enzymes because of their different geometries. This novel selectivity is one of the important reasons for preparing such catalysts.

Molecular models suggested that cyclodextrin bisimidazoles should be able to catalyze the hydrolysis of compound **9**, a cyclic phosphate based on *tert*-butylcatechol. Such a cyclic phosphate resembles the intermediates in the cleavage of RNA, and the process we are examining is then very similar to the second step in the reaction of ribonuclease with RNA itself. We found that this was indeed the case. The cyclodextrin derivative **8** was an effective catalyst for the hydrolysis of the cyclic phosphate **9**, and by the same mechanism as that used by the enzyme. This catalyst also showed a rate optimum near *p*H 6, indicating that one of the imidazoles was acting as a base catalyst while the other one, in its protonated form, was acting as an acidic catalyst. Perhaps even more striking is the fact that the catalyst showed a selectivity closely related to that expected for an enzyme-like mechanism.

When the simple cyclic phosphate **9** is submitted to vigorous chemical conditions, it hydrolyzes to form two products, **10** and **11**, because attack by water can lead to departure of either of the oxygen atoms of the cyclic phosphate. By contrast, when this same substrate undergoes the much faster hydrolysis catalyzed by **8**, it forms a single product, **11**. This selectivity is caused by the geometry of the catalyst-substrate complex. In the attack by water on a cyclic phosphate ester, it is expected that the attacking water oxygen atom will come in on exactly the opposite side of phosphorus from the leaving oxygen atom. That is, the geometry of the activated complex at the transition stage for the reaction has five groups around the phosphorus (the original four plus the attacking water oxygen) arranged in the form of a trigonal bipyramid. The three oxygens that are not attacking or leaving are arranged in an equilateral triangle around the phosphorus, while the attacking and leaving oxygens are arranged above and below the plane of this triangle so as to form a 180° alignment of O–P–O. Molecular models of this mechanism for the cleavage of substrate **9** bound into the cavity of catalyst **8** indicate that this geometry is possible only for one oxygen as leaving group, delivering the water and ejecting that oxygen so as to form the observed product **11**. If instead, a molecular model is constructed in which water is aligned with the other P–O bond, in a mechanism aimed at producing product **10**, the water is too far away from the cylcodextrin catalyst and cannot be delivered by one of the imidazole groups. Thus as Fig. 5 shows, the observed selectivity of this process is precisely what is expected from the geometry of the system.

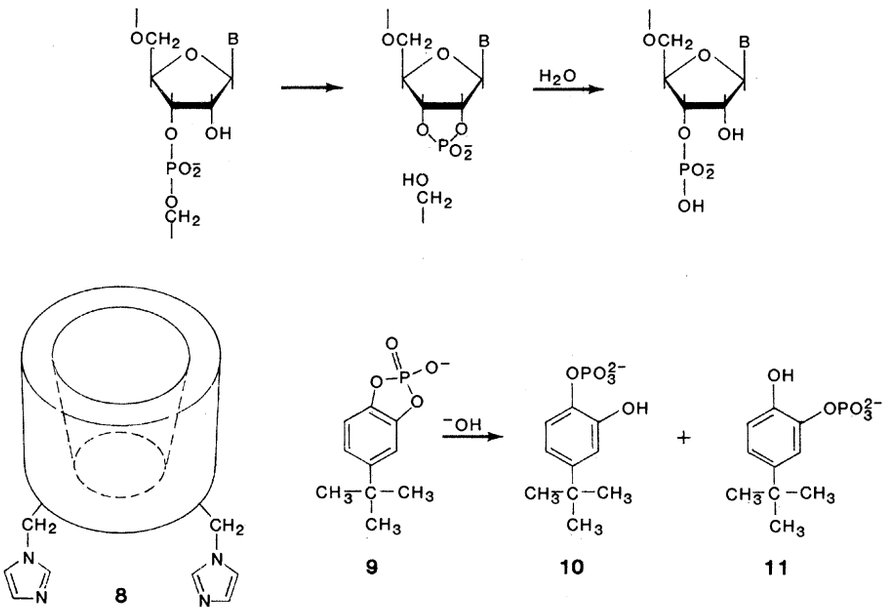

Fig. 4. The sequence by which ribonuclease hydrolyzes RNA (top). Compound **8** is a cyclodextrin bisimidazole artificial enzyme that catalyzes the hydrolysis of substrate **9** by an enzyme-like mechanism. Under simple hydrolysis, **9** yields a mixture of **10** and **11**.

This geometry can be changed by constructing a new catalyst in which the catalytic groups are further from the cavity. This was done with catalyst **12** carrying two imidazole rings attached to cyclodextrin through a short chain. Molecular models show that this species can reach out to a water molecule attacking substrate **9** so as to form product **10** and should be able to catalyze an alternative selective cleavage of the same substrate. This was indeed the case. This new catalyst **12** uses a mechanism similar to that of **8**, with a *p*H optimum indicating that the catalyst must be present with one group in the basic form and the other one in the protonated acidic form. However, this catalyst is completely selective for the alternative mode of cleavage of substrate **9**, producing only product **10**. Figure 5 shows that this is to be expected from the geometry of the system, as judged from molecular models.

The catalytic function of the protonated imidazole in this new system **12** is apparently not to protonate the leaving group, but to protonate the phosphate anion, thus resembling the catalytic function of lysine-41 in ribonuclease itself. This can be determined from a detailed consideration of the *p*H-rate profile and its comparison with the titration curve for the catalyst. However, the selectivity is a reflection of the location of the base catalytic groups in catalysts **8** and **12**, respectively. Cyclodextrins with only one catalytic group, acting as a base catalyst (*11*), have been prepared. These are less effective because of the loss of the additional acidic catalytic function, but they show the expected selectivity. A cyclodextrin monoimidazole related to compound **8** gives only product **11**, whereas a similar monoimidazole derivative related to catalyst **12** gives only product **10**.

Although artificial enzymes like **8** and **12** combine catalytic and binding groups and show enzyme-like mechanisms, with enzyme-like selectivities for a substrate (**9**) that fits the cavity, it would be of interest to develop artificial enzymes that are effective at cleaving RNA itself. We have approached this problem by considering geometrical questions related to those outlined above. In the transition state for the first step of the ribonuclease reaction, in which a hydroxyl group is attacking the phosphate to form a cyclic phosphate intermediate, the ar-

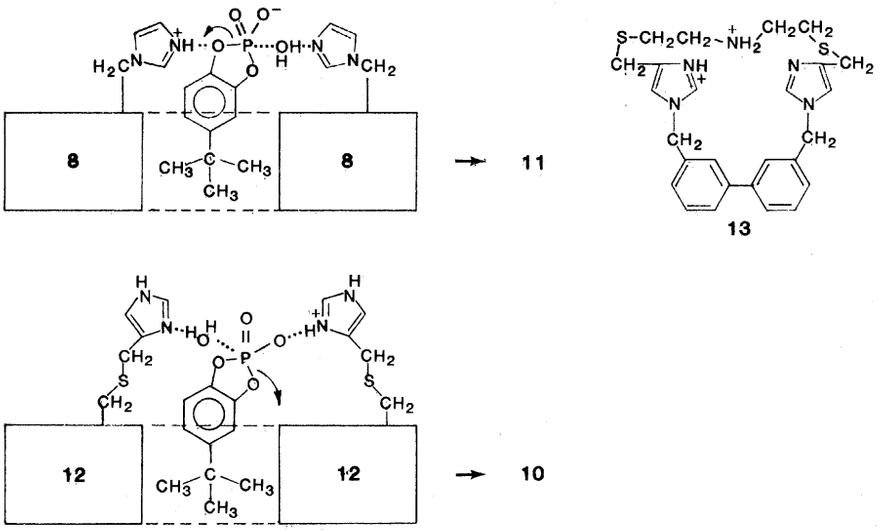

Fig. 5. The geometry of the complexes is such that artificial enzyme **8** hydrolyzes substrate **9** to afford only **11**, while artificial enzyme **12** converts the same substrate only to **10**. Both use ribonuclease-like mechanisms. Compound **13** is another artificial enzyme that catalyzes the hydrolysis of RNA itself.

rangement must again be a trigonal bipyramid with the attacking and leaving groups aligned at 180°. Furthermore, these attacking and leaving groups must be coordinated to the catalytic imidazoles that are adding or subtracting protons, and one of the equatorial oxygens on the phosphorus must be ion-paired with the lysine-41 ammonium ion. We thus set out to construct a molecule that would carry three such catalytic groups—two imidazoles and an ammonium ion—in the correct position to establish the required coordination. Molecular model building made it clear that the desired arrangement could be achieved in compound **13**, a structure that looked synthetically accessible. We have synthesized this molecule (*12*) and find that it is indeed a catalyst for the cleavage of RNA, making a cut every 20 minutes or so in dilute (20 m*M*) solution. Selective catalysts based on this structure may become useful biological tools.

Enzyme-Like Reactions with Very High Velocities

In all of the cases we have been discussing the reactions have been accelerated by the catalysts and in most of the cases have resulted in interesting new selectivities. However, the rate accelerations are modest by enzymatic standards. For instance, in the transamination mimic, the binding to a cyclodextrin group led to a 200-fold acceleration. While this acceleration is enough to make the reaction highly selective, because it occurs only for some substrates and not others, it does not produce a very large velocity. Similarly, in the cyclodextrin ribonuclease mimics, very high selectivities were observed, but the rate accelerations for hydrolysis of the substrate **9** were at most 50-fold as a result of bifunctional catalysis by the two imidazole groups. If artificial enzymes are to be considered truly successful, they must produce rate accelerations of similar magnitudes to those achievable with natural enzymes.

Many research groups have studied reactions of simple cyclodextrin complexes (*1*). When a substrate is bound into the cyclodextrin cavity, parts of the substrate are held near hydroxyl groups of the cyclodextrin glucose units, and this proximity might lead to high intracomplex reaction rates. Such reactions would be mimics of enzymatic reactions in which a bound substrate is attacked by an enzymatic hydroxyl group. For instance, in proteases such as chymotrypsin, attack by the hydroxyl group of the amino acid serine on an amide bond of a bound substrate leads to the formation of an ester. Part of the substrate becomes attached to the serine hydroxyl while the other part has been broken off and can depart. In a second step, the intermediate ester is then hydrolyzed, breaking off the other piece of the original peptide and regenerating the enzyme. This two-step sequence is reminiscent of a similar sequence for ribonuclease, described above, except that with the serine proteases, the hydroxyl group is derived from the enzyme, not from the substrate. Other enzymes also use a serine group to attack carbonyl and phosphoryl groups. Thus, early studies on cyclodextrin chemistry focused on an imitation of this first step, namely, attack by a hydroxyl group on a bound substrate.

Attack by a hydroxyl group within the same molecule can be very rapid (*13*). An intramolecular reaction, with a well-placed hydroxyl attacking a neighboring group, can occur at rates 1 million or more times as fast as the rate of attack on a substrate by solvent molecules that completely surround the substrate, but are not directly attached to it. Such very fast reactions by groups within the same molecule reflect the entropy advantages of an intramolecular attack (*14*). These examples suggested that the very high reaction rates seen in many enzymatic processes, compared with simple chemical reactions between unassociated reagents, similarly reflect the entropy advantages of chemical reactions between species bound together in a tight complex. For this reason, it was disturbing that in all of the examples (*1*) of cyclodextrin-promoted reactions, the accelerations did not exceed 300-fold.

When an ester substrate is bound into cyclodextrin and is then attacked by a cyclodextrin hydroxyl group to make a new ester, the rate of this attack by the cyclodextrin hydroxyl can be compared with the rate of attack by a solvent water hydroxyl under the same conditions in the absence of the cyclodextrin. Because, over the normal region, both the cyclodextrin process and the simple hydrolysis reaction have the same dependence on base, the relative reaction rate ratio is independent of *p*H. When the comparison is done, it turns out that for many bound ester substrates, attack by cyclodextrin is only 100-fold or less faster than simple hydrolysis of the corresponding substrate. The best rate acceleration with β-cyclodextrin when we started our studies was 250-fold, achieved with the substrate *m-tert*-butylphenyl acetate which had been tailored to fit the cavity well (*15*).

Molecular models show that when the *tert*-butylphenyl group of this substrate binds into the cyclodextrin cavity, the ester carbonyl group of the acetate can be in contact with one of the hydroxyl groups of the cyclodextrin. Thus, good proximity seems to be present. However, molecular model building also indicates that when the hydroxyl group attacks the ester carbonyl, the normal geometry of the resulting chemical bonds requires that the *tert*-butylphenyl group be pulled significantly up out of its most stable binding position. Thus, the prediction from these models was that the binding of the substrate is better than the binding of the transition state for the intracomplex reaction in which the hy-

droxyl is attacking the carbonyl group. Ideally an enzyme should bind the transition state more strongly than it binds the starting material (*16*); loss of binding in the course of the chemical reaction should seriously slow the reaction rate. We therefore set out to design systems in which not only the substrate but also the transition state would be well bound in order to optimize the velocity effect of complexing.

One approach to this is to modify the cyclodextrin cavity. We have prepared several derivatives of cyclodextrin carrying groups that can penetrate part of the way into the cavity to produce an intrusive floor (*17*). Studies of the binding of various substrates to the modified cavity show that this intrusion does occur; the cyclodextrin open cavity is turned into a closed pocket that is shallower than the cavity of the unmodified system. Under these circumstances, the original substrate must bind less deeply, although the binding is still strong because the new floor on the molecule contributes to the overall binding interaction with the substrate. As expected from this, the velocity of reaction of a substrate with the modified cyclodextrins was improved because the new binding geometry is closer to that required for the transition state. Increases in rate by an additional factor of 10 or so were achieved with this kind of modification. In another approach (*17*), we constructed substrate molecules carrying additional projecting groups that prevented them from binding deeply into the cyclodextrin cavity. Again this change brought the geometry of binding of the substrate much closer to the geometry required for the transition state, and the reaction rate with a cyclodextrin increased to 4000 times the reaction rate with solvent. However, the most dramatic improvements (*17*) occurred with novel substrate classes whose geometries were optimized so that there would be little loss of binding in proceeding to the transition state from the bound starting material.

One set of substrates was based on the adamantane nucleus (*17*). A reaction rate 15,000 times the rate with solvent at the same *p*H was observed for the reaction of one such substrate with the cyclodextrin. The substrate carried some projecting groups to guarantee that it would adopt the productive geometry rather than an alternative inactive complex. The most striking rate accelerations were seen with substrates based on the ferrocene nucleus. Ferrocene (the sandwich compound between two cyclopentadienyl rings and an iron atom) fits β-cyclodextrin well and is strongly bound into the cavity. Since substituents come off at essentially right angles to the axis of the ferrocene system, we expected (from models) that the transition state for attack by hydroxyls would also be strongly bound. In one substrate based on a simple ferrocene nucleus, the attack by a hydroxyl within the complex was 750,000 times as fast as simple attack by solvent under the same conditions (*17*). Improved fused-ring derivatives of the ferrocene system, with better geometric definition, have led to accelerations of as much as 6 million–fold (*18*), and in one instance, the optical activity of the cyclodextrin nucleus led to a selection of one of the two mirror-image isomers of the substrate in the ratio 65 to 1.

A 6 million–fold acceleration, achieved by complexing a substrate to put it near cyclodextrin hydroxyl groups, is quite substantial. In fact, the reaction is performed in a mixed organic-water solvent, which imitates the interior of an enzyme. The rate is 150 million times the rate of an uncatalyzed reaction in water (the comparison used for enzymes themselves). The enzyme chymotrypsin achieves much less than this rate acceleration in attack on esters, although most enzymes operating on their natural substrates achieve somewhat higher rate accelerations. However, the simple reaction between cyclodextrin and a bound substrate does not involve any of the other catalytic groups normally present in an enzyme. If such catalytic groups are placed correctly in enzyme mimics, so as to assist the intracomplex reactions, even larger accelerations can be expected. Furthermore, in the best substrate examined so far, the transition state for the chemical reaction seems to be almost as well bound as the substrate. A more interesting class of substrates would be those in which the transition state is bound more strongly than the substrate, mimicking the situation believed to exist for many enzymes. In such cases, accelerations even larger than those already achieved can be expected.

Conclusions

The field of the synthesis and study of artificial enzymes is in a sense in its infancy. Most of the work with molecular complexing has involved the use of cyclodextrins, and only recently has attention begun to focus on new artificial cavities that could bind substrates in other ways (*19*) and with different kinds of selectivity. However, it is already clear that with appropriate molecular design it will be possible to achieve very large rate accelerations, comparable to those typical of enzymatic processes. More exciting, the application of the principles learned from the study of enzymatic reactions permits us to design novel chemical processes that can achieve desirable selectivity of the sort not otherwise available outside of biochemistry. The selective, accelerated reactions achieved with artificial enzymes that mimic the natural catalysts have the potential to play an important role in chemical synthesis, and such substances may even prove to have therapeutic utility. Thus one can look forward to continued vigorous growth of this field.

References and Notes

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20. Supported by the National Institutes of Health and the National Science Foundation. I thank my co-workers, some of whom are named in the references, for experimental and intellectual contributions.