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Bifunctional binding and catalysis

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Cyclodextrin dimers can show very strong binding of appropriate substrates, and also significant geometric recognition. When the link between the two cyclodextrin rings carries a catalytic group, good turnover catalysis can be observed. The binding and catalytic properties are comparable with those of some catalytic antibodies. When a cyclodextrin binding group carries two catalytic groups, co-operative bifunctional catalysis can be observed. The details of this process are best understood in a mimic of ribonuclease, but other reactions that show bifunctional catalysis by these enzyme mimics are also being explored. The design of these catalysts was strongly influenced by detailed mechanistic studies of bifunctional catalysed cleavage and isomerization of RNA. A brief description of this evidence for the mechanism is presented. Recent findings on the properties of an isomer of DNA are also discussed.

BINDING BY CYCLODEXTRIN DIMERS

In contrast to most simple chemical catalysts, enzymes are polyfunctional. They generally carry out a chemical process using more than one catalytic group, often using the multiple catalytic groups simultaneously. In addition, they often bind the substrate with more than one interaction. Thus in a first approach to this type of catalysis, we have studied molecules whose binding can be bifunctional, and others whose catalytic mechanisms are bifunctional. The future target is of course to develop enzyme mimics that use multiple binding interactions to direct the geometry of the enzyme–substrate complex precisely, and to provide multiple catalytic groups acting in concert to provide the best speed and specificity.

In our early work, we simply linked two α-cyclodextrins together on the secondary face with a terephthalate tether, and saw that they were particularly effective in acylation reactions with substrates that could bind to both cavities.[1] Since then a number of other groups have made cyclodextrin dimers, and examined them briefly.[2,3] In later work[4] we used a number of chemical links to hold two cyclodextrins together, and saw that the resulting bis-cyclodextrins bound dimeric substrates (those with two appropriate hydrophobic units held in the correct positions) with good binding constants. Most of this work involved substrates having t-butylphenyl units with which to bind into the β-cyclodextrin cavities.

Monomeric t-butylphenyl substrates such as t-butylphenol typically show binding constants with β-cyclodextrin of 10^4 M^{-1} or so in water. We found that binding constants for the best dimeric cases approached 10^8 M^{-1}, or a little higher. It could be thought that the free energy of binding of a dimer would simply be twice that for the monomeric case, so the binding constant would be 10^8 M^{-1}; however, this ignores the chelate effect. The essence of the chelate effect is that translational entropy affects the energy of monomeric binding, but its contribution is not doubled in the dimeric case since binding one end of a dimer ties down the other end. In an ideal case we should see considerably more than 10^8 M^{-1} binding constant for a substrate with two t-butylphenyl groups. However, flexibility, such as that found in a singly linked β-cyclodextrin dimer, in which the two units can twist into many different relative conformations, works against binding.

We turned our attention to cyclodextrin dimers with two linking groups, to better define the geometry of the molecules. Molecular models suggested that the best cases would be those in which the linkages involved two neighbouring positions on a cyclodextrin. With bridging functionalizing reagents it is possible to activate the primary hydroxy groups of two neighbouring glucose units in β-cyclodextrin, leading to the 6A,6B di-iodide.[5] Then two bridging units can be put between them, and in two ways. If the link is between the 6A position of one cyclodextrin and the 6B position of the other (lettering the glucose units A, B, C, etc. clockwise around the ring), and *vice versa*, the result is what we call a clamshell, in which the two rings can co-operatively bind simple dimeric substrates.[6] By contrast, if the two cyclodextrins are linked 6A to 6A′, and 6B to 6B′, then the arrangement is what we call the 'loveseat', in which the link separates the two binding domains.

We have done some studies with symmetric systems in which the linkages are identical (S. Halfon, unpublished work). Thus reaction of β-cyclodextrin–6A,6B–di-iodide with imidazole affords β-cyclodextrin–6A,6B–di-imidazole, a compound that will be discussed

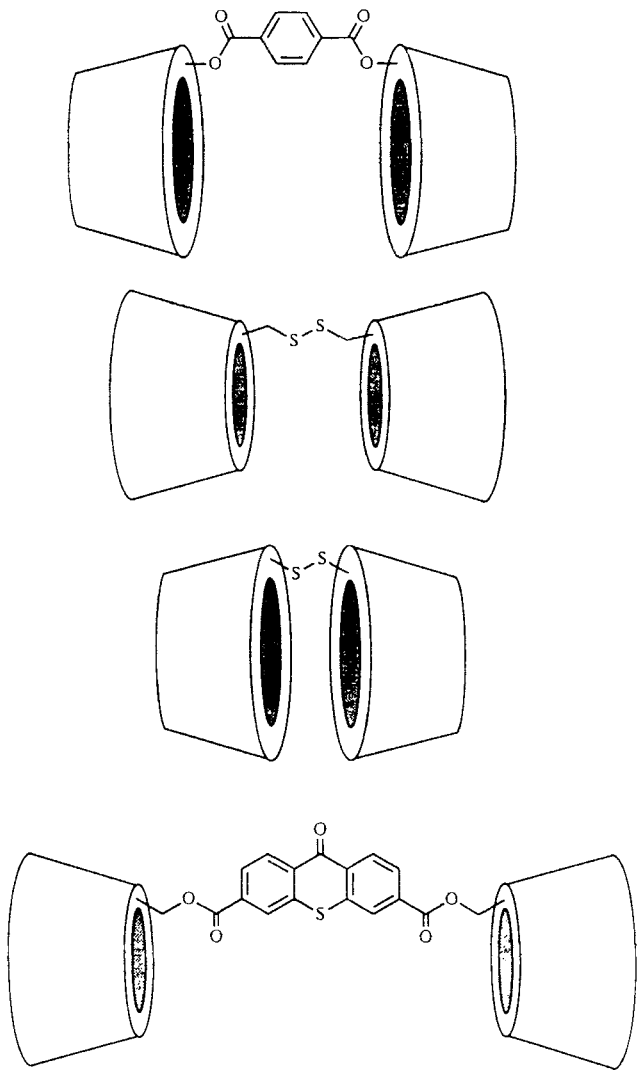


Figure 1 Some cyclodextrin dimers linked on the secondary or the primary face.

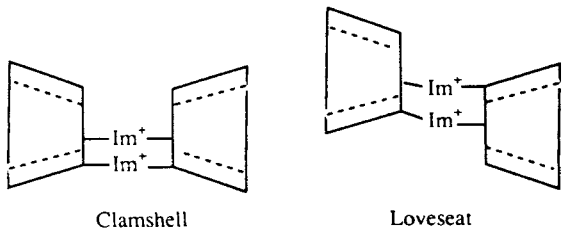


Figure 2 Clamshell and loveseat arrangements for doubly linked cyclodextrins. The ones shown have imidazolium links attached to neighbouring glucose units, and are derived from structure 7.

again later. Reaction of this with the β-cyclodextrin-6A,6B-di-iodide affords a mixture of two dimers linked by imidazolium groups—the clam shell and the loveseat. As expected, binding of ditopic substrates by the clamshell was considerably stronger than by simple cyclodextrin, while binding by the loveseat isomer was comparable with that by simple cyclodextrin. Because of this (expected) difference, it was easily possible to separate the two isomers by affinity chromatography. We covalently attached a substrate to a chromatographic material, and preferentially retarded the stronger binding clamshell isomer (S. Halfon, unpublished work).

Structural evidence came from NMR studies. The C-2 hydrogen of the imidazolium ring is easily seen at low field. In the clam shell the two linkages are equivalent and a single signal is seen. In the loveseat the A,A′ link is not equivalent to the B,B′ link, and two signals are seen. Of course the binding behaviour made the structures evident in any case.

Enzymes can use their binding energy in part to distort the substrate geometry toward that of the transition state. Put another way, enzymes frequently bind the geometry of the transition state more strongly than they bind that of the substrate. Of course all catalysis is a form of transition state binding: binding is the interaction of two species with a resultant lowering of energy, and catalysis is the interaction of the catalyst with the transition state, leading to a lowering of energy. However, enzymes often achieve this not simply by binding directly at the reacting centre, but also by preferring shape changes quite far from the point at which chemistry is occurring. This is the basis for many enzyme inhibitors based on transition state analogues. As an example, reaction of an amide through a transition state that resembles the tetrahedral intermediate can lead to bending of the substrate that can be recognized quite far from the reaction site.

It seemed to us important to learn how to mimic this kind of special binding effect. Thus we synthesized two cyclodextrin dimers in which one linkage was long and the other one short.[6] Again one is the clamshell structure, and the other the loveseat arrangement. Again the loveseat compound, whose structure was established by NMR, showed only modest affinity for dimeric substrates. (With two different linkages, the long one could in principle be between either the 6A,6A′ positions or the 6B,6B′ positions. Apparently the 6B position of β-cyclodextrin-6A,6B-di-iodide is more reactive toward nucleophiles [N. Kumabe, unpublished work], so when the long linkage is attached first we get predominantly the 6B,6B′ linkage.)

Binding by the clamshell isomer **1** was extremely strong in some cases.[6] As shown, binding of the fluorescent probe **2** to **1** had a binding constant of over 10^8 M^{-1}. This was so high that only fluorescence methods are convenient for the measurements; only they are sensitive enough to pick up a signal at concentrations near 10^{-8} M at which the complex is

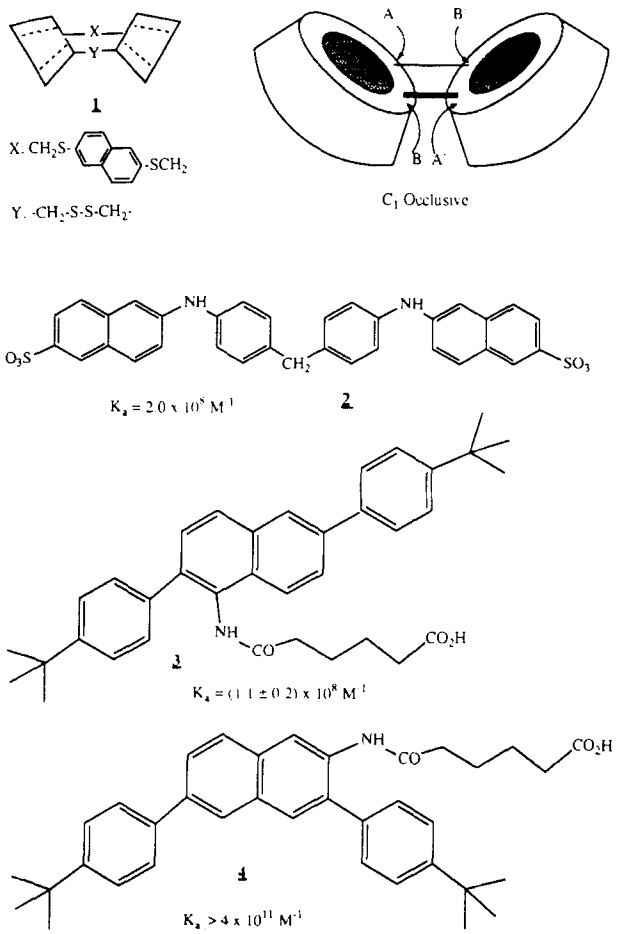


Figure 3 A doubly linked cyclodextrin clamshell with linkages of different lengths, and some substrates to which it binds. The binding constants in water are shown for each substrate.

partially dissociated. Binding leads to a large change in fluorescence, and so it can be measured. Some non-fluorescent systems are bound even more strongly, but their binding constants have been determined so far only by competing them with the fluorescent **2** for the host.

Using such competition fluorescent methods, we saw that substrate **3** had a binding constant of 10^8 M^{-1} for dimer **1**, while substrate **4** had a binding constant greater than 10^{11} M^{-1}.[6] New more strongly bound fluorescent probes are needed to enable us to determine more precisely the number in this very high binding range, but it is already clear that this binding behaviour is very attractive. The hoped for shape selectiviey has been achieved, in the contrast between **3** and **4**, and the binding constants are higher than those of most antibodies for their substrates.

We have described **1** as a clamshell, and the comparison is apt. With the two linkages on neighbouring glucose units, models show that they act as a hinge and permit the molecule to open and close. Thus **1** has one of the most fundamental properties of a biological receptor such as an enzyme or antibody–the ability to adopt an open structure to permit access of the substrate, and then to close up around that substrate. The process has been termed 'induced fit', in contrast to the old lock and key idea.

This is undoubtedly happening with substrate **4**. Models show that the central naphthalene unit is really too large to pass through the cyclodextrin cavity, so substrate binding does not occur by threading the substrate into the already closed **1**. Instead it seems most likely that **1**, in an open geometry, permits approach of the two t-butyl units to the cavity, and that **1** then snaps shut around the substrate. The next goal is to turn the observed geometric preference for bent structures into a useful catalytic factor. That is, we need to incorporate catalytic groups into such a geometrically biased host, so that the preference for binding the geometry of the transition state can contribute to catalysis.

CATALYSIS BY CYCLODEXTRIN DIMER DERIVATIVES

We have synthesized a number of cyclodextrin dimers that incorporate metal catalytic groups in the linkages. Actually the simplest involve using the metal ion to build the bridge; β-cyclodextrin–6A,6B–bis-imidazole can form complexes with metal ions in which four imidazole ligands from two cyclodextrin units are co-ordinated to the metal (S. Halfon, unpublished work; R. Zarzycki, unpublished work). However, these compounds have not yet proven to be as interesting as some others.

The best catalytic system so far was prepared by linking two β-cyclodextrins at their primary C-6 carbons with a bipyridyl unit.[7] This compound **5** binds appropriate dimers well, and it also binds metal ions to the bipyridyl unit. In the best case, the Cu^{2+} complex catalyses the hydrolysis of substrate **6** with a very high rate and good turnover. We see at least 50 turnovers without any sign of product inhibition, and at 25°C and pH 7 we obtain an acceleration corresponding to approximately 225,000 over the uncatalysed hydrolysis rate under the same conditions. The pH dependence of the rate suggests that the mechanism involves bifunctional attack, with the Cu^{2+} acting as a Lewis acid while a metal-bound hydroxide ion acts as a nucleophile.

The rate is quite similar to that of one of the best catalytic antibodies which also cleaves a *p*-nitrophenyl ester.[8] However, in contrast to the situation with some antibodies, our system does not show product binding

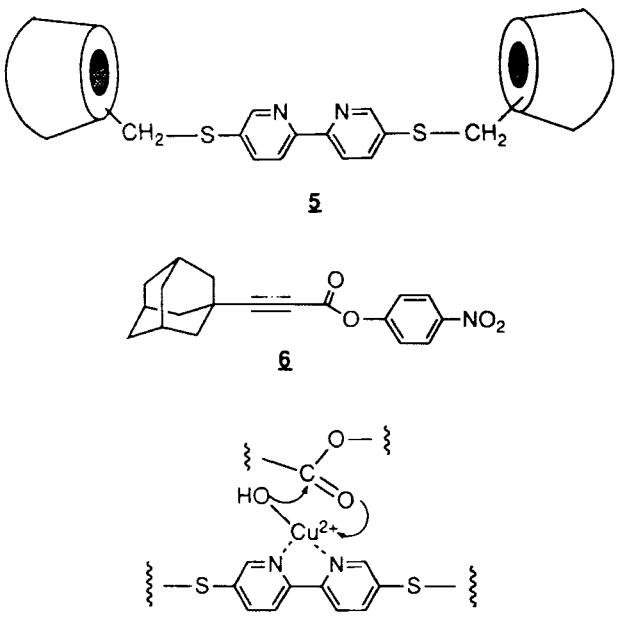


Figure 4 A cyclodextrin dimer **5** whose Cu^{2+} complex is a highly effective catalyst for the hydrolysis of substrate **6** as pH 7.0 and 25°C. The likely mechanism is shown. The observed rate is similar to that of a related process catalysed by an antibody.

and inhibition. We have also made related catalysts by attaching the bipyridyl unit to the secondary face of β-cyclodextrin (S. Halfon, unpublished work). These are also very promising.

SEQUENTIAL AND SIMULTANEOUS BIFUNCTIONAL CATALYSIS BY ACID AND BASE GROUPS

Most hydrolytic enzymes use two functional groups (acting as an acid and a base) as catalysts; in a few cases, more are involved. Metal ions can play one or both roles, as in **5**, but for non-metal enzymes it is often found that the imidazole sidechain of a histidine in the enzyme is a catalytic group. Because the pK_a of imidazole is near 7.0, imidazole is the strongest base that can be available unprotonated at near neutrality. At the same time, the imidazolium ion is the strongest acid that can be available still protonated in this pH region. The pK values of individual imidazole units in enzymes are often perturbed by the environment, so one imidazole unit may be fully free and able to act as a base while another may be fully protonated and able to act as an acid. This is the situation in the enzyme ribonuclease A.

In this enzyme, RNA is cleaved by a 2-stage process in which the first stage is conversion of the linear phosphate diester to a cyclic phosphate diester (a 2′,3′ cyclic phosphate). This leads to cleavage of the chain. In the next enzymatic stage this cyclic phosphate is hydrolysed to form the 3′-monophosphate ester. In both of these processes the principal catalytic groups are the imidazole rings of His-12 and His-119, although the ammonium group of Lys-41 also plays a role.

The classical mechanism for the cleavage–cyclization step has the imidazole ring of His-12 acting as a base to deprotonate the attacking 2′-hydroxy group, while the imidazolium ring of His-119 acts to protonate the leaving group. Some years ago we initiated mechanistic studies in this area. We investigated the ability of imidazole buffer alone to catalyse the cleavage of RNA;[9,10] of course we realized the reaction would be slow and require high concentrations of the catalyst to make up for the lack of enzymatic binding. However, we thought that if we saw catalysis we could learn some important things. It is generally possible to elucidate reaction mechanisms in detail for simple chemical processes, while for enzymatic reactions the mechanisms proposed are often just educated guesses.

We saw that imidazole buffer does indeed catalyse the cleavage of polyuridylic acid (polyU), and with a mechanism that involves both imidazole and imidazolium ions.[9,10] That is, the rate was at a maximum when both buffer species were present. In this sense it is like the enzymatic case, where the pH vs. rate profile shows that both the imidazole (Im) and the imidazolium ion (ImH^+) sidechains are needed for catalytic activity. However, in solution it was quite unlikely that both catalytic species were operating at once, since there was no enzyme to hold them in place. This was confirmed by the finding that in the kinetics of the reaction the buffer concentration appeared to the first power, while if the transition state had both an imidazole and an imidazolium ion in it, the buffer concentration would appear to the second power.

Various controls established that no complexing was hiding a higher order dependence. Thus the two buffer species Im and ImH^+ must be operating in different steps; the cyclization involves two steps with an intermediate. The intermediate in phosphate substitution chemistry is well known to be a phosphorane, the 5-co-ordinate phosphorus equivalent of a tetrahedral intermediate in carbonyl reactions. Apparently one buffer component catalysed the formation of this intermediate; the other catalysed its forward conversion to the cyclic phosphate product.

This situation was further clarified by a study of the catalysed reactions of simple dinucleotides. Most of the work was done with uridyluridine (UpU), which cleaves to give uridine and uridine-2′,3′-cyclic phosphate,[11] but the results were confirmed with the adenosine analogue ApA.[12] Imidazole buffer was again a catalyst, and again we saw evidence that the

mechanism involved two steps with a phosphorane intermediate, in which Im catalysed one step and ImH^+ the other. However, now it was possible to fix the sequence of catalysis. We saw that the substrate 3′,5″-UpU was not only cleaved by the buffer, but was also converted to the 2′,5″-UpU isomer. However, in contrast to cleavage, the isomerization was catalysed by ImH^+ only.[11]

Such isomerizations must also involve phosphorane intermediates, since the geometry of phosphate reactions requires that an intermediate form undergoes pseudorotation as part of the migration. Thus it seemed certain that the cleavage and the isomerization proceeded through the same intermediate, and that the two reaction paths branched off after the phosphorane was formed (Fig 5). If this were so, then ImH^+ must be the catalyst for the first step (it is the only catalyst observed for isomerization), and the cleavage path from the intermediate must involve Im catalysis. On this basis a detailed mechanism was proposed for these processes (Fig 6).

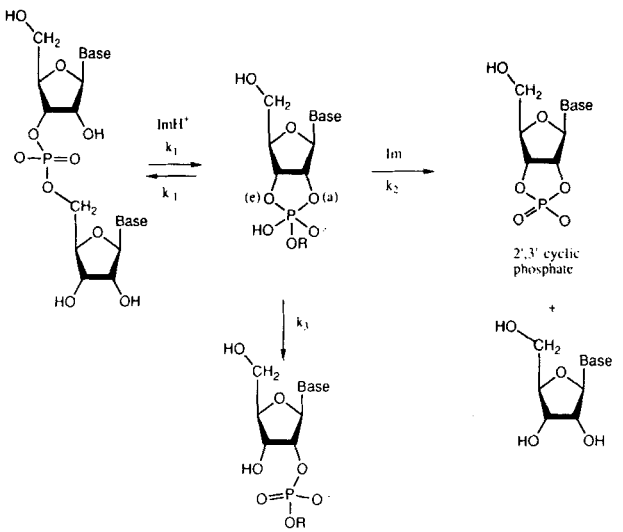


Figure 5 The general kinetic scheme for the cleavage and isomerization of ribodinucleotides catalysed by buffers. Because there is a common intermediate from which the two paths branch, and only the cleavage path shows catalysis by imidazole, an increase in the imidazole concentration is seen to slow the isomerization rate.

Further evidence for this mechanism came from studies with buffers rich in the basic Im component.[12] As Figure 5 makes obvious, an increase in the concentration of Im speeds the conversion of the intermediate to the cleavage product. This decreases the concentration of the intermediate and thus slows the observed rate of formation of the isomer, whose formation is not catalysed by Im. We observed exactly this—basic buffers were still normal catalysts for the formation of the cleavage products, but they slowed the rate of isomerization. Furthermore, when the ImH^+ concentration was held constant but Im concentration was increased, the rate of cleavage went up but the rate of isomerization went down, all corrected for the effects of changing pH.[12] As expected from all this, with a more acidic acetate buffer we saw that isomerization is faster than cleavage, in contrast to the faster cleavage seen with imidazole buffer.[11]

The mechanistic idea involved in these kinetics is quite simple and rather classical—if an unstable intermediate is formed that can then react in two

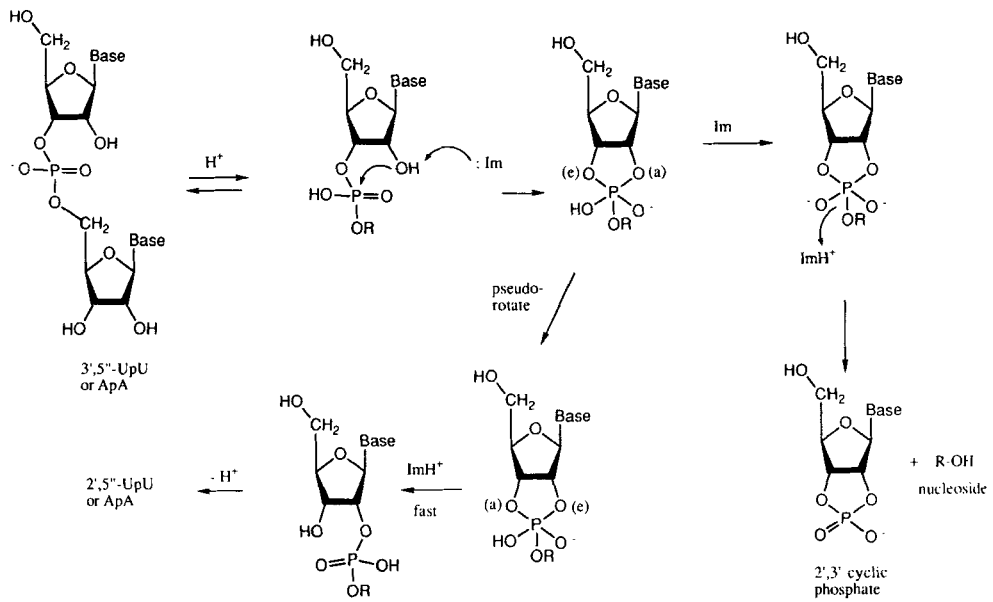


Figure 6 The detailed mechanism for the process outlined in Figure 5. At high buffer concentrations there is evidence of an additional minor catalytic process.

different ways, speeding up one of the paths from that intermediate will be seen to slow the formation of the other product. For instance, in solvolysis reactions that produce an unstable carbonium ion intermediate, trapping the carbonium ion with a nucleophile will slow the rate at which other products are formed, such as the olefin or the alcohol. The branching must come from an unstable intermediate whose concentration is lowered by speeding up one branch. Our studies were performed with initial rates, in which only a small percentage of substrate was converted, so that it was not the substrate whose concentration was being lowered by the Im catalysis. It must have been the intermediate.

This work really established the mechanism involved in these processes. However, because the mechanisms involved are quite important, and because some critics misrepresented the situation and clouded the picture, we did a further detailed quantitative study.[13] The striking mechanistic findings came with basic buffers; thus we selected morpholine as catalyst, which is even more basic than imidazole. We examined the isomerization of UpU, and its simultaneous cleavage, catalysed by morpholine buffers of fixed base/acid ratio 9 to 1, and with ionic strength held constant, so that the pH was unchanging. We saw again an increase in cleavage rate as the buffer concentration was increased, and a decrease in isomerization rate. However we now had many data points and were able to fit the observed rates to the detailed kinetic equations for the mechanisms (Fig 7). In the earlier work the data had also fit the theoretical equations, but there were not enough points for a good quantitative test.

The new work, with detailed quantitative fitting of the observed data to the mechanistic predictions, absolutely confirms our previous mechanistic conclusions. It also adds some solid evidence for particular details of the mechanism that we have previously only suggested, because there was not enough data to establish them definitively.

As we have described elsewhere,[14] the detailed mechanism that this work implies led us to re-examine the evidence about the enzyme ribonuclease A. The preferred mechanism for imidazole catalysis in solution was really not consistent with the 'classical' mechanism for the enzyme. We were interested to see that the structural data on the enzyme were actually more in line with the requirements of our new mechanism than of the classical one. We thus suggested a new mechanism for the enzyme: trajectory calculations on ribonuclease by the Karplus group[15] indicate that our new mechanism seems to be better than the classical mechanism! Thus the mechanistic study with imidazole buffer has furnished real insight into the enzyme itself.

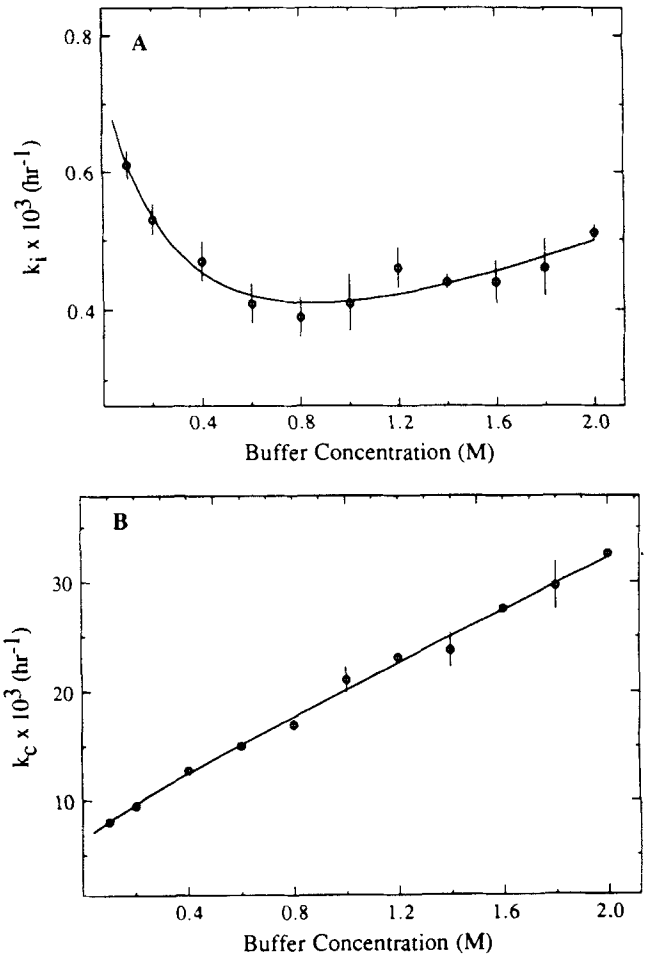
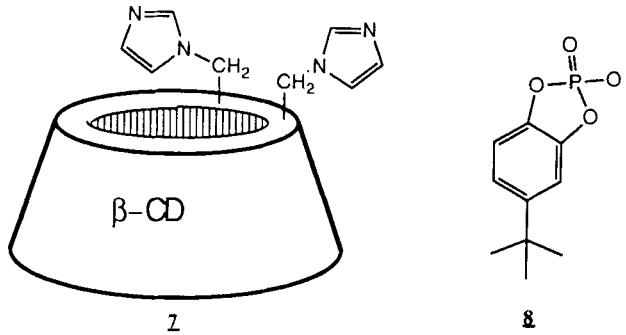


Figure 7 Experimental data for the isomerization of 3',5''-UpU to 2',5''-UpU (curve A) and for its cleavage to form uridine-2',3'-cyclic phosphate and uridine, catalysed by a 9/1 morpholine/morpholinium buffer at constant pH and ionic strength. The curves are theoretical lines for the proposed detailed mechanism of these processes. The excellent agreement with experiment completely confirms earlier more qualitative work which gave the first indication of the unexpected mechanism used. This mechanism has led to re-consideration of the enzymatic process, and has stimulated the synthesis of a particularly effective enzyme mimic.

We also realized that some work we had done constructing an enzyme mimic for ribonuclease needed to be re-examined. We had attached two imidazole rings to β-cyclodextrin, on the primary face, and guided by the classical enzyme mechanism had placed the two on opposite sides of the ring.[16] However, our new mechanism suggested that they would better if placed near each other, on the 6A and 6B positions. We then made this isomer (**7**),[17] and were gratified to see that it is considerably better as an enzyme mimic catalyst for the hydrolysis of substrate **8** than are others with the imidazoles further apart.

Of course catalysis by separate imidazole buffer components differ in one important way from catalysis by a bis-imidazole compound, an enzyme or an



7 8

Figure 8 The 6A,6B β-cyclodextrin–bis-imidazole catalyst **7** and one of its substrates **8**.

enzymic mimic; when both imidazoles are part of the same molecule, the sequential catalysis seen with the buffer can be replaced by simultaneous bifunctional catalysis. This is apparently true of the enzyme, and it is certainly true for our catalyst **7**. The evidence comes from a technique known as 'proton inventory'.[18]

When the enzymatic reaction is carried out in D_2O solvent, all the exchangeable protons are deuterated. Since some of those protons are moving in the reaction, an isotope effect is seen to slow the reaction. The interesting effect is seen with H_2O–D_2O mixtures. If a single (deuterated) proton is moving in the transition state, the isotope effect for H_2O/D_2O mixtures is expectted to fall on a straight line between the H_2O and the D_2O points. However, if two (or more) protons are moving in the transition state, the line is curved. The fundamental idea is that the isotope effect appears to the second (or higher) power. Such a curved line has been seen with ribonuclease,[19] and it fits the theoretical curve for a transition state with two protons moving, each with an isotope effect near 2.0. This evidence argues for a concerted bifunctional mechanism, which is what we proposed and what the Karplus group calculated.

We expected a similar result with our bifunctional catalyst **7**. Here too the two imidazoles would be expected to perform simultaneously the reactions that had been sequential with the simple imidazole buffers. Proton inventory again indicated that this was true;[20] we saw a curved line in the hydrolysis of **8** corresponding to the motion of two protons, each with an isotope effect near 2.0 as in the enzyme. However, in our case we could check the method.

β-Cyclodextrin-6-imidazole, with only one catalytic group, can also catalyse the hydrolysis of **8**.[16] However, it acts only as a base catalyst, delivering the attacking water molecule. If no solvent protons substitute for the missing imidazolium ion, we do not expect to see a second proton moving in the transition state—the second proton was surely the acid proton of the imidazolium ion moving onto the phosphate group. In fact, the proton inventory method showed a straight line, indicating one proton 'in flight' in the transition state.[20] Thus the molecular and kinetic interpretation of the proton inventory method seems to be confirmed, at least in this case.

The mechanistic picture that emerged from our kinetic studies on RNA cleavage by buffers such as imidazole has thus lead to two clear payoffs: we have been able to propose a new mechanism for the enzyme itself that is more solidly based than was the classical picture, and we have been guided to the synthesis of a superior enzyme mimic by the new mechanism. Since that mechanism has also been completely confirmed by recent careful quantitative work, it is probably not only useful but also true. Certainly it has stood up against determined attacks.

One interesting feature of the mechanistic work needs further comment. A key to it was the findings with respect to catalytic interconversion of 3′,5″-UpU and 2′,5″-UpU. This leads naturally to the question: what are the properties of 2′,5″-linked nucleotides? Some aspects of this question have been addressed in the past, but remarkably there was no study of the biophysical chemistry of 2′,5″-linked DNA. We have recently looked into this.[21] While the story is too long to repeat here, we find that base-pairing association of two DNA strands, which is the key to molecular genetics, is much weaker with the 2′,5″-isomers than with the natural 3′,5″-analogues.

Molecular graphics methods indicate that the hydrogen bonding needed for base pairing is still possible, but that the bases do not stack as well in the rather open helix that results. Since base stacking is a major factor that promotes strand assocation, it is not surprising that the 2,5-linkage is not as good in DNA as is the natural 3,5-linkage. Perhaps in the transition from an RNA environment to a DNA environment some organism tried removing the 3′-hydroxy group of a ribonucleoside. If so that organism would not have survived, judging from the properties we see for the 2,5-isomer of DNA.

The catalyst **7** has been studied principally in the cleavage of **8**, a model of a cyclic phosphate nucleotide. However, it of course has many other potential applications, particularly since we have all three isomers—A,B and A,C and A,D—available. We have recently seen that it performs the bifunctional catalysed synthesis of some Schiff base derivatives (A. Graff, unpublished work). With a binding site and two catalytic groups in controlled positions, **7** has a lot of potential. Even more potential can be imagined for compounds that combine two catalytic groups with a dimeric binding site. However, that is the subject for future research.

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REFERENCES

1 Chao, Y.; Ph.D. thesis, Columbia University, **1972**.
2 (a) Harada, A.; Furue, M.; Nozakura, S.-I.; *Polym. J.* **1980**, *12*, 29. (b) Tabushi, I.; Kuroda, Y.; Shimokawa, K.; *J. Am. Chem. Soc.* **1979**, *101*, 1614. (c) Fujita, K.; Ejima, S.; Imoto, T.0; *Chem. Lett.* **1985**, 11.
3 Fujita, K.; Ejima, S.; Imoto, T.; *J. Chem. Soc., Chem. Commun.* **1984**, 1277.
4 Breslow, R.; Greenspoon, N.; Guo, T.; Zarzycki, R.; *J. Am. Chem. Soc.* **1989**, *111*, 8296.
5 (a) Tabushi, I.; Yamakura, K.; Nabeshima, T.; *J. Am. Chem. Soc.* **1984**, *106*, 5267. (b) Anslyn, E.; Breslow, R.; *J. Am. Chem. Soc.* **1989**, *111*, 5972. (c) Breslow, R.; Canary, J.W.; Varney, M.; Waddell, S.T.; Yang, D.; *J. Am. Chem. Soc.* **1990**, *112*, 5212.
6 Breslow, R.; Chung, S.; *J. Am. Chem. Soc.* **1990**, *112*, 9659.
7 Breslow, R.; Zhang, B.; *J. Am. Chem. Soc.* **1991**, *114*, *in press*.
8 Wirsching, P.; Ashley, J.A.; Benkovic, S.J.; Janda, K.D.; Lerner, R.A.; *Science* **1991**, *252*, 680.
9 Corcoran, R.; Labelle, M.; Czarnik, A.W.; Breslow, R.; *Anal. Biochem.* **1985**, *144*, 563.
10 Breslow, R.; Labelle, M.; *J. Am. Chem. Soc.* **1986**, *108*, 2655.
11 Anslyn, E.; Breslow, R.; *J. Am. Chem. Soc.* **1989**, *111*, 4473.
12 Breslow, R.; Huang, D.-L.; *J. Am. Chem. Soc.* **1990**, *112*, 9621.
13 Breslow, R.; Xu, R.; *J. Am. Chem. Soc.* **1992**, 114, submitted for publication.
14 Breslow, R.; Huang, D.-L.; Anslyn, E.; *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 1746.
15 Haydock, K.; Lim, C.; Brünger, A.T.; Karplus, M. *J. Am. Chem. Soc.* **1990**, *112*, 3826.
16 Breslow, R.; Doherty, J.; Guillot, G.; Lipsey, C.; *J. Am. Chem. Soc.* **1978**, *100*, 3227.
17 Anslyn, E.; Breslow, R.; *J. Am. Chem. Soc.* **1989**, *111*, 5972.
18 Schowen, K.B.; Schowen, R.L.; *Methods Enzymol.* **1982**, *87*, 551.
19 Matta, M.S.; Vo, D.T.; *J. Am. Chem. Soc.* **1986**, *108*, 5316.
20 Anslyn, E.; Breslow, R.; *J. Am. Chem. Soc.* **1989**, *111*, 8931.
21 Dougherty, J.; Rizzo, C.; Breslow, R.; *J. Am. Chem. Soc.* **1992**, *114*, 6254–6255.