WHITHER ENZYME MECHANISMS?

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The ubiquity of papers in the biochemical literature that claim to present the 'mechanism of action' of particular enzymes might suggest to the uninitiated that we understand how these catalysts accelerate the rates of the reactions that they mediate. We do not. Being able to suggest one of a number of possible post hoc rationalizations of a phenomenon is not the same as understanding it. But what do we mean by 'mechanism,' and at what level of information are we going to be prepared to say that we 'understand?'

For the bio-organic chemist, the mechanism of a reaction, catalyzed or uncatalyzed, implies knowledge of the sequence of reaction intermediates, the energetic relationships amongst them, and enough structural information to clothe each of the kinetic steps with chemical reality. Further, it is normally assumed that there exists good chemical precedent for each of the elementary kinetic steps, deriving from our knowledge of the physical-organic chemistry of small molecules. Does knowledge at this level amount to understanding? Certainly it has been, and is, the laudable aim of most enzymologists to obtain such information, but understanding includes the capacity to predict, and we should not be satisfied with our understanding unless it provides the basis for prediction about the pathways and rates of enzyme-catalyzed reactions. Since predictive capacity requires generalization (of the kind that in physical-organic chemistry derives from the work of such people as Hammett, Brønsted, or Woodward and Hoffman), we must continue both to expand the body of factual material concerning enzyme mechanisms, and to search for the generalizations upon which predictability will have to be based. In this essay, we shall discuss in necessarily brutal summary, (a) the study of reaction intermediates and energetics, (b) the investigation of enzyme structures, and (c) the extent to which the synthesis of our knowledge about dynamics and structure, together with results from 'model' systems involving small molecules is leading us towards a genuine understanding of the mechanism of enzyme catalysis.

Reaction intermediates

Essentially all enzyme-catalyzed reactions obey the Michaelis–Menten equation, and the pathway can therefore be formally divided into complexation (the formation of a non-covalent enzyme–substrate complex), reaction (which involves, inter alia, the covalency changes that constitute the catalytic act) and desorption (the loss of products from the enzyme). Each of these processes may occur in more than one elementary step, and proceed via more than one discernible reaction intermediate. This section is concerned with the detection and characterization of these intermediates.

Despite the fact that increasing numbers of intermediates in enzyme-catalyzed reactions are being shown to partition rather evenly between progress towards products and return towards starting materials (i.e. no single elementary step is cleanly rate-limiting: this point is discussed later), it is useful to consider the two extreme situations, (i) of an intermediate that occurs before the rate-determining step, and (ii) of an intermediate that occurs after it.

Intermediates that occur before the rate-limiting transition state

Reaction intermediates in this category are often more easily characterized, since they may accumulate to steady-state levels that permit their detection directly

(e.g. by their visible or ultraviolet absorption [1]) or their stability may be great enough to permit their isolation by quenching the reaction from the steady state [2]. However, to obtain reasonable levels of intermediate species, high enzyme concentrations are required, and at these concentrations the catalyzed reaction rates are too fast to be followed by classical techniques. Rapid reaction methods have therefore been developed, and these methods in both equilibrium and pre-steady state situations [3] continue to show that even quite simple enzyme-catalyzed reactions often involve many kinetically distinguishable steps.

For multi-substrate enzyme reactions, much can be gleaned from the study of the incomplete system. For instance, the rapid exchange of the 1-pro-*S* hydrogen of dihydroxyacetone phosphate with tritium from tritiated water catalyzed by muscle aldolase satisfies both chemical propriety (the formation of a carbanion for subsequent condensation with D-glyceraldehyde phosphate is just what the organic chemist would expect) and the requirement that the partial reaction be at least as fast as the rate of the overall reaction [4]. The further characterization of this intermediate as a ketimine–enamine by trapping with borohydride [5] adds to its attractiveness. Not all intermediates behave so well, however. Many phosphokinases that catalyze the transfer of a phosphoryl group from ATP to an acceptor molecule to yield the phosphorylated-acceptor plus ADP have been shown to catalyze the partial isotopic exchange reaction between [^{3}H]ADP and ATP in the absence of acceptor [6]. This normally leads to the proposal of an intermediate phosphoryl-enzyme that is then presumed to lie on the reaction pathway. Yet very often these partial exchanges are very much slower than the overall reaction [7]. Here, one faces two difficulties peculiar to enzyme-catalyzed reactions. First, is it possible that the full complement of bound substrates is required before even the partial reaction can reach its proper rate (this possibility has been encapsulated in the phrase 'substrate synergism' [8]); and secondly, since all intermediate species are enzyme-bound, can the absence of ADP–ATP exchange rule out a phosphoryl-enzyme intermediate, if ADP is very tightly bound? We shall not attempt to answer these questions here, but merely point to the obvious importance of facing the consequences of not finding what may in fact be the reaction intermediate.

Intermediates that occur after the rate-limiting transition state

Traditionally, reaction intermediates that occur *after* the rate-limiting transition state are harder to detect, and much harder to characterize. The classical approach of 'product diversion with no change in rate' simply requires that the intermediate be amenable to trapping by a reagent that will not enter the reaction scheme prior to the formation of the intermediate in question. Such intermediate-capture experiments have been successful in trapping the acyl-enzymes formed during peptide hydrolysis by the neutral proteases, for instance, but it is in general more difficult to divert the course of a reaction intermediate tightly bound at the active site of an enzyme, than it is to capture an intermediate occurring in free solution. Sometimes, however, information about late steps in an enzyme-catalyzed reaction may be forthcoming from the use of isotopes. Whereas the use of a deuterium-labelled substrate in a reaction involving hydrogen transfer can provide information about rate-limiting steps (i.e. the extent to which the hydrogen transfer step is rate-determining), the use of a tritium-labelled substrate can tell one about a product-forming step in which the competition between tritium and hydrogen is manifest in the tritium content of the product. (The recent suggestion of Northrop [11], who has combined the kinetic simplicity of V_{max}/K_M with the relationship between tritium and deuterium isotope effects, is an elegant example of the power of this approach.)

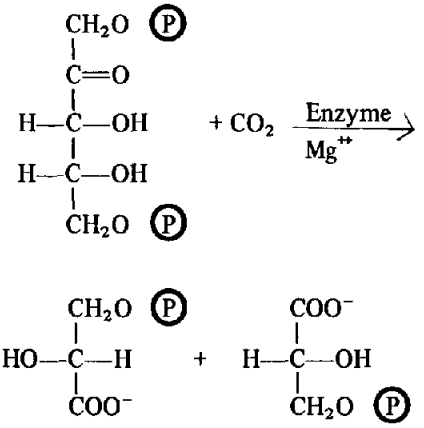
Finally, some chemical transformations that are enzyme-catalyzed have ΔG_0 values that are small enough to allow the reaction to be studied in the reverse direction. In these cases, an intermediate that occurs *after* the rate-determining step in the forward direction, becomes a pre-equilibrium intermediate for the reaction studied in the reverse direction. In the example of aldolase mentioned above, whereas the cleavage of D-fructose 1,6-bisphosphate is the direction of flux in glycolysis, and the ketimine–enamine intermediate with dihydroxyacetone phosphate occurs after the rate-determining cleavage step, the reaction is thermodynamically uphill in the cleavage direction and the intermediate between enzyme and dihydroxyacetone phosphate can be easily investigated.

In general, intermediates in enzyme-catalyzed reactions may be easier to find *or* more difficult to detect, than their counterparts in, say, acid-catalyzed reactions

in free solution. The enzyme's involvement in catalysis may include covalent bonds between substrate and enzyme (for an impressive list, see [9]) which can facilitate the detection and characterization of the intermediate. But by the same token, the ability of an enzyme to hold on tightly even to a proton without exchange into the solvent (e.g., [10]) should make one skeptical of deductions from partial exchange experiments that are negative and appear to point to a concerted process. When any reaction intermediate is postulated, there is one thorny (and therefore frequently ignored?) problem: is it kinetically obligatory? Almost the only criterion for judgement aside from the use of chemical common sense, is whether the intermediate is kinetically competent. That is, are the rates of its formation and breakdown consistent with the demands of overall turnover? This criterion can only rule *out* a possible intermediate, of course, and one may still be faced with a number of feasible intermediates to choose from. Further, the kinetic competence of an intermediate says nothing about its actual involvement in the reaction. In the event, this has not proved to be as helpful in eliminating putative intermediates in enzyme chemistry as it has in the reactions of smaller organic molecules where the intermediate may be amenable to synthesis by another route.

An example of the search for reaction intermediates in an enzyme catalyzed reaction

To illustrate a number of the points discussed above, consider the enzyme ribulose 1,5-bisphosphate carboxylase, which catalyzes the first step of carbon fixation in photosynthesis:



In the cleavage of the five-carbon unit, the C_2–C_3 bond is broken [12], and Calvin, many years ago, suggested the pathway [13] shown in fig.1 as a chemically reasonable sequence for the transformation.

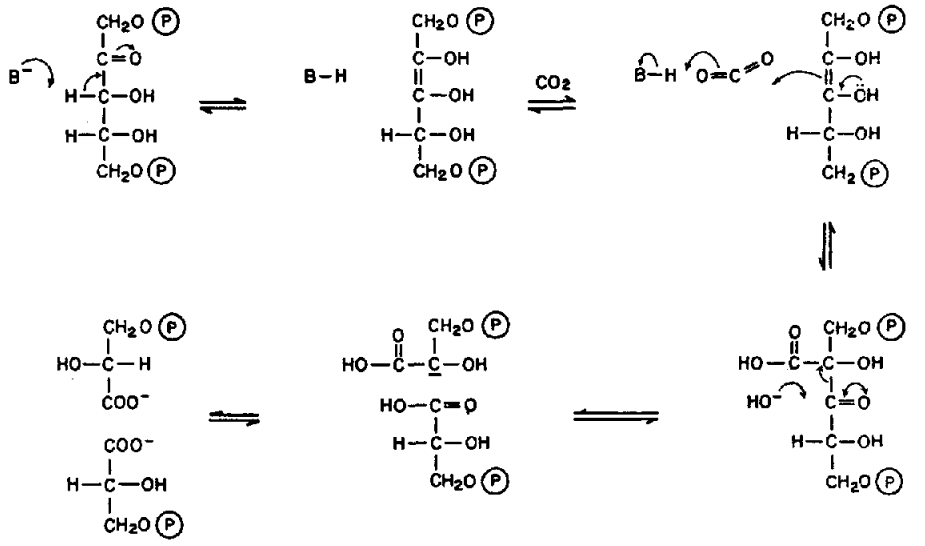


Fig.1. The possible pathway of the reaction catalyzed by ribulose 1,5-bisphosphate carboxylase.

Curiously, perhaps, these proposed reaction intermediates have been very elusive. If ribulose 1,5-bisphosphate is incubated in tritiated water with the enzyme in the absence of CO_2 in an attempt to see tritium exchange into the 3-position of the substrate, essentially no incorporation is found [14,15]. Similarly, when 3-[^{3}H]ribulose 1,5-bisphosphate is incubated with the enzyme in the absence of CO_2, essentially no tritium is exchanged out into the solvent [16]. If the Calvin scheme is correct, we must postulate either that no enolization occurs in the absence of CO_2 (a 'substrate synergism' explanation), or that enolization does occur but the abstracted proton remains firmly bound to the protein as part of a monoacidic base (e.g. carboxyl or imidazolium, but not ammonium) without exchange with protons from the medium. As mentioned above, 'sticky' protons do occur in enzymology, but analogous enolization processes catalyzed by aldolase, triose phosphate isomerase and glucose 6-phosphate isomerase all involve rather free exchange between the carbon-bound proton of the α-hydroxycarbonyl system and the solvent. When CO_2 is added to the substrate and enzyme in tritiated water, label does appear in the product 3-phospho-D-glycerate, which has a specific radioactivity of about one fifth that of the solvent. Here is an example of the implicit detection of an intermediate that occurs after the slow step, where one sees the competition between ^{3}H and ^{1}H in the collapse of the (formal) carbanion of the molecule of 3-phospho-D-glycerate derived from C_1 and C_2 of the substrate with CO_2 in the last, product-forming step. When the reaction is run in ^2H_2O, one molecule of the resulting 3-phosphoglycerate is 'substantially' deuterated, as expected [12,15].

If 3-[^{3}H]ribulose 1,5-bisphosphate is allowed to react partially in the complete system, the radioactivity appearing in the solvent shows that the tritiated material is consumed about five times slower than the protonated substrate. This is consistent with the lack of a pre-equilibrium formation of the enediol intermediate, and suggests that the first, enolization, step of the sequence is indeed the slowest one. Attempts to study the reaction in the R to L direction, with 3-phosphoglycerate as substrate, have not yielded useful information, since no isotope exchange between 3-phosphoglycerate and tritiated water, or 3-phosphoglycerate and [^{14}C]CO_2, has been observed in the presence of the enzyme. This may be due, of course, to the large negative free energy of the reaction in the L to R direction ($\Delta G_0 = -12.4$ kcal/mol), being reflected in very slow rates of the early steps in the R to L direction.

Two further approaches of those mentioned above have been taken to try and pin down the intermediates in the carboxylase reaction. On the addition of ribulose 1,5-bisphosphate to the enzyme, an ultraviolet difference spectrum is observed that disappears on the addition of HCO_3^- and Mg^{++}, which suggests that the spectrum is due to an intermediate in the reaction [17]. The authors proposed on the basis of these changes and of the existence of an essential thiol group whose alkylation abolished parts of the difference spectrum, that the spectrum was due to an interaction (possibly a thiohemiketal) between bound substrate and the enzyme thiol group. This example of an attempt to detect a reaction intermediate directly may be valid, though the kinetic competence of the detected species was not quantitatively demonstrated. Finally, attempts have been made to prove the existence of the carboxylated ribulose 1,5-bisphosphate as an intermediate, by direct quenching from the steady state [18]. The expected lability of this hydroxylated β-keto acid makes its isolation and characterization difficult, but a material possessing a number of the properties expected was detected after electrophoretic separation from other reaction components. For this enzyme, therefore, many of the approaches that can be used for the detection of reaction intermediates have been taken, and the fact that our knowledge of the course of this reaction is still so rudimentary, emphasizes the difficulties in this area.

Energetics

The above discussion has focussed upon the problem of detecting reaction intermediates, while for a complete description of the dynamic events we must define the energetic relationships amongst these intermediates. No attempt will be made here even to summarize the methods by which these energetic relationships may be established. What does seem to be clear, however, is that even for a partial solution to the problem, one has to combine the kinetic information derivable from more than one source. Thus steady state rates, transient kinetic results from stopped-flow and relaxation

methods, rates of partial exchange reactions, and measurements of intermediate partitioning may all contribute to the determination of the rate constants for the elementary steps of the reaction and allow the quantitative construction of the sequence of kinetic events that is sometimes rather curiously called a 'kinetic mechanism.'

Earlier, we suggested that it was useful to consider two extreme situations, where the intermediate in question occurs either before or after a transition state that is cleanly rate-limiting. It must be recognized, however, that things are not likely to be so simple. For an increasing number of enzyme-catalyzed reactions, it is becoming clear that there is not one transition state of very much higher free energy than all others. Many reaction intermediates apparently partition rather evenly between the two paths open to them (i.e., forward towards products, or back towards starting materials). This feature may well be an inevitable consequence of the evolution of catalytic function. Consider a catalyzed reaction for which one free energy barrier is much higher than all the others. Assuming a selective pressure exists to improve the efficiency of the flux between substrate(s) and product(s), then mutational events that result in the lowering of this rate-limiting transition state will be selected for. Every 1.4 kcal/mol that the barrier is lowered will provide an enzyme ten times more effective. At the point where the barrier has been lowered to the level of some *other* transition state in the multistep reaction, then the most that can be gained in rate terms by lowering the energies of either of the two transition states, is two-fold. One may expect then, that for enzymes whose function is just catalysis (e.g. those for which no catalytic advantage is being sacrificed to the possibly higher good of control), two or more transition states will be rather close in free energy. That this intuitive view is correct is suggested by a number of investigations. For example, the kinetic isotope effect observable for a substantial number of enzymes involving hydrogen transfer is rather low for a full primary kinetic isotope effect involving a reasonably symmetrical transition state (for which $k_H/k_D \approx 7$), yet still significantly higher than an equilibrium isotope effect (for which $k_H/k_D \approx 1.2$). Further, in a situation where one knows the free energy profile of the enzyme-catalyzed reaction (e.g. [19]), it is clear that there is indeed rather a fine balance amongst all the transition states.

This feature of enzyme-catalyzed reactions leads to two important changes from the view of the energetics of catalysis that was current a decade ago. First, it is not universally true (and may well not even be frequently true) that the covalency changes which constitute catalysis have higher transition state free energies than the substrate-handling ('on' and 'off') steps. Indeed, a substantial number of enzyme reactions have k_{cat}/K_M values in the region of 10^8 M^{-1} sec^{-1} [19,20] which is near the diffusion limit, and indicates that the substrate handling steps are—or are close to being – rate-limiting. Secondly, it is even more obviously unsafe in 1976 than it was in 1966 to presume that K_M is a measure of binding and k_{cat} is a measure of the rate of covalency change within the enzyme–substrate complex. This simplification of the proper kinetic expressions for K_M and k_{cat} that is so often blithely indulged in (largely in attempts to 'improve' the usefulness of the steady-state parameters), can now be seen as a dangerous and misleading practice.

Structure

A bald collection of rate constants does not constitute a mechanism, and long before we may even ask the question of how a chemical reaction is being accelerated, these kinetic events must be given chemical reality. Obviously the most dramatic impact on mechanistic thinking in enzymology has come from the availability of the structures of enzymes determined by X-ray crystallography. This information has focussed the mind marvellously and – leaving aside the occasional skirmishes about how closely the structure in the crystal resembles that in solution – has most profitably limited the imaginative, sometimes almost vitalist, theorizing of enzymologists. The information from protein crystal structures has not all been mechanistically restricting, however, and it has opened avenues in such areas as protein folding, protein stability, and the nature of the enzyme's catalytic apparatus. In this last field, a most challenging finding has been the discovery of the same quartet of amino acid side-chains at the active site of two totally different proteins each responsible for peptide bond hydrolysis: α-chymotrypsin and subtilisin [21]. Despite a number of suggestions, the physical-organic basis of the particular constellation of Ser–Asp–His–Ser in

these two enzymes is still uncertain. It will not be surprising if there are more chemical surprises in the nature of the catalytic groupings used by enzymes, and we must hope that the kinetic consequences of such constellations will be properly explained at the chemical level.

Rather oddly, there are as yet (summer 1975) no crystallographic data on productive complexes of enzymes and their proper complement of natural substrates (though one promises to be solved in the near future [22]). For instance, abortive ternary complexes of dehydrogenase have been studied, but not the productive ternary complex (in, of course, the state of oxidation–reduction of lower free energy). This has meant that we have structural detail only on enzymes in the absence of bound substrate, and have to rely on model building based on data from non-productive complexes, for information about reaction intermediates. This is a non-trivial point, since to comprehend the relationships between atomic position and reaction rate may well need much more careful analysis at much higher resolution than we currently have. The fact that a very small movement of the iron atom out of the plane of the heme in hemoglobin [23] is coupled with much more major changes in protein structure should be a warning to bioorganic chemists wielding their curly arrows [24]. The stereo-electronic restrictions on reaction pathways as exemplified by Dunitz's view of the attack on a carbonyl group [25] or Deslongchamps' theory of how a tetrahedral intermediate collapses [26] will surely prove crucial to our understanding not only of the pathways of catalysis but also of the rates of the steps involved. It may be that, to discern such stereoelectronic restrictions, we shall need structural detail at *very* high resolution. This problem notwithstanding, the crystal structure of an enzyme under scrutiny has properly become a sine qua non for any detailed picture of the reaction mechanism.

The synthesis of structural and kinetic information

The synthesis of the pure structural information on isolated enzyme molecules and the rates of the individual steps of the catalyzed reaction is not – fortunately – a purely cerebral process. A number of spectroscopic methods and chemical modification techniques provide something of an experimental bridge between the atomic coordinates of the protein and the rate constants for the substrate transformation.

Spectroscopic methods

The magnetic resonance spectra of enzyme:substrate complexes are proving increasing informative in delineating the changes in substrate and in enzyme when complexation occurs. For the substrate, chemical shift and coupling constant data can provide information about the conformation of the bound material, and allow discussion of the degree to which the active site of the enzyme sacrifices binding free energy in order to select a substrate conformation that more resembles the transition state of a subsequent catalytic step. For enzymes that have appropriate metal ion requirements, distances between the metal and carbon, hydrogen and phosphorus atoms of the substrate have been derived from relaxation rate measurements [27]. For enzymes whose structure is not known crystallographically, these experiments can lead to somewhat disembodied pictures of substrate structure, but in combination with the static structure of the native enzyme, this kind of n.m.r. study should provide important and useful structural features of enzyme–substrate complexes. Considerable ingenuity has gone into experiments designed to look at the enzyme itself by resonance methods. Aside from studies of proton resonances, efforts to use n.m.r. to look at *single atoms* in a protein are progressing rapidly. The ^{13}C-enrichment of the C_2 carbon atom of the only histidine residue in α-lytic protease [28], and the labelling of the 11 tyrosines as *m*-fluorotyrosine in *E. coli* alkaline phosphatase [29], exemplify this approach, which results in cleaner and more interpretable spectra and provides potentially sensitive probes of events during catalysis. For the study of non-bacterial systems (each of the above examples was produced by appropriate feeding of the bacteria), subtle chemical modification will be required. This can be done both by labelling the protein with a small reporter group (though this, as with any chromophoric or odd-electron reporter, suffers from the fundamental objection that the act of creating an observable phenomenon will perturb what one is striving to observe), or – more delicately – by introduction of the reporter without changing the chemical structure of the protein. An elegant example is the recent

experiment of Gurd [30] who has partially enriched the methionine methyl groups of myoglobin with ^{13}C using a relatively gentle exchange reaction. Magnetic resonance methods will not solve all the problems associated with the interaction of substrate with enzyme, but the information content in appropriate cases is clearly high.

In the study of a liganded micromolecule, electronic absorption spectra and even fluorescence emission spectra have not the potential of magnetic resonance, since it is hard to disentangle the contributions of what can be lumped together under the term 'solvation' (i.e. environmental effects) and of conformational (or geometrical) effects. Such spectra often provide useful handles, of course, in the sense of giving signals that change as a consequence of changes in external variables. But the level of information we are striving to obtain relates to the geometrical and bonding arrangements of atoms or groups of atoms in a molecule. For this purpose vibrational spectra have more power, but relatively very little work has been done in the infra-red, because the solvent water absorbs massively, only leaving restricted windows in which spectral signals from substrate or proteins can be observed. Raman spectroscopy suffers much less (water is rather a poor Raman scatterer) and the very large number of observed vibrations can be reduced dramatically by using the resonance Raman method, in which the incident light is on or near an electronic absorption maximum [31]. This technique seems likely to extract much more detailed information from systems with accessible electronic absorption maxima, than it has been possible to gain from the study of these absorptions themselves.

Chemical modification

The second major area that relates structure and function is chemical modification [32]. While it must be said that the literature contains a number of exaggerated claims for reagents that are purportedly amino acid-specific, the minimal modification of single residues in enzymes continues to yield useful information about the groups essential for catalytic activity. Some caution has to be exercised in deciding upon the reason for activity loss following modification, since our knowledge about protein motility and about the transfer of information through proteins is still very sketchy. But it is encouraging how well chemical modification studies have generally meshed with the findings of the X-ray crystallographers. It is, however, unfortunate that the analysis of the factors that govern the chemical reactivity of individual groups in proteins has received so little attention. For instance, so many electrophilic reagents of really very low chemical selectivity, discriminate remarkably amongst the large number of nucleophiles that all proteins contain. This discrimination undoubtedly arises from the local environment, but how? If we can properly rationalize the differences in chemical reactivity of groups in proteins, we shall be nearer to understanding the trickier and more complex problem of enzyme catalysis. One rather dramatic example of the importance of the local environment is the observation of a lysine residue at the active site of acetoacetate decarboxylase that has a pK_a of 5.9 [33]. Westheimer has demonstrated that this remarkable perturbation – of some four and a half log units from the expected value – probably arises from the existence of a neighboring cationic centre that can be tentatively ascribed to another lysine residue adjacent in the sequence. This lowered pK_a ensures that a substantial proportion of the free base form of the critical lysine amino group be available for Schiff base formation with the substrate, and the existence of the neighboring lysine also ensures a substantial proportion of the *protonated* Schiff base required for the catalysis of the decarboxylation itself (see W. P. Jencks, in the discussion following ref [33]).

Stereochemistry

One of the most powerful tools in the delineation of organic reaction mechanisms and a very important feature of our mental grasp of chemical transformations, is stereochemistry. In nucleophilic substitution reactions, in addition and elimination reactions, and in skeletal rearrangements, the stereochemistry severely limits the number of acceptable mechanisms, and is a much less equivocal foundation for any mechanistic thinking than, say, the effect of altering the substrate structure on the reaction rate. An elegant example of stereochemical restriction on possible mechanisms is the study of the stereochemical course of each of the two steps of the reaction catalyzed by ribonuclease (fig.2). By introducing chirality into the cyclic phosphate using sulphur, and into the phosphate monoester product using sulphur and ^{18}O, Usher and

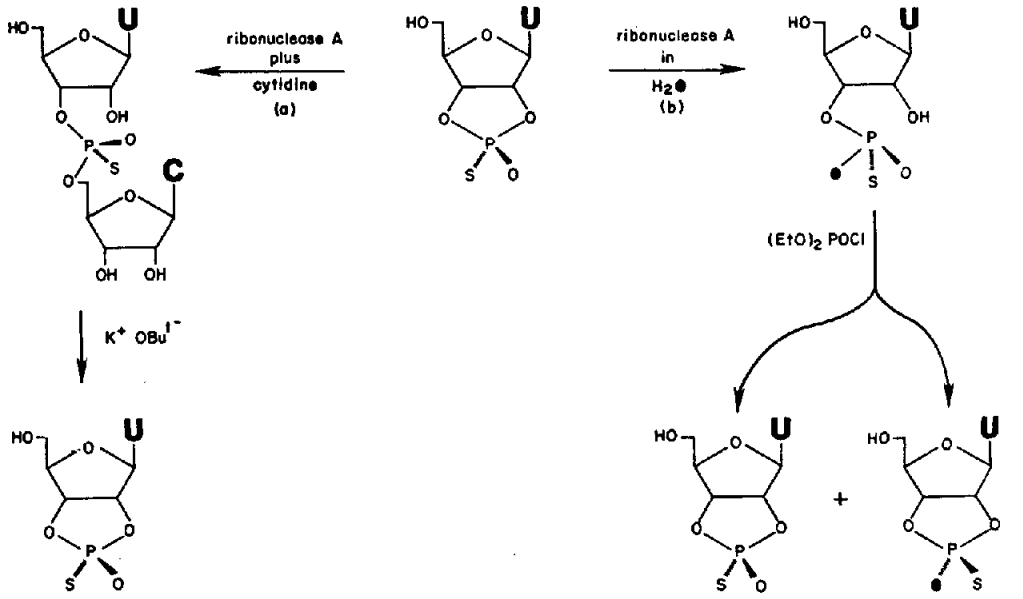


Fig. 2. The stereochemistry of the two steps of the reaction catalyzed by ribonuclease A. (a) One diastereoisomer of the 2′,3′-cyclic phosphorothioate was allowed to condense with cytidine, catalysed by ribonuclease A. The dinucleotide Up(S)C product was cleavage by a known in-line procedure to give the same diastereoisomer of the cyclic phosphate. This demonstrated the in-line nature of the enzyme-catalysed step. (b) One diastereoisomer of the 2′,3′-cyclic phosphorothioate was hydrolysed by ribonuclease A in $H_2{}^{18}O$ (H_2●). Chemical ring closure of the product by a known in-line procedure gave the two diastereoisomers shown, the ● content of which demonstrated the in-line nature of the enzyme-catalysed step.

Eckstein [34] were able to prove that each of the two steps occurred via an 'in-line' path, rather than an 'adjacent' displacement necessarily involving pseudorotation. This unequivocal statement constrains the model-building of the crystallographers, and sharpens the kinetic problem to one of how the enzyme accelerates an in-line displacement so effectively.

Even more important, though, than the contribution of stereochemical analysis to our understanding of particular enzyme reactions, is the possibility of uncovering stereochemical imperatives. For reactions between small molecules in solution, we have only limited access to discovering optimum reaction trajectories and the ideal geometrical arrangement of species for rapid reaction. With enzyme-catalyzed processes, however, there are already enough data that point to the existence of as yet unformulated stereoelectronic preferences, if not actual stereochemical imperatives [35]. For instance, why do all the aldose–ketose isomerases appear to involve a *cis* enediol intermediate? Why do the aldolases all go with retention at the carbanion-oid center yet all the enzymes handling acetyl-CoA go with inversion at the carbanion-oid carbon? Why do all the biotin-dependent carboxylases go with retention? It may be as Hanson and Rose [35] have discussed, that some enzyme systems that share a common stereochemistry have simply derived from the same ancestral protein, and their mechanistic similarity is due to nothing more than divergent evolution. But it is clear that there are also groups of enzymes that have evolved separately, and converged to a common, energetically favoured, stereochemical course. From these considerations, there will surely be mechanistic lessons to be learned. Nature, after all, has been perfecting the exploitation of minimal motion, antiperiplanar orbitals, neighboring groups, push-pull catalysis, and the rest, for more than 10^9 years, and we have only been working at the problem for 10^2.

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