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THE ROLE OF FLEXIBILITY IN ENZYME ACTION

Daniel E. Koshland, Jr.

Biology Department, Brookhaven National Laboratory, Upton, New York

and

The Rockefeller Institute, New York, N. Y.

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The role of flexibility in enzyme action has been a continuous concern of our laboratory in recent years since we concluded that the specificity of enzymes required that the substrate induce a measurable conformation change in the structure of the enzyme (Koshland (1958)). Sufficient evidence has now accumulated so that it seems appropriate to discuss: a) the reasoning which led to the conclusion that the template theory was inadequate, b) the evidence for a flexible active site and an evaluation of the conclusiveness of that evidence, and c) the molecular consequences of a flexible active site which can be useful in the explanation of control mechanisms. The emphasis in this paper will be on the rationale rather than the specific data with the hope that the references will prove adequate for those desiring to examine the detailed data.

A. Evidence conflicting with the template hypothesis.

The classical template hypothesis enunciated by Fisher (1894) successfully explains the vast majority of the specificity patterns of enzymes. There are however some anomalies which do not seem to be explainable on the basis of the template hypothesis without invoking extreme and unrealistic mechanisms for the action of enzymes.

One type of evidence which is illustrative of the reasoning is shown in Table 1. This table shows a specificity pattern of 5'-nucleotidase. The normal substrate for this enzyme is adenylic acid which has a Michaelis constant of less than 10^{-4} and a relative maximum velocity of 100. If the nicotinamide moiety is substituted for adenine the K_m , which in this enzyme is probably a rough measure of binding, increases, indicating a decreased affinity for the surface of the protein. This is reasonable on the basis

of the template hypothesis since the nicotinamide moiety is smaller and of a different over-all shape than the adenine moiety. Moreover, the reactive bond between ribose and phosphate is not changed and, therefore, one would expect on the basis of the template theory that the maximum velocity (i.e., at saturation of the active sites) should be roughly comparable to that of the adenylic acid. Such is observed to be the case, the drop of 33% being readily explainable by perhaps a slight difference in the geometry at the active site.

If now the nicotinamide moiety is removed entirely, producing ribose phosphate, one might expect a precipitous decrease in binding. No such decrease is observed, however. If the enzyme is saturated with ribose phosphate, the ribose phosphate should certainly react as readily as either of the former two substrates if the template theory is correct. The substitution of a hydrogen for the nicotinamide moiety certainly could not prevent access of the ribose phosphate to the active site and the bond to be split is identical in the two compounds. Yet the maximum relative velocity is found to be <0.01 that of adenylic acid. Something other than access or binding to the active site must be essential for enzyme action. Many anomalies of this type, in which the modified substrate contained the reactive group unchanged, was smaller, was bound to the enzyme surface, and did not react, were found (Koshland, 1958, 1959a).

Other examples of a quite different nature were also found. For example, the classical template hypothesis explains the failure to react of a compound which is obtained by adding a bulky group to the substrate by presuming that the bulky group prevents access of the new compound to the

active site. In some cases, for example that of beta amylase, the bulky compound was found to be bound as tightly as the substrate itself and yet reaction did not ensue (Thoma and Koshland, 1960). In other examples compounds containing groupings of essentially the same size but different chemical properties such as the 6-amino and 6-hydroxyl group of ATP were found to cause dramatic changes in enzyme action (Koshland 1959). This dramatic change occurred despite the fact that the bond to be split between the terminal and second phosphate groups of the ATP molecule was many atoms away from the amino and hydroxyl groups which played such a role in the specificity pattern.

Another phenomenon exists in the literature which is difficult to explain with the template hypothesis. It is illustrated in Figure 1. This represents the classical picture for noncompetitive inhibition. From the kinetics of this process it is known that noncompetitive inhibition involves binding at a site other than the active site since it does not affect the binding of either or both substrates. The structure of many noncompetitive inhibitors is different from that of the substrates themselves and hence they would not be expected to compete with their respective substrates at the active site. The anomaly, therefore, does not lie in the kinetics but rather in the attempts to explain how a molecule far from the active site can affect the reactivity of two substrates whose binding has not been influenced. At one time, when less was known about the action of enzymes, the noncompetitive inhibition illustrated in Figure 1 might have been explained by some semiconductor theory of enzyme action. It might be

argued that a circulating current traveling through the protein is responsible for enzyme action and that the introduction of an insulating group would prevent this conductivity and hence prevent enzyme action.

Many facts argue against an alternative of this sort. In the first place, simple chemical analogues of all of the enzymatic reactions are known. Today essentially no anomaly exists which requires a statement that a reaction occurs on an enzyme which cannot be duplicated by the organic chemist in the laboratory. It is true that enzymes because of their high specificity give higher yields and fewer side products than the nonenzymatic analogues. However, isotopic tracer techniques, the preparation of analogues, the well-known model systems developed in many laboratories indicate that simple analogues for acylation, dehydration, dehydrogenation, etc., can be found and that in many cases the exact chemical reaction can be performed, in the absence of enzyme. It would be presumed therefore that some mechanism of organic catalysis is required to explain enzyme action.

Moreover, the concept that the high molecular weight of the protein is essential for its reactivity is no longer considered very likely. Hill and Smith (1956) have removed two thirds of the papain molecule without any noticeable change in its activity. Myosin can have a substantial part of its molecule removed by proteolytic enzymes with only minor change in its specificity properties (Gergely 1953, Szent-Gyorgyi 1953). Surface groups on proteins such as the amino groups of chymotrypsin and beta amylase, the lysine residues of ribonuclease, many of the histidine residues of phosphoglucomutase, etc., can be modified with essentially no loss in enzyme activity.

It seems clear therefore that the total protein molecule is not necessary for the catalytic action of the enzyme. It is probable that a considerable portion of the protein outside the active site is necessary for maintaining tertiary structure of the active site and perhaps to give the active site the kind of flexibility which it needs for its specificity properties. This could explain the observation that there are some amino acid residues whose modification leads to loss of activity even though they are not directly at the active site. However, the observation that modification of extensive portions of the protein molecules can exist without loss in activity suggest that an interlocking network involving physical systems such as semiconductors is highly unlikely.

Finally, we come to the problem of sequential absorption of substrates. A possible mechanism for sequential absorption can be constructed based on a template hypothesis using the idea that the first substrate absorbs contains a critical binding feature necessary to aid in binding of the second substrate and so forth. Although this hypothesis may be tenable in some cases, it seems improbable for those cases in which sequential binding has been most firmly established, i.e. for the DPN enzymes. In these cases the orientation of DPNH in respect to other substrates is more or less prescribed by the fact that a hydrogen must be transferred from the para position of the nicotinamide ring. Since DPNH acts on many substrates of widely different chemical structures, the idea that this molecule can play a key role in binding and in addition have a very special geometry for the 4 position of the nicotinamide ring seems improbable (Koshland, 1963a).

If the template theory is inadequate, what proposal can take its place? The phenomenon of fit between the substrate and enzyme seemed too useful to discard. Moreover, it seemed the only way one could explain the enormous effects exerted by modifications of the substrate many bonds removed from the bond breaking and bond forming groups in the substrate molecule. Inductive effects drop off with distance very rapidly, particularly in saturated systems, and the inductive effect on the terminal phosphate group of ATP by the 6-amino group in the adenine ring cannot be very great. Some ^{steric} fit, therefore, seems essential. We therefore retained one feature of specificity proposed by Fisher that there must be an intimate relation between substrate and enzyme.

We modified two features. The assumption that the active site was already formed as a relatively rigid negative of a portion of the substrate molecule was modified to postulate that a fit finally resulted but the fit occurred only after a change in shape of the enzyme molecule had been induced by the substrate. (It is clear that this theory leads to the possibility that the substrate molecule may have its shape changed also. But this does not need emphasis since it exists as a corollary of the template hypothesis as well. In any molecule containing single bonds about which free rotation can occur, the enzyme must select a particular conformation regardless of whether the active site is flexible or rigid.) A second feature of the "induced fit" hypothesis was the assumption that more than one catalytic group would have to be involved in the enzyme action and therefore the precise alignment of these groups with the substrate would be necessary for enzyme action. This argument followed from the evidence

that free amino acid residues have in no case shown catalytic velocities even approaching those catalyzed by enzymes. If two or more amino acid residues had to be brought into appropriate juxtaposition and orientation, then it is possible to explain why velocities approaching those of the enzyme were never observed by mixing free amino acid residues with the substrates in solution.

Combining these two postulates meant that it was possible to explain the fact that substrates containing the appropriate bond could be absorbed to the enzyme surface and still fail to react. As shown in Figure 2, a substrate approaching a portion of the active site induces the alignment of the catalytic groups A and B so that they are in appropriate relation to each other and to the bond to be broken. As indicated in Figure 3, increasing or decreasing the size of the substrate may lead to thermodynamically stable conformations which do not have the appropriate alignment of A and B and hence will not lead to reaction.

It may be asked what, precisely, the differences are between a template hypothesis and an induced-fit hypothesis. Clearly it would be illogical to expect that a protein would be completely rigid. Molecular vibrations exist and some minor flexibility of the molecule also would be expected. The differences involve changes in the shape of the protein and in the prediction of observable kinetic and specificity patterns. For example, lack of reactivity of larger substrates in the template hypothesis is based on steric hindrance preventing access to the surface. In the case of the induced-fit hypothesis equally tight binding may occur for a bulkier

substrate as indicated in the figure. In this case the changes are not simply molecular vibrations but a significant change in the geometry of the protein.

Exactly how great the changes in conformation would have to be in angstroms was difficult to deduce a priori. In fact, when we first proposed the hypothesis there were some who believed that a precise alignment might mean the precision of only a few angstroms in which case the chemical and physical tools available might never distinguish between a template and a flexible site hypothesis. As the work unfolded, however, it was possible to say that the changes in structure were sufficiently substantial to be observed chemically, as indicated by reactivity of amino acid residues, and physically, as indicated by difference spectra and therefore a significant change in nuclear positions undoubtedly occurs.

The fact that a significant change in protein structure occurs is also an argument against the objection that the flexibility theory itself is "too flexible", i.e. that it can explain anything and hence explains nothing. The very ease with which a wide variety of phenomena can be explained by this theory are a cogent argument for it. More important, however, is that the detailed arguments lead to predictions of correlation between protein structure and specificity pattern which are verifiable by experiment. In the next section the evidence for one particular case will be examined in detail as typical of the kinds of correlations that can be obtained.

B. Evidence for a flexible active site in phosphoglucomutase.

Since the initial postulation of a flexible site, work has proceeded in our laboratories and other laboratories as well to attempt to obtain evidence for a change in conformation induced by substrate. Much of this work has been reviewed previously in the references cited and therefore it would seem appropriate to summarize the evidence on one enzyme phosphoglucomutase which has been studied intensively in our laboratory (Koshland, Yankeelov and Thoma 1962; Yankeelov and Koshland, In press). The types of evidence which have been used to indicate flexibility at the active site are as follows:

1) Kinetic stability of the phospho enzyme. Phosphoglucomutase exists in both the phospho and dephospho forms. The phospho enzyme phosphorylates either the 6 or the 1 hydroxyl group of glucose phosphate. The hydroxyl group of glucose is essentially of the same order of reactivity as water and therefore in the absence of water one would expect to observe hydrolysis of the phospho enzyme. No such hydrolysis is observed and therefore it seems logical that a lack of access of water to the phospho site caused by a folding which protects the phospho group is a logical explanation for the stability of the phospho enzyme.

2) Increased reactivity of SH groups in the presence of substrate.

If the glucose phosphate causes an unfolding of the molecule so the phosphate on the protein is capable of phosphorylating the hydroxyl group of the glucose it is possible that an amino acid side chain is exposed during the interaction of the substrate with the enzyme. Treatment of the protein with iodoacetamide

in the presence of substrate and in its absence showed indeed that this was the case. A sulfhydryl group was more reactive in the presence of glucose phosphate than in its absence. Such an enhanced reactivity could be explained in two ways: a) that the unfolding of the protein exposed an SH group, or b) that the addition of glucose phosphate so changed the environment of the SH group that an enhanced reactivity was observed because of medium effects. The latter alternative was eliminated by adding glycerophosphate which is also a phosphate ester of the same pK and refractive index as glucose phosphate, but which is not a substrate for phosphoglucomutase. The control run with the glycerophosphate showed an SH reactivity equal to that of other dinegative ions and noticeably different from that of the substrates of the enzyme.

3) Increased inactivation of phosphoglucomutase in the presence of substrate. The classical template hypothesis would predict that substrates should protect the enzyme against inactivation or should have essentially no effect on the rate of inactivation. However, an unfolding of the protein leading to the exposure of new reactive groups might lead to the opposite phenomenon and indeed this was observed with phosphoglucomutase. The rate of inactivation in the presence of glucose-6-phosphate was noticeably greater in the early phases of reaction than in the presence of the nonsubstrates glycerophosphate or inorganic phosphate.

4) Absorption difference spectra. Difference spectra have been shown to register changes in the environment of tyrosine and tryptophane residues and, a conformation change caused by substrate might lead to an observable change in these two residues. Such a change was indeed observed.

In the presence of sulfate ion, inorganic phosphate ion, and glycerophosphate ion, all non-substrates, essentially identical difference spectra were observed. This difference spectra however was notably enhanced and characteristically different in the presence of the substrates glucose-1 or glucose-6-phosphate.

5) Fluorescence spectra. When fluorescence spectra of the protein were examined by exciting at approximately 270 $\text{\AA}$, a peak in the 320-330 region was observed and this peak was markedly enhanced in the presence of glucose-1 and glucose-6-phosphate in contrast to the spectra observed in the presence of non-substrate negative ions, e.g. phosphate and beta glycerylphosphate. The fluorescence spectra correlate with a change in the environment of the tyrosine and tryptophane residues and, as in the case of the difference spectra, the change is one which is characteristic of a change from an aqueous to a hydrophobic environment.

6) Correlation of spectral changes with specificity. The difference and fluorescence spectra of phosphoglucomutase were further correlated with the specificity pattern by using a series of compounds, finally involving the true substrates. With phospho enzyme, as one goes from chloride ion to phosphate glycerophosphate ion to glucose_A ion, a steady progression in both the absorption spectra and the fluorescence spectra is observed. The logical deduction from these observations is that the final conformation is achieved by having all of the ingredients of the substrate and enzyme together at the active site. Removal of part of the substrate, such as leaving three of the carbons off the glucose molecule, produces a change in conformation from that

of the native protein but not a sufficient change to lead to the proper conformation at the active site. This conclusion is further supported by the fact that the fluorescence spectra for dephospho enzyme were found to be different from those of the phospho enzyme. In this case little enhancement was observed as one goes from chloride to phosphate to glycerophosphate to glucose phosphate in contrast to the similar fluorescence observations with phospho enzyme. Presumably the absence of the phosphate at the active site which plays its part in the final conformation changes prevents the change which was detected by fluorescence. A difference spectrum is observed, however, with absorption, indicating that absorption and fluorescence measure different, though similar, effects.

7) Numbers of amino acids involved. The previous arguments for difference spectra can be questioned on the basis that environment of tyrosine and tryptophane is affected by direct proximity of the substrate rather than by inducing a change in the protein conformation. This is a pertinent argument but it is believed to be excluded by the number of residues which have been shown to undergo changes in the presence of substrate. As mentioned previously at least one cysteine residue is exposed to alkylation in the presence of substrate. This observation is made at short time intervals, i.e. approximately 10 minutes at pH 7 at room temperature. If the reaction goes for several hours, a classical protection effect is observed. Alkylation by iodoacetamide of methionine, lysine and cysteine residues occur and these alkylations are diminished in the presence of glucose phosphate. At first glance one would conclude that these residues were at the active site and protected in a classical template

manner. However, it should be mentioned that we have already implicated a cysteine residue which is more reactive, tyrosine and tryptophane residues whose environment is affected and a serine residue which is the repository of the phosphate at the active site. Since each of these amino acid residues is approximately the same size as the glucose phosphate molecule, it seems highly improbable that a glucose phosphate molecule is protecting 7 amino acid residues. Rather it seems probable that a refolding which is exposing a cysteine residue is causing a burying of some other residues leading to the protection affect. Which of the residues, methionine, lysine or cysteine, is being protected by refolding and which by direct protection at the active site remains to be determined. That all of the protected residues, the enhanced SH and the tyrosine and tryptophane cannot be in contact with the substrate seems unequivocal. Some of these phenomena, therefore, must be occurring away from the active site and must indicate a general change in the structure of the molecule.

8) The thermodynamic stability of phosphoenzyme. Equilibrium measurements have shown that the phosphoenzyme is more stable than characteristic phosphate esters of the "low energy" type. Glycerophosphate, glucose-6-phosphate, ethyl phosphate, etc., all show hydrolysis equilibria of approximately the same order of magnitude. When compared with these, the phosphoenzyme is notably more stable by approximately 2400 calories. It seems improbable that there should be this deviation between the serine phosphate of the protein and the similar phosphate groupings of a wide variety of compounds. A neighboring positive charge might cause such a change but this seems unlikely in view of the small ionic dependence of this equilibrium.

A more plausible conclusion is that a conformation change correlated with the phosphorylation of the protein leads to an enhanced stability of the phosphoenzyme. A conformation change of 2400 calories for example caused by the phosphorylation of the protein would together with the 1600 calories to be expected from hydrolysis of the phosphate bond give a net positive ΔF of 800 calories for hydrolysis of phosphoenzyme which is just that observed.

Extensive studies of this sort have not been performed on other enzymes but nevertheless the occurrence of a protein conformation change has been shown to be very probable in several systems. Penicillinase (Citri and Garber 1961) and chymotrypsin (Foster 1961; Wooton and Hess 1962) are illustrative examples of enzymes which probably show such a change. It appears, therefore, quite certain that phosphorylation shows a substrate-induced conformation change correlated with specificity and that this is a widespread, if not universal, feature of enzyme action.

C. Relation of Flexible Site to Control Mechanisms.

The flexible active site has already been useful in explaining a variety of phenomena which could not be explained at all or only with great difficulty using the template hypothesis. One of the most exciting areas of application has been in the field of control mechanisms. The suggestion that this flexibility might provide an explanation for hormonal action (Koshland 1959) has yet to be established for a particular hormone although modification of enzyme activity by small molecules has been established in a number of systems (Krebs and Fischer 1963, Frieden 1959, Tomkins and Yielding 1961). In the field of feedback inhibition, however, the brilliant studies of

Umbarger, Pardee, Monod, Ames, Martin and others have provided a particularly exciting new development. Gerhart and Pardee (1962), Monod, et al. (1963) and Martin (1963) have applied the concept of a flexible site to the control mechanisms and postulate the existence of a "regulatory" or "allosteric" site at which the feedback inhibitor acts. Although direct protein structure studies have not yet been made on these proteins the kinetics and specificity evidence strongly supports this hypothesis. It would seem, therefore, appropriate to examine molecular models based on our protein structure studies which are capable of explaining control of intracellular enzymes by means of a flexible active site.

In Figure 4 a mechanism for sequential absorption and for the preservation and control of a highly reactive intermediate (Koshland 1962b) is shown. In this case, the intermediate formed is an acyl phosphate which then reacts with an amine to form a peptide bond. Acyl phosphates are reactive intermediates which can react nonenzymatically with many of the constituents of a cell. It seems appropriate, therefore, for the cell to make reactive intermediates of this sort with the acceptor compound in situ, ready to react with the activated carboxyl group as soon as it is formed. In Figure 4 the amino compound induces a change in conformation leading to the absorption of the carboxylic compound. The presence of the amino derivative is a necessary condition for the absorption of the carboxylic acid and hence reaction between the carboxylic acid and ATP cannot ensue in the absence of the amine. This action of a flexible site ^{is} a type of control function of enzymes, i.e., the prevention of side reactions of highly reactive

intermediates. The mechanism also explains sequential absorption which has now been observed in a number of enzyme systems and which is very difficult to explain on the basis of a template hypothesis.

The relation of this mechanism to previous considerations of specificity and to hormone action is shown in Figure 5. If we replace the amino compound of Figure 4 by a buttressing group attached to the carboxylic acid, we see that this added substituent induces the proper conformation and is, therefore, the mechanism that was used to explain the specificity of enzymes such as 5'-nucleotidase and phosphoglucomutase. The amino compound in Figure 4 is thereby performing a role in the initial step no different than the role of a bulky substituent in the reaction specific for a substrate of minimum molecular weight. It is only a short further development to the action of a group which is not attached to the substrate, and which does not undergo a stoichiometric reaction during the enzyme action. The molecule labeled R in Fig. 5 demonstrates this relationship.

Such a molecule may have arisen by accident and caused reactivity of a small compound having the same functional group as a large substrate for which an enzyme already existed. Its usefulness in control may have developed because of the added permutations available when the R group of the hormonal type molecule was separated from the substrate. This mechanism provides a tool for inhibition as well as activation since the replacement of the activator R by an inhibitor could obviously inhibit enzyme action.

In Figure 6 the role of an inhibitor acting near the active site is shown, using models similar to those of Figures 2 and 3. If the inhibitor, I, attracts a peptide chain as indicated in the left-hand side of the figure,

it may block enzyme action by causing a misalignment of one of the key catalytic groups, in this case, B. If B is only involved in catalysis and the inhibitor will not affect the binding of substrate and hence noncompetitive inhibition would be observed. If, on the other hand, the group B is essential to binding as well as catalysis, competitive inhibition will be observed.

On the basis of such models the effect observed by Martin (1963), i.e. that a reagent such as a mercurial can prevent the action of an inhibitor without preventing the binding of the inhibitor, can be explained. On the right-hand side of Figure 6 is the presence of group, R, which acts to prevent juxtaposition of the group C in the protein chain to the inhibitor I. As a consequence the inhibitor would not be able to attract the chain containing B and hence the inhibitor would have no effect on the substrate reaction even though it were bound to the protein precisely as before. It is easy to develop models of this sort to explain the damage of the action of the inhibitor site by heating or other forms of degradation reactions which would destroy the effectiveness or the binding affinity of the inhibitor site without in any way affecting the binding at the substrate site.

Carrying this model one step further one can see as in Figure 7 that the binding of a hormone or of an activator could offset the effect of an inhibitor.

Finally, it is perhaps appropriate to speculate on the way in which "regulatory" or "allosteric" sites arose. It is, of course, quite possible that the allosteric site arose purely by chance. The resultant

value to the organism would allow only these organisms containing control mechanisms to survive. A more directed mechanism for the appearance of such proteins would seem desirable. In Figure 8 is shown a possible mechanism for competitive inhibition at different sites. When the substrate reacts to induce a conformation change it would so modify the protein that the inhibitor site would no longer attract inhibitor. Conversely, when the inhibitor is attracted to its site on the protein, it would change the protein conformation so that the C, D site would no longer attract substrate. Since both substrate and inhibitor could not be on the protein at the same time, the kinetics of competitive inhibition would exist. This would be inefficient in the absence of a desirable feedback control mechanism since the presence of the second substrate, here designated as I, would merely act as a competitive inhibitor of the first reaction with the substrate S, and vice versa. If, on the other hand, the appearance of this inhibitor site coincided with the first enzyme in a biosynthetic chain, the feedback inhibition which is observed would be particularly beneficial to the organism and would have survival value.

Summary

Kinetic and specificity data lead to the conclusion that a classical template specificity mechanism is inadequate. A model involving an "induced-fit" site can, however, explain the anomalous specificity patterns, non-competitive inhibition, sequential absorption and other phenomena not explainable on the basis of a template. This theory predicts changes in

protein conformation which have been verified using a variety of techniques and protein reagents. The theory allows the construction of molecular models which are capable of explaining the observed phenomena of feedback inhibition.

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Figure 1. Schematic illustration of noncompetitive inhibition based on a classical template mechanism.

Figure 2. A substrate induced conformation change leading to an active complex based on an induced-fit specificity theory.

Figure 3. Explanation for lack of reactivity in compounds larger or smaller than the true substrate based on an induced-fit specificity model.

Figure 4. Possible mechanism for synthetase enzyme action. (a) Step leading up to absorption of all reactants, (b) Catalytic steps involving bond changing. Heavy black line indicates protein chain with binding groups, Groups B_1 , B_2 , B_3 indicate catalytic groups.

Figure 5. Possible role of a hormone and a specificity requirement for a bulky group on a substrate. Hormone, indicated by crosshatched R, is necessary to induce proper alignment of protein chain. The same is true for the group R in the substrate.

Figure 6. Noncompetitive and competitive inhibition based on a flexible active site mechanism.

Figure 7. The effect of hormone in overcoming competitive inhibition in a flexible active site mechanism.

Figure 8. Competitive inhibition at a site distant from the active site in a flexible active site mechanism.

SPECIFICITY PATTERN OF 5'-NUCLEOTIDASE

SUBSTRATE	Km	Vm
ADENINE -- RIBOSE -- PHOSPHATE	$< 10^{-4}$	100.
NICOTINAMIDE -- RIBOSE -- PHOSPHATE	2×10^{-3}	67.
RIBOSE -- PHOSPHATE	3×10^{-3}	0.8

Table 1

INHIBITOR DOES NOT
AFFECT BINDING OF
 S_1 AND S_2 BUT
PREVENTS REACTION

NON-COMPETITIVE INHIBITION

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

NON-COMPETITIVE - IF ② INVOLVED IN
CATALYSIS AND NOT BINDING.
COMPETITIVE - IF ② ESSENTIAL TO
BINDING

REAGENT R NULLIFYING EFFECT
OF I WITHOUT AFFECTING BINDING
OF I

Figure 6

NON-COMPETITIVE - IF (B) INVOLVED IN CATALYSIS AND NOT BINDING.
COMPETITIVE - IF (B) ESSENTIAL TO BINDING

HORMONE OVERCOMING EFFECT OF INHIBITOR

Figure 7

COMPETITIVE INHIBITION AT DIFFERENT SITES

Figure 8