The Key-Lock Theory and the Induced Fit Theory

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It is a great pleasure for me to contribute to this symposium honoring the great scientist Emil Fischer. My graduate thesis required me to synthesize [1-^{14}C]glucose, which introduced me to the famous Fischer-Kiliani synthesis of glucose and mannose from arabinose and HCN.[1] I was also particularly intrigued with his classic key-lock (or template) theory of enzyme specificity,[2, 3] which like all great theories seemed so obvious once one understood it.

This symposium in his honor allows me to pay tribute to Fischer's great contributions to biochemistry varying from natural products chemistry to the key-lock theory, to review some of the history and significance of our induced tit theory, to illustrate the ramifications of those theories in our present era of protein-ligand interactions, and to discuss recent work in our laboratory which is helping to clarify conformational changes and their function. These theories have assumed again a central role in modern health research where the need for drug design requires taking into account the complementarity of tit of Fischer's principle and the flexibility and regulatory implications of the induced fit theory.

The induced tit theory is no more a refutation of Fischer's key-lock principle than the Heisenberg atom was of the Bohr atom or the modern DNA sequences are of the one gene-one enzyme hypothesis. A new theory must explain all the existing facts that pertain to it at the time of its enunciation. Gradually the new theory becomes accepted and then acquires anomalies due to the new facts uncovered after its enunciation. That in turn generates a newer theory which elicits new techniques to test it and its predictions. These new techniques then uncover facts which eventually require further new theories and so on. The new theories are built on components of the old principles. It is said that each scientist stands on the shoulders of the giants who have gone before him. There can be no more honored place than to stand on the shoulders of Emil Fischer.

Limits of the Key-Lock Theory

My first inkling that the Fischer key-lock model needed modification really arose from my consideration of the role of

water in biological reactions. I was preparing a lecture for a scientific meeting and decided to consider why some proteins were kinases and others ATPases. The more I thought about the protein, the more astonishing it seemed that water could be prevented from reacting at the active site of a kinase.

In hexokinase, which I took as a typical kinase, the OH group of water was known to be as good a nucleophile as the OH group of a sugar. If glucose is bound very tightly then it could exclude water, and a basic group on the protein would generate a glucosyl oxyanion nucleophile which could attack the ATP. But glucose would not normally saturate the site and could in many physiological circumstances fall to very low levels. Water, at 55 M, would fill up an empty site, and therefore water would be constantly competing with glucose in the nucleophilic attack on ATP. The existence of kinases in the absence of substrate or with only partially filled template type active sites would result in great ATPase activity and an enormous waste of energy.

Once I started thinking along these lines other anomalies came to mind. One example was "noncompetitive inhibition", which was explained by saying that the inhibitor blocked enzyme action but did not affect the binding of the substrate. No key-lock concept was available to explain such a result.

The key-lock (or template principle) could explain why smaller sugars might not react: they would not be attracted to the active site strongly enough to form significant amounts of the ES complex. However, we found that cc-methylglucoside was not a substrate but was a tightly bound competitive inhibitor of the enzyme. Thus it was tightly bound, could fit into the site, had the right chemical stereochemistry, but did not react. ("Substrate analog" was used for those chemicals whose chemistry is similar to a substrate but fail to react on the enzyme's surface as in a-methylglucoside, a substrate analog of the enzyme amylomaltase.)

Other reactions raised the same question of smaller chemically logical molecules that nevertheless did not react. And there were also cases in which a bigger substrate analog did not react. As another example, we found that cyclohexaamylose was an inhibitor of β-amylase (an enzyme that cleaved glucosyl bonds in long amylose chains). One could try to explain this on the basis of the key$-$lock principle by saying that the cyclic amylose was too big and couldn't bind, but we showed it did, in fact, bind (and tightly) but failed to react.[4]

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Introduction of the Induced Fit Theory

So the induced fit theory[5] was proposed in the following terms "a) the precise orientation of catalytic groups is required for enzyme action, b) the substrate causes an appreciable change in the three-dimensional relationship of the amino acids at the active site, and c) the changes in the protein structure caused by the substrate will bring the catalytic groups into the proper alignment, whereas a nonsubstrate will not."

Pictures to illustrate this concept and how it could explain the previous anomalies are shown in Figure 1 taken from papers published at the time.[6] The theory of Emil Fischer was deep in the hearts of scientists and journal editors, so I had great difficulty getting the original ideas published or convincing skeptics, but we did obtain more evidence from my own laboratory, and soon others joined in. One of the predictions that results from the assumption of a flexible enzyme, namely that a small nonreactive molecule could make up for a structural deficiency in a nonsubstrate (Fig. 2), was established for us by two
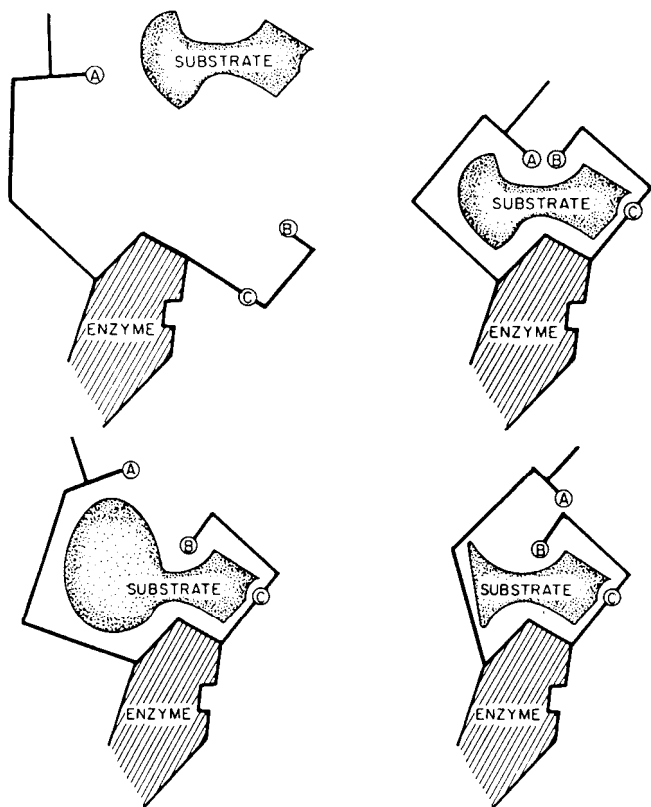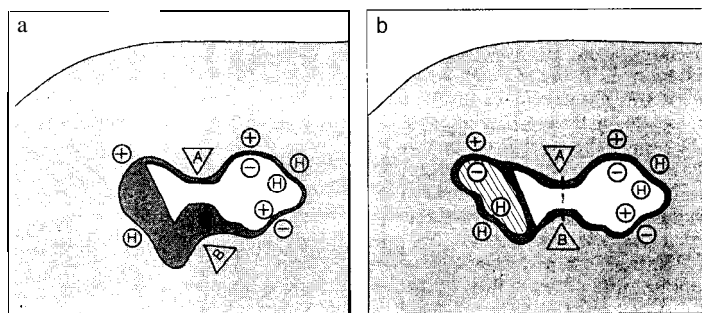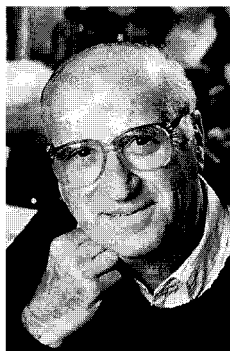

Fig.1. Schematic model of the induced fit mechanism. Black lines indicate protein chains containing catalytic groups A and B and binding group C. Upper left: substrate and enzyme dissociated. Upper right: substrate with induced change of protein chains to bring A and B into proper alignment for reaction. Lower left: bulky group added to substrate prevents proper alignment of A and B. Lower right: deletion of a group eliminates buttressing action on the chain containing A, so the thermodynamically stable complex has incorrect alignment of A and B.

Fig. 2. Activator **molecules can,** according to the flexible model of enzyme action, help to make a deficient molecule act as a substrate by altering the shape of the enzyme. For **example, in** the case of a molecule (unshaded) that by itself is too small to induce the proper alignment of catalytic groups, A and B [shown in a)]. a second molecule (shaded) can bind immediately adjacent to the deficient molecule [shown in b)] or (not shown here) to a more distant site. thereby inducing a stable shape with the proper alignment of catalytic groups.

laboratories. Sols et al. showed that xylose, a pentose (similar to glucose but lacking the $6\text{-CH}_2\text{OH}$ group), made hexokinase a better ATPase,[7a] and Murachi et al.[7b] showed that the nonsubstrate for trypsin, glycine ethyl ester, could react appreciably if ethylamine was added to the incubation mixture. These "regulatory" molecules which did not themselves undergo chemical changes could induce the further conformational changes in an enzyme needed for reaction (as illustrated in Fig. 2).

These indirect chemical assays added to the credibility of the hypothesis, but we needed direct evidence for the predicted induced conformational change in the protein (a proof which was easy later when protein crystallography became available). So Yankeelov and I said we must get a result with protein reactivity

Daniel E. Koshland was born in New York City in 1920. He earned his B. S. degree from the University of California, Berkeley, in 1941, and Ph. D from the University of Chicago in 1949. After two postdoctoral years at Harvard he joined the staff of Brookhaven National Laboratory, and later also of Rockefeller University. In 1965 he joined the faculty of the University of California, Berkeley, where he is currently Professor of Biochemistry and Molecular Biology. He became Editor of Science in 1985. Among his honors are the National Medal of Science, the Edgar Fahs Smith and Pauling Awards of the American Chemical Society, the Rosenstiel Award of Brandeis University, the Waterford Prize, and the Merck Award of the American Society of Biochemistry and Molecular Biology. Included in his fields of interest are the role of conformational changes in enzyme regulation and the elucidation of the catalytic power of enzymes. He demonstrated that bacteria have short-term memory and that purified mammalian cell lines show rudimentary memory. His recent work has emphasized the chemical mechanism of short-term and long-term memory, and the structure–function relationship of receptors.

that tested the key-lock template hypothesis and induced fit. We argued that adding a ligand to a template type enzyme can bury groups but it cannot expose them, whereas an induced fit conformational change could bury some groups and expose others. We picked the enzyme phosphoglucomutase (whose reaction had similarities to hexokinase, and thus we expected it to be an induced fit enzyme)[8] and used the reactivity of its SH group as a test. The experiment illustrated in Figure 3 gave the result we wanted.[8] Ligand binding induced the exposure of an SH group, a result incompatible with the key-lock theory. We likened it to the flexibility of a "hand in glove", which included Fisher's idea of a fit but added the flexibility concept.
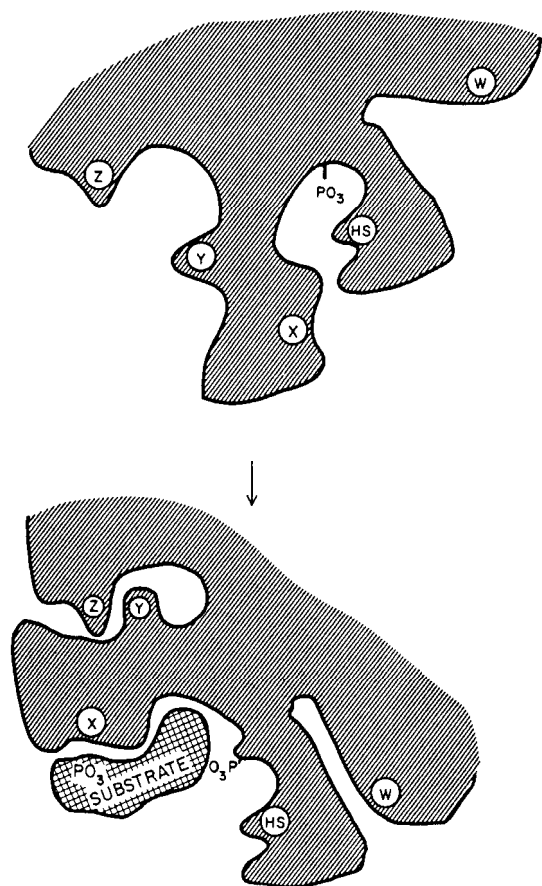

Fig. 3. Schematic illustration of flexibility in the action of phosphoglucomutase. The upper part of the figure represents the enzyme molecule in the absence of substrate. The lower part of the figure represents the change in conformation leading to exposure of −SH and burying of X, Y, Z, and W.

Further support came when the structures of lysozyme[9] and ribonuclease[10] were published because there were definite conformational changes; however, these were small and did not impress many. (Many biologists forgot that C−C and C-O bonds are only 1.5 Å long, so small changes can easily disrupt a catalytic alignment needed to catalyze changes in the bonds.) Then Steitz et al. with carboxypeptidase[11] and Steitz et al. with hexokinase[12] showed conformational changes that were breathtakingly large and highly convincing. Steitz showed that the engulfing of the substrate glucose by hexokinase occurred precisely as the induced fit predicted, thus giving visual proof that ligand-induced conformational changes were real and significant.[12]

Today almost every enzyme has been shown to undergo significant ligand-induced changes. A recent review by Gerstein, Lesk, and Chothia[13] divides these changes into "hinge domain" and "shear" motions and lists 42 enzymes that illustrate major conformational changes. The enzymes that show the least conformational changes are the hydrolases such as the proteases and nucleases—and they are precisely those one might expect to fall in this category, since they do not need to exclude water. The finding of extensive conformational changes in many enzymes is logical, since most enzymes exist in a cytoplasm with many pathways that contain many smaller substrate analogs, for example trioses, which must be prevented from reacting at sites of larger analogous substrates, for example hexoses. If the specificity failed to exclude smaller analogs, poor yields and bad side reactions would occur.

The question then arises as to how big the conformational changes have to be in order to be considered "significant". Some recent evidence indicating some answers to this problem is discussed below.

Isocitrate Dehydrogenase and Small Conformational Changes

We have recently been studying isocitrate dehydrogenase to obtain some clues to the size and significance of conformational changes. We found, for example, that the enzyme was inactivated by phosphorylation,[14] but this phosphorylation, unlike the case of glycogen phosphorylase,[15] involved phosphorylation right at the active site with little resulting change in conformation of the protein.[1619] We also found very little change in conformation induced by the substrate isocitrate on binding to the protein and were about to conclude that the enzyme was one of those that approximated the Fischer key−lock model. However, we did one more experiment and tested the protein in the presence of the product, a-keto glutarate.[20] In that case the ligand-induced conformational changes were wide spread. Many atoms moved though each movement was rather small. The protein did not tit into the "hinge domain" category of Gerstein, Lesk, and Chothia nor even into the "shear" category, but rather into what might be called a "spider web" category, that is, small interconnected changes occurring over an extensive surface. The changes in each atom were less than an angstrom but many atoms moved, which suggested that subtleties in alignment were capable of turning an enzyme off or on.

We have also measured the changes in the aspartate receptor of chemotaxis in collaboration with Sung Hou Kim et al.[21] This case fits the shear model more closely, as we postulate that the small changes at the binding site for aspartate can cause a sliding of one helix past another.[22−24] The changes that are generated in the cytoplasmic domain are relatively small   maybe an average change of 0.5 Å—but the conformational change is transmitted from one side of the dimer to the other. In addition we have shown that the receptor shows negative cooperativity[25,26] in which binding of the first aspartate to a dimer completely blocks aspartate binding to a second aspartate site. The two sites are initially identical. but the ligand-induced changes in the second site reduce the size of the second site so it becomes too small to bind aspartate. The changes are quite

Table 1. Distances between side chains in binding sites in the *Salmonella* aspartate receptor-ligand binding domain.

Amino acids	Separation [A] in unbound receptor [a]	Separation [A] in empty site of Asp-bound receptor[a][Å]	Reduction in distance
Ser-68, Thr-154	8.9	8.1	0.x
Tyr-149, Arg-73	6.9	6.0	0.9
Tyr-149, Arg-64	4.1	3.2	0.9
Phe-150, Arg-73	4.8	3.5	1.3
Ser-68, Arg-69	7.4	6.6	0.8

[a] Distance between closest non-hydrogen atoms.

small in Å (Table 1) but are enough to prevent binding of the aspartate molecule.[26]

Our conclusion is that big movements are important but so are small ones. The important feature from the induced tit theory is that the alignment of catalytic groups and binding groups must be optimized for the transition state, and the attainment of the state is unfavorable energetically unless it is supplied with the energy of the substrate binding. If the protein movements were easy to attain, they would occur spontaneously often enough to have little effect on catalysis. However, a small movement can also be energetically unfavorable, as in the shift of a ferrous atom 0.7 Å into and out of the plane of the heme in hemoglobin.[27] When the small movement needed for catalysis, in the case of an enzyme, is generated by the binding of the substrate, enzyme action occurs.

A second conclusion is that the conformation of the protein is undoubtedly selected during evolution to optimize both the unliganded state and the liganded state. Allosteric sites are often distant from the active site by 20 Å or more, and the conformational changes are far larger than can be explained by a distortion that propagates from one site to another by pure chemical torsions. Those nonbonded forces dampen out too rapidly. Therefore, the conformational change induced by the substrate binding has a long-range effect because it generates and catalyzes the transition from one evolutionarily selected conformation to another.

Summary and Outlook

The basic concept of Emil Fischer's key-lock theory, which explained enzymatic properties of specificity and action for 60 years, required modification to explain discrepancies such as the lack of hydrolytic activity of kinases, noncompetitive inhibition, and other apparent inconsistencies. The new theory, the induced fit theory, incorporated Fisher's concepts of the complementarity of enzyme and substrate but introduced the concept of a flexible enzyme, likened to the tit of a hand in a glove. The flexible enzyme concept not only explained the discrepancies but set the stage for further understanding of *regulation, cooperativity*, and *specificity* as described in papers by Pardee,[28] Monod,[29] and our own laboratory, as well as many others. Thus the great work of Emil Fischer lives on in an extension of the theory and application to new problems of chemistry and biochemistry that were impossible to visualize in the 1900s. The new studies focus on the importance of conformational changes, both large and small, and the manner in which they control enzymatic reactions. The findings from modern X-ray crystallography that essentially all enzymes undergo conformational changes induced by substrate binding has made the induced fit theory universally accepted in textbooks and by scientists.

These theories are of increasing importance because of the rise in drug-resistant strains of organisms. Computer-assisted drug design is what we and many others are now developing to prevent the ravages of the new virulent organisms. For that purpose the key-lock theory with a relatively rigid enzyme would be an easier basis for computer designs, but unfortunately the evidence that induced fit theory is closer to reality means that computer programs will have to be a little more sophisticated. However, the modern computer seems clearly up to the challenge, and a rigid enzyme is a good starting point for initial assumption. The flexibility can then be built into subsequent calculations. Moreover, the flexible enzyme allows binding to a "regulatory" or "allosteric" site, which may be a better target for drug therapy in many cases. The finding that very small changes can "turn on" or "turn off" an enzyme is very encouraging in this regard.

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