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Conformational changes: How small is big enough?

DANIEL E. KOSHLAND, JR.

The finding that small conformational changes are important in protein function is the ending of an old controversy and the beginning of new applications. The induced-fit theory proposed[1–5] that protein flexibility is an essential characteristic of enzymes, in contrast to the rather rigid key-lock or template theory of Emil Fischer—that is, a hand-in-glove type of flexible fit versus the jig-saw puzzle type of rigid fit. The induced-fit theory was stated in the following terms: the precise orientation of catalytic groups is required for enzyme action; the substrate causes an appreciable change in the three-dimensional relationship of the amino acids of the protein; and the changes in the protein structure caused by the substrate will bring the catalytic groups into the proper alignment, whereas the non-substrate will not. The induced fit theory explained many anomalies–such as the ability of enzymes to exclude omnipresent water, regulation outside the active site and non-competitive inhibition–but it was at first greeted with skepticism, as are all theories that confront long-established concepts.

When the first two structures of enzymes (lysozyme and ribonuclease) were solved by x-ray crystallography[6,7], small conformational changes were found between the structures of the enzyme in the absence and in the presence of substrate. I was surprised that these small changes were discarded as unimportant, rather than being cited as a confirmation of the new theory. Later, large changes in conformation of the enzyme carboxypeptidase (Lipscomb and co-workers[8]) and of hexokinase (Steitz and colleagues[9]) were observed, and the induced-fit theory is in all biochemistry textbooks today. However, the question I set out to answer in the beginning (which is still relevant today) was: "How small of a conformational change is big enough?" Because the induced-fit theory was proposed before crystallography had been successfully applied to proteins, the size of the conformational change was defined in functional terms; that is, a change big enough to produce the desired catalysis. With many X-ray structures of enzymes today, almost all of which show conformational changes[10], the question has become: "Are all of the changes important? And if not, which ones are?" Fortunately, the tools are now available to answer that question, as it has become even more relevant as we explore ways to apply enzymology to solve medical problems of therapy and chemical problems of new materials.

Correlation of small structural changes induced by ligand binding with protein function is described here in two typical proteins—one an enzyme, the other a receptor. In the case of the enzyme, isocitrate dehydrogenase, the kinetic alterations of many orders of magnitude of the enzyme action are related to changes of a fraction of an angstrom of its amino acid side chains at the active site. Figure 1 (reproduced from the original article by Mesecar and Koshland) shows the very small conformational changes in protein structure that cause differences in the catalytic constant of the enzyme[11].

In the case of the receptor—for example, the aspartate receptor expressed on the cell surface of bacteria which provides the signal for chemotaxis—small conformational changes in the extracellular domain of the protein occur upon binding its ligand aspartate[12] and this binding causes large functional changes in the cytoplasm that trigger a change in the swimming pattern of the organism[13,14]. The small changes in the extracellular domain of the receptor measured by both X-ray crystallography[12] and electron spin resonance[15] are transmitted as small conformational changes in the cytoplasmic domain[16], and these are then amplified by enzymes in the cytoplasm to achieve large changes in function. Small conformational changes are responsible for other important functions of the receptor—for example, its negative cooperativity[17–19]. Changes in the unoccupied site of the receptor are induced by binding of the first molecule of aspartate at a distant occupied site[19] (see Table). These small differences are sufficient to prevent the binding of a second molecule of aspartate, despite the fact that this site was quite capable of binding aspartate before those small changes occurred.

The consequences of these small conformational changes are profound. They are responsible for the enormous amplifications of stimulus to response that is essential in biological systems. They are at the root of feedback inhibition, enzyme activation, cooperativity, specificity and evolutionary selection. A bacterium can detect the binding of a single molecule of nutrient to a receptor on its surface[20]. That requires a great amplification because the binding of a single molecule does not provide enough energy to generate the subsequent signal that the sensory system activates. Similarly, the eye can detect biochemically a single photon[21], but it requires six simultaneous photons to leave a record in the cortex of the brain[22]. The initial photon or even the combination of six photons have too little energy to produce the big muscular movements that are the proper response to the external stimuli. In both cases the conformational changes alter the enzymological properties of the proteins with consequences to the organism.

In chemotaxis, the aspartate receptor becomes an inhibitor of the kinase cascade. The aspartate receptor is also induced into becoming a better substrate for methyltransferase, thereby allowing the adaptation that is essential for bacterial memory. In the case of the enzyme isocitrate dehydrogenase, it is in-

Distances between side chains in binding sites in the Salmonella aspartate receptor ligand-binding domain

Amino acids	Separation (Å) in unbound receptor[a]	Separation (Å) in empty site of Asp-bound receptor[a]	Reduction in distance (Å)
Ser-68, Thr-154	8.9	8.1	0.8
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.

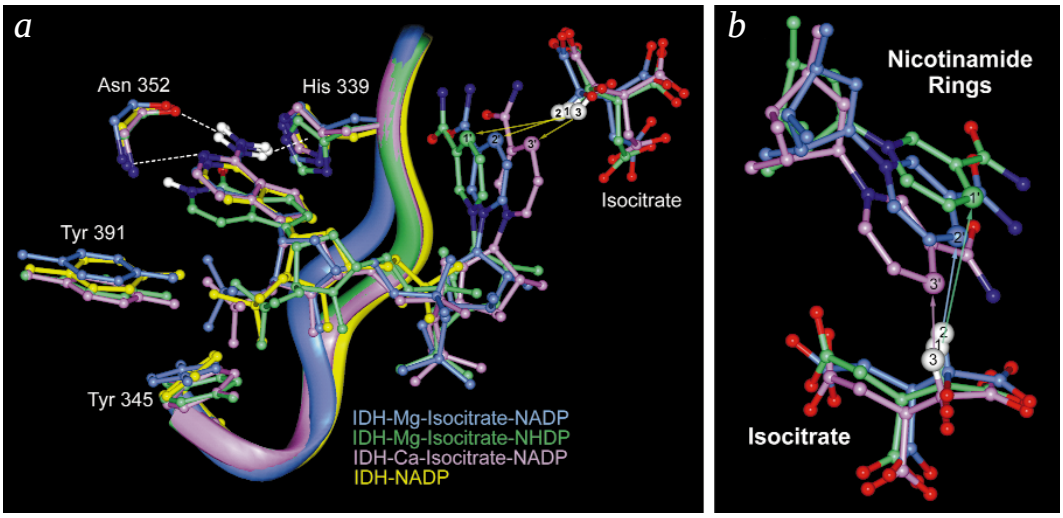

Fig. 1 Superpositions of various isocitrate dehydrogenase complexes, including isocitrate-Mg^{2+}-NHDP (green) and isocitrate-Ca^{2+}-NADP (pink), Y160F-IDH-isocitrate-Mg^{2+}-NADP (blue) solved by time-resolved Laue crystallography, and IDH-NADP (yellow). ***a***, The rotation of the adenine ring, which occurs on mutation to a hypoxanthine derivative, is evident and differs from both the Mg^{2+}- and Ca^{2+}-NADP structures. The driving force behind this movement seems to be the formation of a pair of interactions between the O_6 carbonyl oxygen and two separate protein atoms in the nucleotide binding cleft at 3.0 Å to the ε-nitrogen of His (position 339) and 3.3 Å to the backbone nitrogen of Asn (position 352). These interactions are aligned in the plane of the electron pairs of the sp^2-hybridized O_6 oxygen when the ring is rotated, as described above, with bond angles through the oxygen of 130 (and 95°, respectively. In addition, the N1 nitrogen of hypoxanthine ring is in contact with the side-chain oxygens of Asn (position 352) and Asp (position 392). The hypoxanthine nucleotide ring is hindered from rotating further in the pocket to completely optimize these interactions by the packing of protein atoms against both faces of the purine ring, and by the electrostatic interactions between the ribose phosphate and Arg (position 395) and Arg position 292, which serve to further anchor the nucleotide. However, the distances between that same phosphate and two neighboring tyrosines that bind NADP (345 and 391) have increased by approximately 1.4 Å each, whereas the contacts to the arginine residues are preserved. These changes in distance presumably contribute to the decrease in the binding affinity of this compound as compared to NADP. Backbone movement of residues 340 to 344 (ribbon representation), observed in the Laue structure, is most likely due to the room temperature dynamics of the protein under steady state turnover. ***b***, An expanded view of the nicotinamide rings and isocitrate, as well as the hydride transfer paths (1 to 1', 2 to 2', 3 to 3'). The nicotinamide ring for the NADP in the binary IDH-NADP complex is not observable in the electron density. Placing the hydride donor atom (C2 of isocitrate) closer to its acceptor atom (C4 of the nicotinamide ring) does not accelerate the rate of the reaction; it actually decreases the rate of the reaction, indicating that orientation (angle) makes a major contribution to the catalytic rate. k_{cat} rates: Mg NADP 80.1, Ca-NADP < 0.2, Mg^{++}NHDP (hypoxanthine) .00305, all with isocitrate and wild type enzyme.

duced into a better catalytic machine. Those conformational changes are barely detectable by the best physical tools available but are easily detected by the discriminatory power of enzymes. This discriminatory power results from the enzyme's specificity that catalyzes a very fast reaction with the right substrate and a very slow or no reaction with a poor substrate or a non-substrate. For example, the catalytic constant (or turnover number) of an ordinary enzyme (moles of product produced per mole of enzyme per second) is typically 10^4 (although some are as high as 10^6). That means that in an ordinary mammalian cell that is about one millionth of a milliliter, the concentration of an allosteric activator could go from zero to 1μM in 0.1 second or from 1μM to zero in the same interval. The small conformational changes are amplified to considerable physiological effects by the high specificity and high catalytic power of the enzyme.

The examples given here illustrate that a shift of 1 Ångstroms is 'big enough' to be functional in a biological signaling system, through either a great decrease (10^4) in an enzymatic rate, a great change in cooperativity (binding at one site in a dimer prevents binding at a second previously identical site), or the initiation of a signal that is effective on the other side of a membrane 100 Ångstroms away. The evidence of widespread conformational changes is good evidence that similarly small as well as big changes will implement many other processes in biological systems.

The finding that very small changes are important in enzyme and protein action has consequences for drug development. Much of pharmaceutical research now is aimed at finding drugs that compete at active sites and block enzymes that have deleterious effects. The disadvantage of a competitive inhibitor is that higher concentrations of the substrate can nullify its effect. Non-competitive inhibitors could be more effective as they cannot be overwhelmed by more substrate. Allosteric effectors that are not competitive can serve as effective activators of weak enzymes or as inhibitors of overactive enzymes. Allosteric activators or inhibitors induce small conformational changes that can either turn on or turn off an enzyme. X-ray crystallography and protein 'docking' experiments are creating new avenues for drug design that depend on very small changes being transmitted by distant effectors to affect the function of the defective enzyme or receptor.

Small changes having a large effect explains the process of the evolution of proteins and why proteins are large[23,24]. Small changes in distant parts of a protein can cause incremental small changes in activity from which a selection system can systematically select improvements and discard unfavorable changes. If the protein were very small and finely tuned, any change in that area would probably be disastrous and the system would oscillate between very good and very bad, with the hope that the bad swings would not go so far that the species would die. It would be far better for the species to have a mechanism for slow progress that proceeds from good to better so the species survives no matter what, and becomes steadily better in the selection process. This explains the slow incremental process that is the mechanism of evolution.

This understanding offers great opportunities for us to mimic evolution and create new molecules that have the ideal proper-

ties that some of the products of evolution have attained. That opportunity is being seized by many in a new area called 'directed evolution'[25]. Perfecting the molecule requires such precision in very small changes that our theory and experiments are strained to make logical predictions that can be used to improve drug design. However, we can use an alternative approach and make many small changes rapidly and at random and then select the ones that work. The areas of combinatorial chemistry and random mutagenesis have made the business of getting many mutations feasible and the techniques for selecting or screening are getting continuously better. Thus the results of evolution are being recreated on a laboratory time scale that is very short, measured in days, not millions of years.

The finding that small conformational changes have important functional consequences offers a great opportunity, but is also a hurdle that must be overcome. It means that the correct product must be very precise. Studies are revealing theories[11] as to why the small conformational changes work, and that helps a lot. Therefore, as the technology continues to improve and the theory becomes more solid, the experimental procedures of directed evolution and drug design should produce new molecules of great therapeutic value and also new materials that may be even more useful than the arrays of polymers, drugs, chemicals, structural materials, and so on that evolution and humans have produced up to now. As usual for all basic research, the initial curiosity of "how small is big enough" turns out to have important societal value.

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Daniel E. Koshland, Jr.
Department of Molecular & Cell Biology
University of California
229 Stanley Hall
Berkley, CA 94720-3206