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The Elastomeric Rack in Biology

(conformational changes/pressure/activation)

HENRY EYRING* AND FRANK H. JOHNSON†

* Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, and
† Department ofBiology, Princeton University, Princeton, New Jersey 08540

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ABSTRACT A model designated "The elastomeric rack" is discussed with reference to the mechanism of enzyme catalysis and the necessity of large protein molecules in the process. This model, which represents an extension of the earlier "Rack mechanism," stems from experimental evidence demonstrating large volume changes of activation or of reaction accompanying various biochemical reactions and more complicated physiological processes. For reasons discussed, the volume changes, as revealed through the influence of increased hydrostatic pressure on reaction rates and equilibria, prove that there are large conformational changes in the protein which, because of numerous cross links in a complex network, lead to additive stress on the substrate–enzyme complex, and thereby to increased reactivity. The manner in which the model serves to account for the influence of changes in the environment (temperature, pressure, chemical composition, electrical fields) on the activity of the protein catalyst, and the modifying action of particular inhibitory or activating substances, alone or in combination, is briefly discussed. The bearing of various considerations on protein denaturation, excitation of nerve, contraction of muscle, specific precipitation, and the divers physiological processes in living cells, including mutation, cancer, and the degenerative diseases of aging, is also briefly discussed.

During recent years the mechanism of enzyme catalysis, the action of inhibitors and activators, and the fundamental role of large protein molecules in these processes have received much thought and discussion, leading to a steady improvement in the understanding of the phenomena involved (1–22). There is one useful source of evidence, however, that happens to be one with which we have been concerned for some years, yet has only rarely been taken appropriately into account by other authors, namely, the quantitative effects of moderately increased hydrostatic pressures (up to a few hundred atmospheres), and those of temperature throughout the range of measurable activity, on rates and equilibria of biological reactions. The volume changes of activation or reaction which through the influence of pressure have been found to amount to as much as 50–100 or more cm^3 per mole (3, 23) provide a valuable insight into the conformational changes involved. It seems especially worthwhile, therefore, to reconsider the whole problem in terms of the presently available evidence. As a result, we have arrived at perhaps a somewhat clearer view, based on a model that stems from mechanical stress. For the following reasons, it is appropriate and convenient to refer to this model as the "Elastomeric Rack Mechanism." As the discussions below indicate, the same concept seems applicable not only to enzyme catalysis in the usual sense, but also to various other processes, including contraction of muscles, excitation of nerves, specific precipitation, and others.

Many kinds of catalysis involve mechanisms not dependent upon the catalyst's being a high polymer. Thus, the unfilled shells in the transition elements of a solid provide a fleeting refuge for the overcrowded electrons of the activated complex and thus lower the activation energy. Withdrawal of electrons from a molecule can make it more reactive toward a nucleophyllic reactant, while donation of electrons can increase the reactivity of a molecule toward electrophyllic reactants. Sherman, Sun, and Eyring (24), from theoretical considerations, pointed out that the best atomic distance for two nickel atoms in a surface was considerably greater than the length of the hydrogen bond of the molecule that is being absorbed. This was verified experimentally by Beeck *et al.* (25–27, see also Twigg and Rideal, 28). Thus, the nickel atoms act like a rigid rack in stretching the hydrogen bond as it attaches itself to the surface and so increases its reactivity as it weakens the bond to be broken in reacting with an approaching molecule.

CATALYSIS BY THE ELASTOMERIC RACK MECHANISM OF ENZYMES

In some reactions enzymes act in ways analogous to the mechanisms mentioned above, but the *elastomeric rack mechanism* depends upon another property, i.e., the ability of the enzyme, because of its high polymeric character, to assume various conformations not differing greatly in free energy from the most stable state of the protein alone. The effects of pressure on reactivity, which is frequently accompanied by large volume changes in forming the activated complex and in undergoing enzyme inactivation, *ipso facto*, indicate large changes in conformation.

These volume changes are promoted conspicuously, but by no means exclusively, by dissolving in the aqueous enzyme solutions molecules such as alcohols, ethers, urethane, and high concentrations of xenon that tend to combine with patches of the enzyme that are otherwise folded inward against each other. The volume change itself is chiefly due to changing amounts of electrostriction resulting from increasing or decreasing the number of solvated ions, but the fact that hydrophobic substances trigger the change, which they are entirely incapable of doing with simple ions, leaves no acceptable conclusion but that the triggering is a conformational change in the highly folded macromolecule (often a very extensive change). The changing number of ions resulting from this conformational change undoubtedly plays a catalytic role in many cases. The enzyme patches, of course, may involve factors other than hydrophobic bonding. Now if a molecule contains two separate patches that become attached to the separate patches of the enzyme, and if these two enzyme patches are at equilibrium at a different distance

(ordinarily greater than the patches on the substrate), the result will be a stress on bonds of the substrate situated between the two attachments to the enzyme. Because the tension in the single strand of the substrate is pitted against the tension of many bonds acting in parallel in the disturbed enzyme conformation, this substrate tension can reach major proportions and substantially activate an otherwise stable bond, resulting in the extraordinary catalytic efficiency of the enzyme. This elastomeric rack picture is in keeping with the earlier rack mechanism of Eyring, Lumry, and Spikes (1). The emphasis here is on the fact that a single strand holding together two metastable networks of the enzyme can be subjected to a magnified stress when the enzyme network tightens, in much the same way as when a muscle contracts. This is important and is only possible for substances like enzymes that embody a network having a great deal of cross bonding through hydrogen, hydrophobic, ionic, and occasional S–S bonding.

Thus, the preliminary step in the elastomeric rack catalysis is the formation of this strained substrate–enzyme complex, which may then be followed by a reaction. Any change (temperature, pressure, chemical composition, and electrical field) in the environment of the substrate–enzyme complex will almost certainly affect the stable conformation of the complex, exerting a stress on the substrate strand and increasing its reactivity. The special effects resulting from the combination of molecules and enzyme at a distance from the combined substrate have been recently emphasized by Monod and others (10, 15, 18, 22) as the allosteric phenomenon. The combination of a molecule with the enzyme may, of course, either enhance or decrease the reactivity of the catalyzed substrate. The antagonistic effect of one inhibitor against another, e.g., that of urethane against the sulfonamide inhibition of bacterial luminescence at low temperatures (3, 29), behaves quantitatively in accordance with the interpretation that these two agents combine with each other. As we noted some years ago, however, the same algebraic formulations apply to "a simultaneous combination of the two drugs with the enzyme to give an intermediate state of the catalyst that is more active than that of the enzyme combined with either durg alone" (3, p. 475; see also p. 29). One or many molecules may add to the substrate–enzyme complex. It is always of interest to find the molecular order of events. Whether the substrate first adds to the enzyme, followed by the addition of the other modifying molecules, or vice versa, however, does not affect the measured rate of reaction provided that the slow step in the reaction comes at a later stage.

Since the resulting molecules from an elastomeric rack catalyst are apt to be formed with the parts that were bonded to the catalyst but little changed, the products should compete quite successfully with the reactants for this critical enzyme area. Thus, the products would tend to inhibit further reaction except as they are removed. Hence, if enzymes for consecutive reactions are near together, there is a channeling of the reactants with very little to be found detached from the enzymes. Also if enzyme B, which acts upon the product from A is incapacitated, A will stop working because of inhibition by its own product, unless that product is otherwise removed. These considerations bear implicitly on the phenomenon of feedback inhibition (9, 15, 18, 19, 22) and the regulation of the sequences of biological reactions in general.

The accelerating effect of modifying molecules may be to speed up reactions either by lowering the free energy of the enzyme–substrate complex, increasing the strain in the reactive strand, or both. The first effect increases the equilibrium constant in the Michaelis–Menten formulation, and the second increases the specific-reaction rate constant of the complex. Clearly it is desirable to study and differentiate between these two effects.

Coenzymes for the elastomeric rack mechanism may either have a modifying effect on the enzyme structure, act to facilitate the formation of one or both of the substrate–enzyme bonds, or finally act directly at the site of the bond to be broken. Specially designed experiments are needed to separate these three effects.

It is important to note that if a number of weak cross-links in parallel are made in an enzyme network by reactions involving energy-rich phosphate bonds, the stresses induced can add up to make a large stress on a single bond linking two such networks together and so cause this bond to become correspondingly reactive. This is then a mechanism by which several energy-rich phosphate bonds can transfer at least a part of their cumulative energy to a substrate through induced stresses in the attached enzyme.

Organic chemists have been torturing primary bonds into chemical reactivity for generations by trapping them into tight rings or by building onto an otherwise simple ring, bulky groups which strain the ring by steric repulsions or a squeeze resulting from tying two atoms together across the ring. Molecules have the same possibility for torture of the bonds in a ring made from an enzyme and a substrate. The requirements are that they be tied tightly enough together by primary bonds or a sufficient number of secondary bonds and that the torture be applied by a conformational change. The torture screws can be tightened by energy-rich phosphate bonds that tie atoms together by extracting water at strategic positions or by the multitude of other influences that are known to set off conformational changes, such as an aldehyde landing on an olfactory receptor causing a conformational change that opens a channel through a nerve membrane (30), or the chemistry that tightens a muscle.

The tying of substrate to enzyme can be highly specific, but high polymers such as cellulose are often tightly bonded by crystalline regions without convalent bonds. The extent of the area of hydrophobic or other bonding between enzyme and substrate puts an upper limit on the amount of torture that can be transmitted, as measured by the chemical reactivity imparted to the substrate bond.

In the foregoing discussion, the emphasis has been on the muscular, boa constrictor-like action of biologically active giant molecules that accompanies their conformational changes. There is no intended downgrading, of course, of the other well established catalytic effects of enzymes such as the stresses accompanying the flow of electricity in the activated complex. Our analogy is not too bad, inasmuch as any victim caught in the writhing coils of a boa constrictor would do anything to get out, including reacting chemically.

THE ROLE OF THE LARGE SIZE OF AN ENZYME

A particularly compelling argument for the importance of elastomeric rack mechanisms is that most enzymes are very large molecules with many possible conformations of comparable free-energy content. While part of this structure is necessary to fasten onto the substrate, it is difficult to see

how most of it can have any useful function except to form conformations that introduce stresses into the substrate, and because of the short range of electrical forces, it seems necessary to assume that, in many cases, these stresses must be transmitted mechanically.

THE ELASTOMERIC RACK MECHANISM

Membrane permeability

A membrane in which contiguous hydrophobic molecules or groups of molecules are attached to the protein molecule at two areas (distance changes with conditions) may be expected to develop water channels as changing conditions develop stresses that pull the hydrophobic groups apart. These changing conditions, for example, may be the addition of one or more molecules at strategic positions such as the so-called receptors in membranes having to do with taste and smell (see above). An applied potential across the membrane acts on an extensive network of the dipoles in the protein molecule, again pulling the contiguous hydrophobic groups apart. The resulting increase in membrane conductance sets off a nerve impulse. A deformation pressure likewise can alter the relative stability of protein conformations and the resulting nerve impusle will be interpreted as pressure or pain depending on the intensity of the stimulus and the receptor affected.

Muscular activity

As we have seen, change in conformation of a protein may result in a magnified stress tending to lengthen or shorten the units that together make up a muscle. These conformational changes are responsive to the same type of stimulus that affects enzyme activity and nerve impulses. There is ample evidence, through the influence of increased hydrostatic pressure on muscular contraction as a whole and on specific reactions involved in the physiology of contraction (31–34), that large conformational changes are fundamental to these processes. The unifying principle running through these considerations is the well-established concept that the relative stability of protein conformations is strongly influenced by temperature, pressure, electrical fields, and chemical compositions of the environment, and the important fact that high polymers with many cross links can act with great force on a single linkage connecting two parts of this squirming network.

These same considerations apply to any high polymer, such as RNA or DNA, that is highly cross linked and has suitable prosthetic groups that can make two or more attachments to a substrate. Consequently, importance of RNA and DNA in mutations and synthetic processes seems apparent.

Protein denaturation

If the principal cause of volume change in enzyme denaturation is due to electrostriction, then volume change, ΔV, and the entropy change, ΔS, for the various processes might well be in an approximately fixed ratio. When ice melts, the molal increase in volume is 1.8 cm^3, while the molal entropy increase is 5.2 e.u. This ratio, $1.8/5.2 = 0.35$, is the same as for the ratio $\Delta V/\Delta S = 64.6/184 = 0.35$, for denaturation of the luciferase of *Photobacterium phosphoreum*. This is at least consistant with the view that the main entropy increase in luciferase denaturation is due to electrostriction. A neutralization such as $CH_3COO^- + NH_3^+CH_3$ increases in volume by 15.8 cm^3 mol, as shown by Linderstrøm-Lang (3, p. 337), so that one can estimate that roughly $64.6/15.8 = 6$ ion-pairs are neutralized in the enzyme denaturation. This neutralization is made possible by hydrophobic bonding with such molecules as alcohols, ethers, etc. or by a rise in temperature.

The denaturation of proteins is often accompanied by breakage of S–S linkages. Since these bonds often connect large networks of proteins, it is to be expected that many kinds of conformational changes will introduce large mechanical stresses into connecting bonds, increasing their reactivity and so hastening their dissolution. Thus, the breaking of S–S bonds by drastic conformational changes is to be expected.

Mutation, cancer, and degenerative diseases of aging

Any changes, other than unphysiological, that cause conformational changes in the molecules of a cell may be expected to speed up chromosome breakage through the induced stresses and so increase the probability that cell division will bring mutations of the kind responsible for cancer and the other degenerative diseases of aging (35, 36). This idea is at least consistent with the frequently observed fact that cells that are continuously under physiological stresses are prone to develop cancer.

Specific precipitation

Finally, although it seems that theories pertaining to the mechanism of antigen–antibody reactions, such as theories involved in specific precipitation (37–41), are not predicated on drastic conformational changes in the process, it has been shown (42) that when a pressure of some 10,000 psi is applied within 1 or 2 min after a simple trihaptenic dye is mixed with homologous antibody, practically no precipitation takes place over a period of an hour or more at room temperature. When the pressure is released, however, precipitation takes place at seemingly the normal rate and in the usual manner. The inhibitory effect of pressure indicates that large conformational changes occur in this process, presumably involving primarily, if not virtually exclusively, the antibody molecules, inasmuch as the relatively small, stable hapten molecules could scarcely undergo large volume changes due to the effects of pressure in this experiment.

It seems necessary to believe that analogous large conformational and volume changes also occur in various other types of antigen–antibody processes. Further, judging from the very pronounced effects of increased hydrostatic pressure on a considerably wider variety of physiological processes in living cells than are briefly discussed above, e.g., cell division (43), synthesis (44), enzyme induction (44), cyclosis (45), rhythmic beating of cilia (46) and of cardiac muscle in tissue culture (47), growth and disinfection of bacteria (48–51), multiplication of bacteriophage (52), respiration (53), mutation (54), action potentials in nerve (55), narcosis of amphibian larvae (56), and others, it appears that the elastomeric rack mechanism is essential to the successful operation of life's most intricate machinery.

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