Chapter 23. Compensation temperatures are the most useful parameters in protein research

Compensation temperatures in small molecule chemistry are not of much use because processes are usually dominated by enthalpy changes. The very large rate enhancements characteristic of enzymic catalysis always well known but in recent years shown by Wolfenden in particular as being even more extraordinary pass through transition states for primary-bond rearrangements the same in as occur in the small-molecule counterparts. However heat activation characteristic of the small-molecule process does not depend on the protein as a thermal reservoir since thermal fluctuations of sufficient size would soon destroy the protein. The so-called "contributing degrees of freedom basis" has been easily excluded since the forties leaving only the "transition-stabilization" possibility and the pretransition-state activation idea. The former best known through Koshland's "induced-fit" hypothesis requires some electronic device but has never gained support from small-molecule devices or from electronic tricks and entropy management found only in proteins. The missing activation free energy must then arise from mechanical devices.

Kistiakowsky and Lumry simply because of the large size of enzymes that the device or devices must depend on conformational changes and Eyring, Lumry and Spikes having little confidence in electronic enhancement proposed mechanical activation like a nut cracker. They hypothesized that the protein conformation serves as a source of mechanical free energy required to elevate the reacting system into a pretransition state from which the step to the true transition state was effected by a small amount of normal thermal excitation. The activation free energies are low because they are computed from the experimental rate using absolute rate theory. The activation enthalpies are also small because they measure only the temperature dependence of the activation

free energy that arises from the thermal activation and has no direct connection to the mechanical activation.

Having little confidence that an adequate new electronic trick would be found Eyring, Lumry and Spikes looked for a mechanical device not based on such tricks and concluded that enzymes depend on entropy changes in conformations as well as enthalpy changes so that one could pay off for the other especially in the transient events of catalysis. This requirement provides the rational, although not the only one, for the large sizes of enzymes and mechanical force is a vector quantity not the pure scalar of heat so direction of force application greatly increases the activation efficiency and direction also increases the varieties of specificity available locking out all those directions not found suitable in evolution of single enzymes.

This hypothesis was first called a "rack mechanism" because it was applied to the regulation of the electronic properties of the iron ions of hemoglobin by mechanical interaction of the two halves of each subunit through the iron ligands. This has been the main theme in hemoglobin physical chemistry and the ligand-field theory was given in 1961 but the conformational details have only become clear in this volume. Once the mechanism was substantiated by application to hemoglobin application to enzymes was easy since there was more information about conformation of proteins such as chymotrypsin and the expansion-contraction process providing the mechanical force was established. That cumulative effort was described by Lumry and Biltonen in 1965 and called by them the "subtle-change" process. It was only in 1985 that the B factors from diffraction studies gave a molecular description. The catalysis process in the trypsin family of enzymes is better called a "nutcracker mechanism" after the suggestion by Corloni et al in their extraction of the mechanism from a priori computational methods.

The conventional activation energy from rate studies measures the temperature dependence of the activation free energy for pure thermal activation to the transition state. When there is transfer of potential energy from matrix to reaction site for mechanical action of the pretransition state, the potential energy raises the potential energy surface at the reaction site and lowers it in the matrix so the net change in potential energy is small. It is probably than the amount can be estimated from the matrix compensation temperature and the passive conformation temperature. If the former is 450K and the latter 300K, the amount would then be `(450-300) times the activation entropy change. The latter is due to matrix contraction and is negative so the estimate is the loss of potential energy by the matrix and the gain in the excited pretransition state. An assumption is that the entropy loss is always proportional to the potential energy loss. That is not an unreasonable first approximation. This estimate applies to chymotrypsin but has considerably generality because the compensation temperatures appear to be the same for many enzymes. The potential-estimate and thus the amount of rate acceleration varies with substrate as the activation entropy varies. The details for chymotrypsin based on linear compensation temperature for a series of ester substrates (Drovoska-Taran and coworkers) are given in Study of enzymes, edited by S. Kuby and will be reproduced in Volume II of the Primer.

It is probable that all enzymes use the same mechanism since in the many we have examined using PDB data all have the same construction features giving evolution limitless opportunities to control conformation regardless of chemical process to be catalyzed. Apparently that is why compensation temperatures are low, not too far from physiological temperatures and that is why they are so important in describing the processes. Obviously the electronic devices found in small-molecule processes are insufficient for biology although electronic supplementation is undoubtedly used where is offers add-on utility. A current

example is the current fad for low-barrier hydrogen bonds. As an equilibrium device they are unlikely because of the cost to protein stability but they are a natural consequence of the mechanical enhancement of pre-transitions states. just as it the formation of unusual electronic states of metal ions in proteins such as carboxypeptidase A.

In biology in general and certainly in most protein-supported processes compensation behavior is very common and it is usually linear within the experimental errors of protein research. Because of their large size and the small advances in structure parameters associated with protein physiology linear-response theory and even mean-field potential functions are often good approximations. Furthermore their compensation temperatures can identify many processes Knots of mesophiles have T_c values of 354K within error. Matrices on the other hand have values from above 450K down to as low as 200K because the matrices of enzymes are transient potential-energy sources arising from their contraction process and thus dependent on the advancement of that process in turn dependent on specific ligation. These changes appear to be the most difficult to comprehend and particularly so because the conformational changes involved are detectable in diffraction studies only in the B factors and not in the coordinate data. Not only do the changes in T_c provide a means for measuring the extent and thermodynamic important of matrix conformational changes but it other kinds of protein-centered linkage systems the y provide a means for describing the free-energy flow among the part processes of such a system. In thermo stated organisms there is usually an operating temperature determined by the environment or by the intrinsic temperature-regulation system of the organism. Then a linked sub process with T_c larger than the operating system losses free energy as the linkage system advances and those with T_c lower than the operating system receive free energy. Linked systems as free-energy machines can be described in this way.