Chapter 21. Mechanical mechanisms in some multi-protein systems (Draft 6-15-03)

The mechanism of enzymic catalysis that emerged in the sixties continues to gain substantiation. At present the least well established details involve the thermodynamic changes in the matrix expansion-contraction process and any general rules relating triggering of that process to the structures of substrates and inhibitors. Modulation by temperature, pH and solvent composition is mediated by changing the matrix parameters of the process, a change in protein activity coefficient. A major exception is the pH dependence of the ionization of functional groups sometimes including support groups like ASP 102 in the trypsin family of proteases since they have substrate contact and direct chemical involvement in catalysis. The total mechanism can be modeled at several levels of which the nut-cracker may be a sufficient model in the sense that the pulsed potential-energy transfer process provides the temporary stress exerted by the nut cracker on the nut.. Previously published discussions of these features suffered not only from insufficiency of information but also from the use of classical formulations of enzyme kinetics for pretransition excitation by variable potential-energy transfer. Major progress beyond discovery of knots and matrices twenty years ago is the ubiquitous enzyme mechanism signaled primarily by the approximate C-2 symmetry. That construction has not been found to be limited to enzymes. Is the versatility of the nut-cracker mechanism a ubiquitous consequence of the substitution of mechanical work for heat in many biological processes beyond those now established in enzymic catalysis?

Enzymes are made reentry cyclic machines because the conversion of substrate to product changes the entropy-enthalpy ratio (phase relation) from that for of the pretransition state to that for the expanded matrix. Passage through the transition state to product is critical. Otherwise the original substrate

becomes a inhibitor and can be removed only by input of work required to return the contracted state to the expanded state. That equilibrium binding process is discussed above as "completing the knot". Immunoglobulins appear to be more sophisticated than enzymes since as illustrated by the digoxin antibody the B values are all small and variable dependent on details of ligand binding. The possible importance of matching of the conformational fluctuations of associating proteins to minimize the potential energy and entropy loss in protein protein interactions has been examined elsewhere. This dynamic matching may be responsible for the stability of the immune association complex but the antibody has two functional domains that may also participate. Such matching by modulating the free-energy change in association provides specificity as well as a device for transmission of information between proteins through modulation of matrix properties. In this way proteins are coupled into larger cooperative units that make higher-order biological behavior possible. The four-chain hemoglobins as the classical example of allosteric linkage illustrate all these features at a relatively simple level. Allosteric coupling among oxygen-binding sites is through sharing conformation changes.

The streptococcus G protein binds to antibodies but by surface-to surface contact and thus perhaps with little domain closure since the two functional domains are connected by several of the short H-bonds reported by Cornilescu et al.. The reasons for it high C-2 symmetry have general importance but are not yet known.

The selection of residues makes all this possible but the degree to which we must understand the subtleties in this selection so as for example to make proteomics a true science seems a daunting task. We have found that the positioning of knots and thus the balancing of the functional domain pairs depends only on the numbers of inter-knot residues and at that relatively simple

level through comparisons of enzymes but the difficulty of the larger goal is estimated from the very small fraction of total residue arrangements found suitable for evolution. In principle we must duplicate the same tedious search by which at least one successful protein has been found. And to do so requires tests of very high precision to measure and correlate the effects of residue change, tests yet to be developed at the required precision level. Atomic free volumes are the raw data but none of the currently popular structural methodologies have the necessary power. Even if research is concentrated on a single protein production testing of mutants is a task not much different in magnitude from determining the residue sequences of genomes. Coordinates from x-ray-diffraction and nmr methods at least as now developed lack the necessary precision and supercomputer results are no better than the potential functions on which they are based. Although varieties of spectroscopic methods will amplify their power only the temperature factors from diffraction studies can provide useful measures of free volumes and their coordinate values at a useful level.