Chapter 14 Effects of independent variables on physiological function.

The separation of total free-energy change in melting by the ad hoc procedure of Murphy et al as confirmed in the previous section makes it possible not only to determine the forward rate constants for melting from equilibrium melting data but provides a simple way to estimate the effects of independent variables such as pH and solvent composition on knot disruption to form the single transition state in two-state melting but and on the step from bubble to the latter state. This is another benefit at least for mesophiles of the very narrow range of allowed constructions. Roughly speaking there are two limiting ways that such variables can alter native states and thus in the case of enzymes the catalytic parameters. The first is estimated by the change in stress applied by matrices to knots. The second is the change in bubbles. If the ratio of the standard enthalpy change to standard entropy change effected by alteration in a variable is 354K within small error, the effect is only on the knot and thus on the native state and measures the change in stress. The pH dependence of the equilibrium melting of two varieties of the G protein from streptococcus given in Table @ illustrates this behavior. At high pH where the standard enthalpy and entropy changes were found to be maximal the test ratio is very close to 354K. At increasing acidity it decreases indicated a pH effect on bubble stability, one never very large with these proteins.

Effects of pH dependencies of proteins in terms of stoichiometric relationships interpreted to mean alter pKa values of acid and base groups on melting alter the protein charge. It is unlikely that this assumption is correct in detail since the entire charge atmosphere cooperates in changing the activity coefficient of the protein. Stoesz and Lumry reexamined the pH dependence of the active-inactive process of chymotrypsin in native states found an apparent pKa near pH 7, close to the isoelectric point, although there are no ionization

processes of the protein's functional groups at this pH. This behavior has been reported chymotrypsin and more recently for a number of other proteins. There is little need to interpret such results as ionization processes of the bubble state. Indeed the bubble is an osmometer dependent only on the activity of water.

A pH titration will see any ionization processes of the functional groups but is overlaid by the indirect effects through matrix change. Idea of total proton gain or loss from protein in the process is ok but not as reflection of ionization from single determinable protein acid or base groups.

Solvent composition is illustrated by urea. Up to at least 8 M the test ratio on the trypsin proteases, elastase and ribonuclease A is 354K within small errors. As with pH the effect is due to change in the surface free energy and probably directly due to removal of the Kuntz "non-freezing water" , a major stabilizer of the native state at least at the subzero temperatures where the effect has been studied. It is possible that there is actual binding of urea by the protein, a classical explanation of its effect, but there is no supporting evidence and binding is not necessary.

Pohl found that changes the position along his compensation plot depended on the number of amino-acid residues. His cited only a few example but the analysis by Murphy, Privalov and Gill generalized the effect by ten examples.

Recall that Morozov and Morozov by application of anisotopic mechanical force showed that the Youngs Modulus of several protein fell to a very low value at 354K consistent with the fact derived from Pohl's compensation plot the stress from the matrices is just equal to the mechanical strength of the knots at this temperature. Their experiments established that the large effects of temperature and pressure are mediated through changes in matrices usually small expansions increasing stress on the knots but also

weakening the secondary binding of the matrices. Pressure effects might be explained in the same way but increased pressure drives water into matrices with several kinds of change. Both temperature and pressure change the expansion-contraction process but in also can favor denaturation of only one of the functional domains of multi-domain proteins. The latter result can be produced by drying as shown by Battistel and Bianchi for example. A difference in sensitivity of the functional domains to independent variables adds to the difficulty in explaining the changes they produce. Mobile-globulin production is a very common example of little physiological importance. Larger effects are produced by some of the other variables because although free energy of formation of matrices and knots is not much changes the matrix expansion-contraction process can receive significant alteration in catalytic parameters since its action is focused on that process.

Much of the above is familiar as interactions of charge cloud usually attributed to interaction with ionized groups of functional residues. Solvent effects take us to Timasheff et all with some comparisons of structure-breakers and structure makers.

Structure breaker cosolvents weaken by breaking up Kuntz B shell but also as concentration increases they shift bubble toward true unfolding. Structure maker increase surface free energy again by reducing water activity but they tend to stabilized both native states and bubbles. There is little tendency toward random-coil states.

Small amphiphiles including methanol, ethanol and acetic acid form mixed micelles with proteins destroying native and bubble states and eventually so solubilizing the polypeptide that random-coil states are produced. Brandts and coworkers selectively denatured one of the two functional domains of ribonuclease A by increasing methanol concentration. Methanol completely

converts bulk water to a clathrate state resembling the lower-density state of pure water but like other small alkanols it forms a sort of labile mixed micelle with denatured parts of proteins.

Larger alkanols and amines form micelles at very low concentrations: 0.05 mole fraction for t-butanol. These deplete the percentages of pure-water states At cosolvent mole fractions rarely larger than 0.2 both macrostates of pure water are eliminated. Since a large part of solution chemistry has been carried out in such mixtures, much reexamination of older data has become necessary. .