Chapter 6 volume 2 Protein substructures

Volume ` 1 of the Protein Primer contains descriptive material much of which includes detail about the two substructures discovered by Gregory and coworkers using proton-exchange data. The protein surface with its hydration forms the third substructure for which little detail was available for that volume. The new facts about pure water and aqueous mixtures described in chapters 4 and 5 have been fully confirmed thus undermining most of the knowledge of proteins extracted using classical ideas. And some important newer ideas about protein structure and function also now require revision to eliminate previous errors in the descriptions of the three major substructures. In turn these are required because of rather dramatic new descriptions of water and aqueous mixtures. Particularly drastic errors in attributing to knots characteristics now known to be general consequences of hydration processes.

Pohl first observed that the heat capacity of activation for melting rates of proteins is zero within experimental errors. According to compensation theory in chapter 2 of this volume the activation enthalpy and entropy pairs will demonstrate liner enthalpy-entropy compensation behavior. This is the first of several heat-capacity problems evolution has had to circumvent to find proteins and protein properties. In this example the compensation temperature is 354K and on the basis of proton-exchznge rate data could be attributed to the knot substructures of Gregory and Lumry which demonstrate the same compensation behavior. One useful consequence has been the ability to attribute several common protein processes to knots using their compensation temperatures of 354K for this identification. However as discussed in the previous two chapters, that temperature due entirely to water and to knots only because knots as they

depend on hydration displace that unique temperature now known to be the temperature at which the enthalpy change in the clathrate formation generally called hydrophobic hydration changes from negative to positive values. To see how this destroys protein folded stability we must take into account the thermodynamic contributions to that stability from all three substructures.

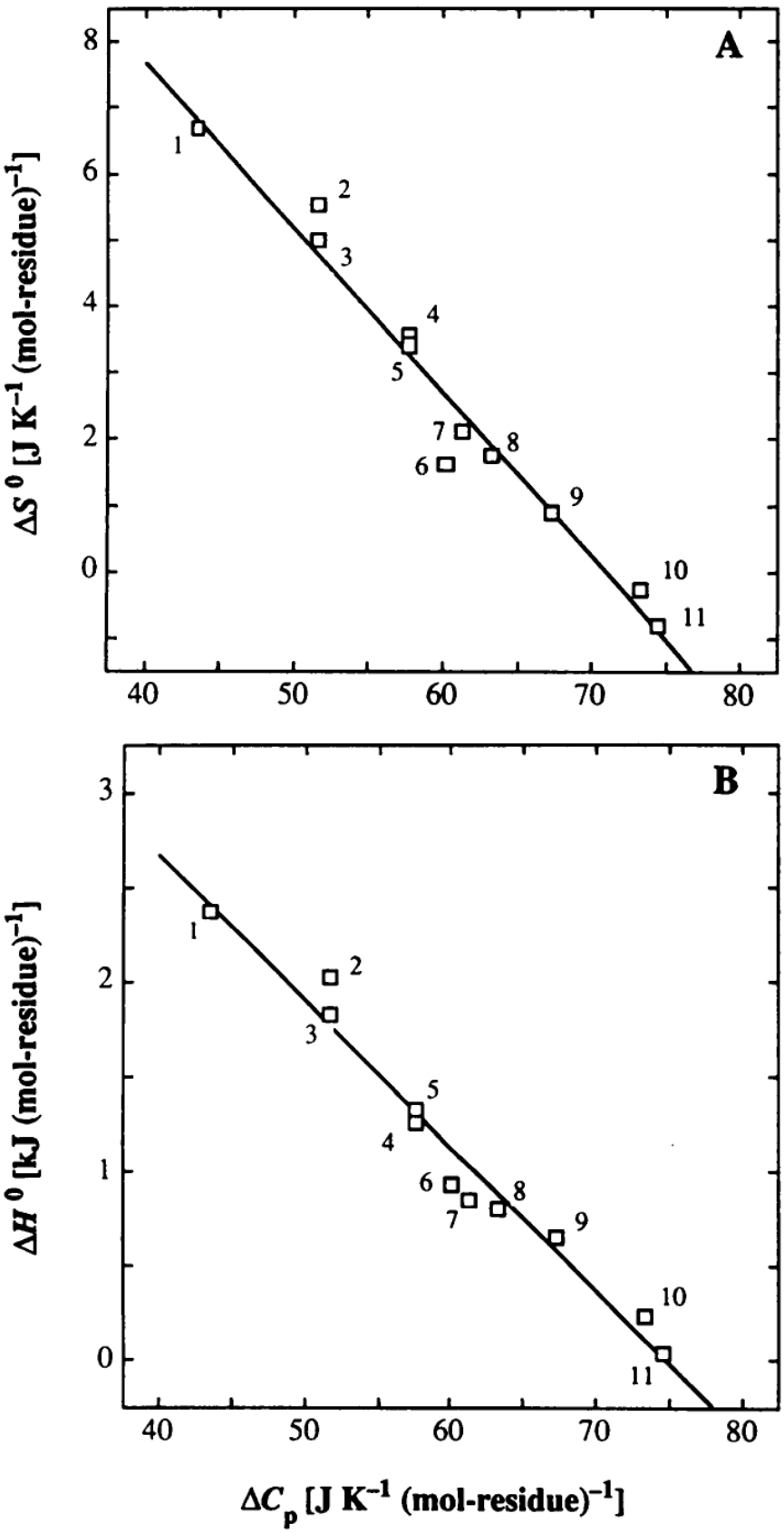
Murphy, Privalov and Gill in 1990 separated the overall process of protein melting .strictly on the basis of their heat-capacity contributions into a sequence of two steps in which the total standard heat capacity change was assigned to the second step in which the highly restricted conformation of the native protein decays into a motile bubble. They attributed the heat capaicity change to the interaction with water now possible because of the expansion and disruption of the native conformation taking place in the second step. That deduction is probably partially correct but neglects the increase in statistical entropy of the polypeptide. The intrinsic heat capacity of the native species is almost entirely due to its hydration rather than conformation expansion and it has been found as with the G protein from streptococcus that the standard heat capacity change in melting is independent of temperature not the expectation for hydration alone. It should be noted again that in the absence of denaturing cosolvents of which urea is the most studied the normal product of thermal denaturation is only slightly larger in volume than the native species. Structure-making cosolvents of which sulfate ion and PEG are now the most used in protein crystallization, compress bubbles to further limit motility. (cf. Gregory using glycerol as structure maker.). What has escaped notice is the reversal of the latter behavior that occurs at 354K. Any contribution to folded stability due to favorable interaction with solvent goes to zero at 354K thus becoming destabilizing at higher temperatures. If that contribution domiunates

the sum of the contributions from the other two substructures: knots and matrices, stability is a minimum at 354K. Thus Morozov and Morozov found that youngs modulus for deformation by application of force to protein crystals vanishes with two degrees of that temperature..

Gregory analyzed the report in Science by Murphy, Privalov and Gill to show that the contribution from what those authors called the conformation part of the total free-energy change in unfolding from native to thermally denatured protein became destabilizing at 354K The authors did not attribute this to hydration change or change in the properties of water but considered it a property of the protein conformation. In contrast they analyzed the second step from transition state to native species in terms of the hydrophobic hydration heat capacity arising from interaction of denatured species with solvent. This has been the popular opinion but thermodynamic data for interaction of water with structure makes such as ethanol, ethylene glycol and PEG show a revision is necessary because the characteristic temperature of 354K is not a property of protein conformation but instead a property of water. Gregory's analysis makes this clear but in the subsequent years from his analysis, from 1996, his clarification has failed to receive attention. This chapter building on chapters 4 and 5 of volume 2 is intended to repeat and enhance Gregory's analysis of the paper by Murphy et al. (Gregory's paper from "Water in foods" (in "The properties of water in foods", ed. D. Reid, ISOPOW 6, Blackie , London 1998 page 57, http://kent.edu.chemistry//moleimage reproduced in the Utilities folder on the Protein Primer web site. . That paper and chapter 2 by Gregory from "Protein-solvent interactions", ed. R. Gregory, Dekker, New York 1998 , 191 are the most complete compilations of experimental information about matrix contraction now available.)

There is a more general significance of the dependence of folded stability on hydration of importance in explaining why stability is sometimes retained up to slightly higher temperatures. When the hydration contribution goes to zero, the sum of the contributions from the knot and matrix substructures need not sum to zero. That they usually do undoubtedly has importance despite the fact that that is not obvious Bur relaxing the dependence of stabilized on knots previously assumed, the melting point can be adjusted by addition of disulfide cross links and other modifications of residue composition.. That is what makers thermophilic and hyperthermophilic enzymes as welll as those functioning most efficiently at sub ambient temperatures possible. (vide infra on exrtremophles). In any event when the melting temperature is 354K or very close to that the contribution of the first step to the overall free energy change is just equal to that for the second step but the latter is negative and the former positive.

Privalov found that the per residue standard heat-capacity change in melting disappears at abut 384K which suggests a dependence on water similar or identical with the loss of "hydrophobic hydration" near 354K and he uses that phenomenon to normalize the second step in denaturation Murphy, Privalov and Gill using denaturation data collected by Makhatadze and Privalov tound considerable phenomenological simplicity when the data are compared per mole of residue regardless of the protein. Their figures are copied again here


A
B
ΔS^0 [J K^{-1} (mol-residue)$^{-1}$]
ΔH^0 [kJ (mol-residue)$^{-1}$]
ΔC_p [J K^{-1} (mol-residue)$^{-1}$]
1
2
3
4
5
6
7
8
9
10
11

The proteins are: 1 ribonuclease A, 2 Hen eggwhite lysozyme, 3 fragment K4 of plasminogen,4 α-chymotrypsin, 5 β-trypsin, 6 papain, 7 *Stapylococcus* nuclease, 8 carbonic anhydrase , 9 cytochrome c, 10 pepsinogen, 11 myoglobin. Data adjusted to 298K. Taken from Murphy, Privalov and Gill, Science 247 (1990) 559.

The two steps of ovrall thermal denaturation are easily separated in these figures (Lumry, Biophysical Chemistry, @) by the common interecept at which since the first step has zero heat-capacity change, all proteins share that step in the same way and there is no significant heat-capacity change because the transition state is formed from native state only by potential energy change, what Frank called a "motive change".. This was verified by the intercept values of standard enthalpy and entropy change per mole of residues proving equal to the values extracted from Pohl's compensation plot for the rate of melting. That result is a consequence of the fact that the melting rate was found to be limited by the first step. That remarkable evolutionary consequence was necessary to form the knot substructures which establish the genetic and thermodynamic stability properties of the entire protein. Furthermore the identification of the melting rate with the first of the two thermodynamic steps of unfolding makes it possible to compute the activation enthalpy and entropy for the melting rate process as well as the enthalpy and entropy contributions for the two thermodynamic steps of melting. The latter are most reliable at the temperature at which the contribution from hydration is zero. but they are never very poor with normal hydration (vide infra).

Aside from the common intercepts of the two plots the data all per mole of residue describe indirectly the variations in construction over a wide range of

protein families and some variation in type. Note in particular that the variations with heat capacity change per residue lie on common lines linear within small errors. The variations arise only from variations in the total number of residues and not from sequence or atom free-volume arrangements. This reveals another remarkable consequence of successful evolution. (Lumry, Parsimony in protein evolution, Biophysical Chemistry 101-102 (2002) 93) The lines of those figures is the best quantitative description of protein variation available at this time quite in contrast the pictorial characteristic of diffraction results. for atom positions but in useful accord with knot palindrome patterns. Note for example the position of myoglobin, a soft protein with very weak knots characteristic of the hemoglobin family and very different from the enzymes.

The figures aside from the intercepts apply to the second step of denaturation, explansion from the compact single transition state as the fine conformational details of the native state give way to the slightly expanded but highly motile bubble product. The *knots are untied* so melting can take place.. The melting rate process gives essential information about the knots and the reverse rate process from bubble to the single transition state gives information about the bubble but only meager information about the third substructure of the normal protein called the matrix because of its adaptability by residue change to support a new physiological function. Tying the knot forces the matrix into the conformation necessary to support such as function. Thus for enzyme catalysis, the functional matrix conformations are expanded from a most-stable conformation to raise their potential energy, In chapter 7 the details of the *nutcracker* mechanism made possible by contraction on triggering by substrate followed after product formation return to the initial expanded state. This all depends on rearrangement of atom free volumes closely resembling a first-order

phase transition. In fact the nutcracker is a good model for the process except for the return to initial state since the work required for that in the nutcracker must be provided from an external source. Any nutcracker concept satisfying biological needs must have a built-in mechanism for return and for enzymes that had been a major requirement for successful evolution explaining why three substructures have had to be fund. Chapter 9 provides an account of the enzyme evolution so it is necessary to describe briefly the cooperativity of the substructures and how it has been adjusted to effect perfect reversibility in enzyme nutcrackers.

Matrices of which there are two in any enzyme are dynamically matched through free-volume design and close association in C-2 rotational symmertry contract to to a closed conformation in which by their contraction the potential energy is moved to the reaction assembly of substrate and direct chemically functional groups of the protein. The net free energy change is small but the potential energy change quite large. Thus for example 15 Kcal of potential energy transfer cn produce 10 orders of magnitude in rate enhancement. The compression destabilizing the pretransition state if relieved by matrix expansion once product leaves and product formation must be spontaneous. What remains for completion is to push the matrices back up their contraction hill not a big undertaking in terms of the work required but nevertheless one required to prevent destruction of the enzyme. This is why the interface has to have had a lot of attention in evolution. Not only must the free energy change in the contraction-expansion process be zero, buy also the net enthalpy change and the entropy changes in the enzyme itself must each be zero. over the entire path. That requires the heat –capacity change to be zero so the process is very unusual and its properties must have been very slowly developed not only in any

one enzyme but apparently in every enzyme. None of the mechanism are in any way similar to those of familiar small-molecule chemistry and although thermodynamics provides the requirements that have made enzyme evolution a most unlikely success, none of the mechanism appear to profit from thermodynamics.

The interface substructure is quantitatively complicated because so many sources of its adjustment are utilized none of which have useful models at this time. Sulfate ion as a major structure maker is frequently found 5e tightly bound thus changing surface hydration. Ionization of acid and base groups has a similar importance because of the changes in the clathrating water and so on. The elegant studies of such changes particularly by Timasheff and coworkers described in chapters 4 and 5 of this volume provide the basis for fuller conversion of any map of the interface into the thermodynamic parameters to rationalize protein structure. That task it formidable because any detail makes some quantitative contribution and each detail of the interface is the product of long adaptation. Such details of construction that have minor consequences and might be labeled passive may not be. Recall from chapters 5 and 9 of this volume that all participants in a common water phase are in competition thus forming a complex linkage system. Contacts among macromolecules at surfaces provide the device for higher evolution.