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Hydrophobicity—getting into hot water

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Abstract

In his famous 1959 review, Walter Kauzmann clarified important features of the thermodynamic stabilities of proteins. The hydrophobic effect is recognised as an important contributor to the stability of proteins and an important determinant of their structural patterns. As generally understood, it depends on the unusual properties of cold water and its interactions with nonpolar solutes. Here we comment on the relationship between this paradigm and the stabilities and structures of proteins from thermophilic organisms.

Keywords: Protein structure; Hydrophobicity; Solvent-solute interaction; Thermophilic proteins

My graduate studies began shortly after two landmarks in the field of protein structure: the publication of Walter Kauzmann's 1959 review in advances in protein chemistry [1], and the solution of the first crystal structures of proteins by John Kendrew and Max Perutz (see, e.g. [2]). What better impetus could one ask for? Nevertheless, although I have worked on protein structure during most of my career, my most intimate interaction with Walter Kauzmann occurred several years later, when I was writing a textbook of physical chemistry [3]. In their wisdom, the publishers asked Walter to read and comment on the book, and it was my very great privilege that he agreed. He took the manuscript (on paper in those days) with him to Nova Scotia that summer, and in the fall I received it back, drenched in red ink. That is when I learned physical chemistry, and also when I learned how to write. My dedication of the book to Walter testifies to my gratitude for this and many other kindnesses, and his acceptance of it leads me to think that he felt he got through to me, in at least some respects. It is very rare for the combination of creativity in research and teaching to be combined to so great an extent in one individual, and I joined his other colleagues in honouring him.

With the availability of protein structures, first from X-ray crystallography and subsequently from NMR spectroscopy, the field developed in two directions. Measurements of thermodynamic properties of proteins in solution continued to give data on the factors stabilizing native structures, notably in the hands of Privalov and his collaborators (see, for instance, [4]). The structure of aqueous solutions and of water itself also continued to interest Walter for some years to come [5] (until he wrote me that he had discovered that liquid SiO_2 was

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even more interesting—in that letter he also mentioned that his interest in proteins had become limited to things to eat, like cheese or meat).

Another approach to protein structure emerged from the laboratory of Fred Richards at Yale [6] and his coworkers, notably Cyrus Chothia [7]. This approach involved geometric analysis of the coordinate sets produced by the crystallographers. It has developed with the growth in the number of solved protein structures and in the power of computers.

It should be emphasised that there have always been many contacts between these two approaches. For instance, Oleg Ptitsyn observed that helices in proteins often have hydrophobic faces that point into the protein interior and hydrophilic faces that point into the solvent. These ideas led to searches for patterns of periodicity in hydrophobicity plots, and Schiffer and Edmundson's helical wheels. Cyrus Chothia calibrated the thermodynamic correlates of buried surface in terms of a 25 cal/Å^2 contribution to the free energy of stabilisation of proteins from the buried area calculated from the coordinates of a structure [8].

We now understand, at least qualitatively, the important contributions to the stabilisation of the native states of proteins [9]. The major effects include saturation of hydrogen bonding potential by buried polar groups, primarily through formation of secondary structure, relatively few deviations from good stereochemistry, dense packing of residues in protein interiors, electrostatics, and of course burial of hydrophobic surface.

We cannot calculate stabilisation energies of native protein structures quantitatively from first principles, because they are small differences between large numbers.

Many geometric studies of proteins have emphasised the nature of the internal structural paradigms, such as standard secondary and supersecondary structures, and geometric regularities in the packing of secondary structures [7]. Others treat evolutionary aspects of structural change and sequence-structure relationships [2]. It is true that thermodynamics does not speak directly about structural details—its strength is that it is independent of them. Nor can thermodynamic principles say anything about the historical accidents that appear to govern in large measure the observed repertoire of protein folding patterns. Thermodynamics provides the rules of the game, but not the blow-by-blow account of any particular match.

Let us consider one area in which thermodynamics may contribute to discussions within the structure/evolution approach. Many biologists (no longer the term of disdain that it was when I was getting started) have suggested that the first life forms resembled contemporary thermophiles [10]. Thermophiles are organisms found in natural habitats in which the temperature is at or even above the boiling point of water. For instance, *Sulfolobus acidocaldarius* was found in a hot, acidic spring in Yellowstone National Park and has an optimal growth temperature of 87 °C; *Pyrolobus fumarii* grows on the walls of marine hydrothermal vents and is happiest growing at 105 °C.

Arguments that thermophilic organisms resemble primeval life forms include geological evidence that their preferred conditions of growth are typical of what the earth looked like when life began, and that in a comprehensive evolutionary tree of life the archaea, the general group to which most thermophiles belong, take their place near the root. It has also been suggested that early organisms might have made use of chemicals formed by interactions of hot water and minerals.

Proteins isolated from thermophilic organisms show that their adaptation to high temperature is inherent in the structures of proteins and not in some complex higher-order structural organisation [11,12]. Therefore, from the point of view of the physical chemist thermostable proteins raise interesting questions. If the structures of ordinary proteins—from ordinary organisms, not thermophiles; that is, those that denature at reasonable temperatures (at which Walter could easily cook them)—are governed by the thermodynamics of water at low temperatures, how are the structures and thermodynamics of thermostable proteins related? In particular, what happens to the hydrophobic effect at high temperatures?

Most colloquial understanding of the hydrophobic effect and its significance for protein stability is based on the properties of water, aqueous solutions, and proteins at temperatures roughly in the range 273–310 K [13,14]. The basic idea is that

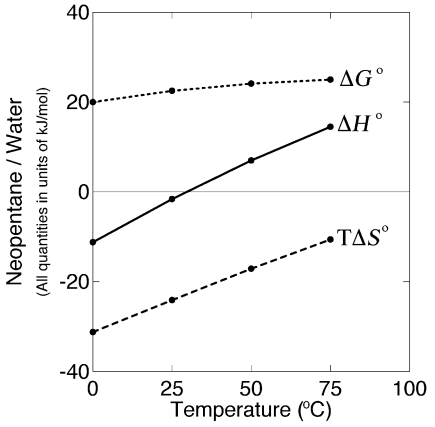

Fig. 1. Temperature dependence of ΔH°, $T\Delta S^\circ$ and ΔG° of transfer of neopentane from pure liquid state into water. Data of Lee [18]. For this solute, extrapolation suggests that ΔS would turn positive at a temperature of approximately 122 °C.

the sequestering of hydrophobic sidechains in protein interiors removes an unfavourable entropic effect arising from the formation of ordered water aggregates around nonpolar solutes. That is, although water is itself a relatively structured liquid, it is even more structured around non-polar solutes than in the pure liquid state, as first suggested by Frank and Evans [15].

The interactions of nonpolar solutes and water have been measured over the entire accessible range of temperatures. At room temperatures, we have the paradigm that the low solubility of nonpolar molecules in water is the result of a large unfavourable entropy change arising from the additional ordering of water around solute particles; in contrast $|\Delta H| \ll |T\Delta S|$. This produces the conventional textbook hydrophobic effect and explains the clustering of nonpolar sidechains in polar interiors. At high temperatures, nonpolar molecules still have very low water solubilities but thermodynamically the situation is reversed. The low solubility of nonpolar molecules in hot water is largely an enthalpic effect: $|\Delta H| \gg |T\Delta S|$ (Fig. 1).

What about the structures of thermostable proteins? Many examples are known of sets of homologous proteins from thermophilic and ordinary organisms. Their structures are generally very similar; indeed if you were given the sequence and structure of two such homologues, it would be difficult to decide which was the thermostable and which the nonthermostable molecule (but not impossible [16]). It has also been shown that by careful design, thermostability of proteins can be enhanced by a few crucial substitutions [17].

Studies of homologous pairs of thermostable proteins and nonthermostable protein structures have turned up some subtle general differences in their structures. Thermostable proteins tend to have more salt bridges, and somewhat tighter packing. Given the greater weighting of the entropy term in ΔG at higher T, sidechains with fewer internal rotational degrees of freedom are preferred by thermophiles (the argument is that this would reduce the entropy of the denatured state) or, in other words, freezing buried sidechain conformations to form the native state costs less.

Let us focus on the question: why do thermostable proteins appear to obey the same basic structural principles as those of nonthermostable ones? If you stopped me on the street and asked me this question, I would have given you without thinking the 'low-temperature chauvinist's' answer that proteins evolved first at approximately 37 °C, in the regime in which conventional hydrophobicity thermodynamics applies, that the form of their structures is in large part a consequence of this thermodynamic regime, and that thermostable proteins evolved from them by cosmetic changes including addition of salt bridges, improved packing, etc. This is inconsistent with the idea that primeval organisms resembled contemporary thermophiles as hypothesised by the biologists.

Alternatively, what is implied by the idea that proteins originally evolved at high temperatures, and organisms subsequently adapted to low-temperature habitats? From the structural point of view, there might be less difference than one might expect. Walter wrote in his 1959 review [1]: 'Since the nonpolar sidechains have a low affinity for water, those polypeptide chain configurations in proteins which bring large numbers of these groups into contact with each other, and hence to remove them from the aqueous phase, will be more stable than other configurations, other things being equal.' This statement is true both in protein

structures stable only at low temperatures, where the low solubility of nonpolar molecules is entropy-driven, and in protein structures stable at high temperatures, where it is to a greater extent enthalpy-driven.

It is therefore not at all inconceivable that proteins evolved initially at high temperature (in total ignorance of the unusual entropy-driven hydrophobic effect in water at low temperature) and made a smooth transition to adapt to low temperature conditions. Indeed, if thermophilic life forms developed intelligence and were given the text of Walter's 1959 article, they would have read without surprise that: 'The unusual heat, entropy and volume effects described above become less unusual as the temperature is raised to 50 or 100 °C; ΔH becomes less negative, and ΔS_u, perforce, also becomes less negative.'

Indeed some proteins are stable over a wide range of temperature. In going from low to high temperature, the burial of hydrophobic surface changes from making a stabilizing entropic contribution to make a stabilizing enthalpic contribution. My conclusion is that although life as we know it depends on the thermodynamics of aqueous solutions, the unusual properties of cold water are not essential. Given the physical chemist's classic maxim, 'like dissolves like,' provided other solvent–solute pairs could provide the same degree of discrimination in solubility that water does between polar and nonpolar solutes, life based on alternative solvents might well be conceivable. It would be nice if there were some weak interaction analogous to hydrogen bonding, but maybe even this is not absolutely essential. Would it not be ironic—thinking as always of Walter's point of view—if someone discovered a planet with a life form containing condensed biopolymers based on liquid SiO_2?

Acknowledgments

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