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Thermodynamics of enzymes in unusual environments

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Abstract

This article describes recent progress in the study of the thermodynamic aspects of enzyme function in unusual environments. The systems considered include enzymes immobilised onto solid supports, incorporated in reverse micelles and suspended in low-water solvents. We also summarise advances in direct thermodynamic methods applicable to the study of such enzyme systems.

INTRODUCTION

In the three decades since the 1950s, the general field of biophysical chemistry has greatly matured, especially in the specific area of enzyme function. Three lines of inquiry have led to this advance. Firstly, the structural studies of enzymes and other proteins have provided a large data base for the primary, secondary and tertiary structures of enzymes and enzyme–ligand complexes. Secondly, a theoretical understanding of enzyme thermodynamic stability and structural dynamics has emerged over this period. Finally, and perhaps most importantly, contemporary biophysical instrumentation can provide quantitative answers to many, if not all, of the questions that can be asked about enzyme mechanisms.

This improved overall understanding permits the consideration of questions that are much more detailed or complex than those which could have been profitably explored even a few years ago. One such area of inquiry concerns the possible activity of enzymes in unusual environments, a topic first raised some 80 years ago [1]. Such systems might be taken to include enzymes immobilised onto solid supports, incorporated in reverse micelles or present in low-water solvents.

There are a variety of reasons why such activity should be of both theoretical and practical importance. Firstly, unusual environments offer the possibility of improved enzyme stability. Globular proteins in solution have been shown to have maximum stability near room temperature [2];

however, even under these conditions, their stabilisation free energies are sufficiently small that a finite fraction of the molecules are unfolded. While this lability is associated with function, it doubtless provides access to paths leading to protein denaturation over time. The possibility of accessing enzyme function in the solid state where stability is enhanced is obviously attractive. Secondly, modification of the solvent might lead to enhanced or usefully modified biological activity. For example, changes in pH, ionic strength, or the presence of polar cosolvents might affect the equilibrium thermodynamics or uncatalysed rate of a reaction, or might alter enzyme kinetics or specificity. In addition, factors such as immobilisation might stabilise enzymes against conditions which would be denaturing, and thus not useful, in aqueous solution. Thirdly, Klibanov [3] has pointed out that water is generally a less than ideal solvent for organic reactions. This is especially true of reactions that are conducted commercially. The possibility of conducting enzyme-catalysed reactions in organic solvents where non-polar substrates are highly soluble, where hydrolysis does not occur, and where solvent and products can be easily separated has obvious attractions. Fourthly, enzymes are expensive catalysts and techniques which facilitate their retention in processes are economically important. Thus methods that permit enzymes to function in a phase separate from that preferred by the reaction products are essential for many commercial applications. Fifthly, as well as denaturation, enzymes in solution are subject to a variety of degradative processes such as bacterial growth, proteolysis, etc. Such processes would be retarded or eliminated in the above environments. Finally, unusual environments might permit the use of structurally modified enzymes which would be inactive in aqueous solution. Such materials might range from proteins modified relatively non-specifically by chemical treatment to "designer" enzymes produced by site-directed mutagenesis.

In the following sections of this article, we summarise selected biophysical studies of enzymes in the three types of environments described above. We then discuss recent progress in direct thermodynamic methods applicable to the study of such enzyme systems.

IMMOBILISED ENZYMES

Enzyme immobilisation can be accomplished in three principal ways: covalent attachment to a solid support; adsorption to surfaces; and entrapment in gels or similar matrices. It is the first of these methods that has found the widest application and is best characterised thermodynamically.

Immobilisation exerts important effects on the enzymes involved. In the first place, protein conformational changes can be expected to follow immobilisation, especially when this involves covalent attachment to solid supports. Such conformational changes, in turn, alter enzyme stability

towards changes in pH, temperature, solvent, etc. Moreover, the effective solvent environment of the protein is altered by the support, especially if this matrix is charged. These factors can lead to various changes in enzyme function, including shifted pH optima, altered reaction rates and changes in substrate specificity.

One particularly important consequence of enzyme immobilisation is the enhanced protein stability frequently encountered. This can arise either from enhanced thermal stability at equilibrium or from decreased rates of irreversible denaturation. Immobilisation may help preserve the functional equilibrium conformation of the protein or it may contribute an unfavourable conformational entropy to unfolding pathways, thus inhibiting denaturation kinetically. Clearly, much more information is needed on the nature and magnitudes of non-covalent forces stabilising immobilised enzymes.

The study of immobilised enzymes vis a vis the corresponding enzymes free in solution has been approached by a variety of methods including fluorescence spectroscopy [4], electron spin resonance [5], hydrogen-exchange kinetics [6], infrared spectroscopy [7] and specific antibodies [8]. Nonetheless these systems pose special challenges for biophysical study. In particular, a suspension of immobilised enzymes is a highly heterogeneous system which resists most forms of optical examination. Calorimetric techniques have therefore proven extremely valuable in elucidating the kinetic and thermodynamic aspects of the function of these proteins.

Isothermal batch calorimetry has been the method of choice for reaction studies. This is because the suspensions such immobilised enzymes provide are ill-suited to flow calorimeters. Particle settling, clogging of plumbing, and large and erratic heats of viscous flow all result from the introduction of enzyme-support slurries into flow systems. Batch methods eliminate these problems; moreover, titration calorimeters afford a full protein–ligand titration curve from a single sample. Studies of protein–ligand reactions yield information on ligand binding affinities and on thermodynamic linkages between solution conditions and the reactions of interest. Moreover, thermal titrations of immobilised enzymes with appropriate inhibitors provide an excellent method for assessing the integrity and functionality of enzyme sites. Finally, enthalpies and other thermodynamic parameters of ligand binding to immobilised enzymes reflect the effects of immobilisation, support environment, etc.

Differential scanning calorimetry (DSC) provides direct information on the effects of immobilisation on protein stability. Measurements of ΔG, ΔH, ΔS and ΔC_p for unfolding of the dissolved and immobilised enzymes gives insight into both the magnitudes and the nature of the forces introduced by immobilisation.

A particularly comprehensive study on the reaction thermodynamics of an immobilised enzyme system is that of Battistel et al. [9]. In this work,

ribonuclease A was attached to aminopropyl–CPC–silica beads by glutaraldehyde crosslinking. The soluble and immobilised enzymes were then compared with each other and with valeraldehyde-derivatised ribonuclease with respect to their binding of 3′-cytidine monophosphate (3′-CMP) using both calorimetric and spectrophotometric methods. Ribonuclease immobilisation had little effect on the binding of this inhibitor, the binding constants being 5.1×10^4, 8.5×10^4 and 7.6×10^4 M^{-1} for the soluble, valeraldehyde-derivatised and immobilised enzymes, respectively. Both the soluble and immobilised enzymes had comparable 3′-CMP binding enthalpies of 38.7 ± 0.8 and 35.6 ± 1.0 kJ mol^{-1}, respectively. Valeraldehyde-derivatised ribonuclease exhibited an appreciably higher 3′-CMP binding enthalpy of 49.5 ± 1.0 kJ mol^{-1}. The number of binding sites per immobilised enzyme molecule was 0.97 ± 0.05, the same as the free enzyme.

DSC measurements of ribonuclease melting were performed in connection with the above study. The soluble and valeraldehyde-derivatised enzymes melted with a single transition near 65 °C. In contrast, the immobilised enzyme showed two transitions at 62 and 66 °C. For all transitions of the three proteins in the pH range 3–7, the ratio of the calorimetric enthalpy ΔH_{cal} to the van't Hoff enthalpy ΔH_{vH} was approximately one, indicating two-state transitions in all cases. Battistel et al. [10] suggest that the higher temperature transition exhibited by the immobilised enzyme arises from stabilisation by the support matrix of the N-terminal domain by which the enzyme is presumably attached.

The preceding study involved proteins acylated at about 1.7 NH_2 groups per molecule. Koch-Schmidt and Mosbach [11] have examined the consequences of variable numbers of protein immobilisation sites for ribonuclease. They show that the ribonuclease activity decreases from 60% to 15% of that of the soluble enzyme as the number of attachment points to a Sepharose matrix increases from 1 to 8. DSC shows an unperturbed protein melting transition with 1 attachment point, but the T_m is elevated by 5 °C with 8 attachment points. Similarly, the ΔH of the melting process is unaffected for one attachment point but falls for more than one. Recovery of enzymic activity after melting is about 80% for ribonuclease immobilised by 1 or 2 attachment sites but falls for greater extents of immobilisation.

Ichijo et al. [12] were able to immobilise invertase on poly(vinyl alcohol) (PVA) fibrous support and found, by DSC measurements, that the temperatures of thermal denaturation were 77 °C and 72 °C for the immobilised and free enzymes, respectively. Although the temperature of denaturation depended somewhat on the enzyme concentration, the authors concluded that a stabilisation of the protein structure occurred after immobilisation due to favourable interactions between the protein and the polyhydric structure of PVA.

ENZYMES IN REVERSE MICELLES

Reverse micelles are aggregates formed by certain surfactants in non-polar solvents. The apolar parts of these detergents are directed outward into the solvent while the polar head groups point inward to produce a polar core which will enclose water. Reverse micelles thus represent an enzyme environment especially attractive for organic synthesis. For example, reactants and products which are soluble with difficulty in water often dissolve in the organic solvent of a reverse micellar system. Diffusion of the small molecules into and out of enzyme-containing reverse micelles is rapid and permits efficient access to the catalytic capability of the protein.

A typical system that has been studied extensively, consists of isooctane–AOT–water; AOT (Aerosol®-OT) is bis(2-ethylhexyl)sodium sulphosuccinate. An important parameter of this and other reverse micelle systems is the water–surfactant molar ratio, w_0. For values of w_0 in the range 5–50, the physical size of the aqueous region of a reverse micelle is 10–80 Å (radius). Given the fairly small size of these water pools, it is not surprising that their water molecules exhibit properties considerably different from those of bulk water. Such differences include lowered freezing point [13], activity [14], local dielectric constant [15], NMR proton chemical shift [16] and IR OH-stretching frequencies [17].

This behaviour arises from interactions of micellar water with the AOT sulphonate group and its counter ion, and a principal question about these systems is the amount of water so bound. This matter has been examined in DSC studies of reverse micellar dispersions containing varying amounts of detergent and water. The heat of ice melting at or below 0 °C was measured and the apparent enthalpy of ice melting decreased linearly with decreasing w_0, having a zero value at $w_0 \approx 6.1 \pm 0.2$. This suggests that about six water molecules are bound to each AOT sulphonate group and accords well with other studies showing approximately 5–15 water molecules bound per AOT head group [18].

The activities of enzymes such as peroxidase and α-chymotrypsin are increased in low-w_0 reverse micelles and their stabilities are enhanced. This raises the question about how the unfolding of such proteins compares with that observed free in solution. This matter has been examined using DSC methods for ribonuclease, cytochrome c and lysozyme-binding NAG_3 (N,N',N''-Triacetylchitotriose) [19]. A number of features emerge from these results. Firstly, the melting transitions appear to be two-state, as the ratio $\Delta H_{cal}/\Delta H_{vH}$ remains near unity for all proteins at all w_0 values examined. Secondly, for all w_0 values, the protein melting transitions occur at lower temperatures in reverse micelles than in water. For example, ribonuclease melts in 10 mM diglycine buffer, pH 3.3, at 48.1 °C but at only 38.8 °C in reverse micelles of $w_0 = 11.1$. Thirdly, this reduced stability appears linked to reduced melting enthalpies. Thus for the preceding

ribonuclease example, ΔH_{cal} is 297 kJ mol^{-1} in H_2O but only 247 kJ mol^{-1} in reverse micelles. There is some evidence that optimal stability in reverse micelles occurs at a definite water/surfactant ratio. For ribonuclease, this value was 11.1. Finally, enzymes in reverse micelles proved much more stable toward denaturation at room temperature than they are in water. For example, the melting enthalpy of ribonuclease in reverse micelles remained constant to within 0.5% over six weeks. The general picture emerging from these studies is that the stability of reverse-micelle-solubilised enzymes is optimised by the presence of just enough water to hydrate both the detergent head groups and the protein surface, the latter to the extent of perhaps 1–2 layers.

A third concern about reverse micelle systems is the thermodynamics of protein solubilisation by these structures. This is a complex problem that has been treated approximately by Maestro and coworkers [20,21] using an electrostatic model. This rather difficult calculation represents the unfilled reverse micelle as a capacitor comprised of spherical, negatively charged AOT ions and positive counter-ions. Protein uptake by the reverse micelles adds a number of positively charged proteins surrounded by corresponding counter-ions. The free energy change for protein uptake in such a case arises from four factors: the electrostatic interaction free energy of the specified ions in their given geometries; corresponding electrostatic entropy terms arising from the possible configurations of the ions present; mixing entropy terms for concentration changes in aqueous phase; and mixing entropy terms associated with hydrophobic phase sites. The free energy change predicted by these four factors can then be minimised to suggest how the micellar sizes, water content, number of surfactants, etc. should be altered by protein uptake.

Both the experimental results and theoretical treatment of proteins in reverse micelles show that these are complex systems. The difficulty in understanding the physical reality of protein-bearing reverse micelles or the function of the enzymes so situated is complicated by the thermodynamically driven nature of reverse micelles. These factors introduce a level of complexity far beyond that exhibited either by immobilised enzymes or by solid phase enzyme systems in organic solvents.

ENZYMES IN LOW-WATER ENVIRONMENTS

As mentioned earlier, the ability of crystalline enzymes to function while suspended in organic solvents is of considerable potential practical importance. To understand this ability two questions must be answered. First, what types of interactions does water have with the enzyme molecules and which of these interactions are necessary for catalytic function? Second, what types and degrees of hydration are available to enzyme crystals suspended in organic solvents?

The first question has been studied since the earliest days of protein biophysical chemistry, but modern DSC methods have greatly facilitated progress in this area. Rüegg et al. [22] examined the thermal denaturation of β-lactoglobulin as a function of water content. They distinguished four different states of water in the system. For water contents up to 0.29 g H_2O per g protein, the water appears non-freezable. In the range 0.29–0.45 g H_2O per g protein, water freezes but with enthalpies and at temperatures different from those of bulk water. From 0.45 to 0.70 g H_2O per g protein, water freezes at about 0 °C but the melting enthalpy again differs from that of the bulk solvent. Finally, at water contents above 0.70 g per g, the thermodynamic properties of the water approximate those of the bulk fluid. Thermal denaturation of β-lactoglobulin decreases the quantity of water in states 2 and 3, and increases that in state 4. These authors also observed that water in states 2 and 3 may represent molecules able to interact with both the tightly bound water of state 1 and the bulk solvent of state 4, thus being the molecules most intimately associated with protein conformational changes.

Fujita and Noda [23,24] used DSC to examine the denaturation of lysozyme and chymotrypsinogen. They found that, for lysozyme, there are at least two types of hydration. Primary hydration occurs up to 0.33 g per g H_2O and 0.23 g per g D_2O, and exhibits similar enthalpies of hydration for both solvents, i.e. about 0.8 kJ per mol water. The degrees of hydration correspond to about 1.0–1.5 molecules of water per hydrogen bonding site on the protein. Secondary hydration occurs for 0.33–0.75 g per g H_2O and 0.23–0.55 g per g D_2O. It appears stronger for D_2O than for H_2O, i.e. 0.43 kJ versus 0.14 kJ; this may reflect the stronger intermolecular interactions of D_2O relative to H_2O. Similar results were obtained for chymotrypsinogen in H_2O.

Combined use of laser Raman and NMR methods enabled Poole and Finney [25] to measure directly the conformational changes occurring at small extents of lysozyme hydration. They concluded that, at about 0.08 g H_2O per g protein, there is a general loosening up of the molecule, followed by local conformational changes at higher water contents. Enzymic activity appears only after these hydration processes are complete.

More recently, Lobyshev [26] modelled the relative interactions of H_2O and D_2O with proteins undergoing thermal denaturation. He showed that various models predicted quite different shapes for plots of T_m against volume fraction of D_2O, and that only one model adequately described the experimental results for ribonuclease and collagen. The successful model requires that two −OH/−OD groups behave independently, as would be expected for molecule bridging pairs of proximal hydrogen-bonding sites in the proteins.

The upshot of these various studies is that a relatively small amount of tightly bound water, perhaps 0.1–0.3 g per g, is required for enzymic

function. Various forms of additional bound water may be distinguishable calorimetrically but do not appear essential for catalytic activity.

A variety of studies on the function of enzymes while suspended in organic solvents have been presented by Klibanov and coworkers [27–29]. The general format of these studies has been to lyophilise enzymes from a particular set of aqueous solution conditions and then to suspend the powder in organic solvents containing small and varying amounts of water. Enzyme-catalysed conversions of substrates soluble in the organic solvents are then examined and compared with the cognate reactions in water.

In reading these studies, it is essential to remember that the wording "in organic solvents", though precise, is misleading. This somehow implies that proteins are dissolved in these fluids and that their activity in such an environment can therefore be compared with that observed in water solution. This is not the case; the appropriate comparisons are with activities of crystal-phase enzymes as observed in connection with crystallographic studies.

From such studies a number of interesting points emerge. Firstly, these are true enzyme-catalysed processes because no reaction at all is observed in the absence of enzyme or when the enzyme is inactivated by site-specific covalent inhibitors [28]. As judged by the ratio v_{max}/K_M, the ratio of maximum velocity to the Michaelis constant, the activity of both subtilisin and chymotrypsin is strongly retained in highly hydrophobic solvents. For example both materials exhibit maximal activity in hexadecane and are virtually inactive in dimethyl sulphoxide. The enzymatic activity of chymotrypsin requires that the protein be able to adapt conformationally to the desired substrate and this is generally impossible in the absence of a small amount of water. The addition of 0.1% H_2O (together with 1 M *n*-propanol to solubilise this water) permits this flexibility to be attained. Alternatively, the enzyme can be lyophilised in the presence of a substrate analogue so that the protein molecules pre-acquire the necessary conformation. In this case no addition of water is necessary. Karl–Fischer determination of the amount of water present in the enzyme solid phase shows that those solvents in which enzymes are most active, i.e. the most non-polar ones, permit the protein to obtain the highest degree of hydration. For example, chymotrypsin in octane contains 2.5% residual water while the enzyme in pyridine attracts only 1.0%. This suggests that the low activity of enzymes in more polar solvents might result from more effective solvent competition for available water, thus making it unavailable for the protein. Such a conclusion is confirmed by observations of increased enzyme activity in polar solvents when the water content is increased appreciably. It should be noted that the amounts of protein-bound water are small; even the 2.5% water bound to chymotrypsin corresponds to only about 50 water molecules per molecule of protein and this amount is about 10 times less than that required to form a monolayer on the protein

surface. One aspect of the problem not discussed by Klibanov is the relation of such degrees of hydration to those present in enzyme crystals where activity has been demonstrated by X-ray crystallographic studies.

The activity of enzymes in these systems has a number of interesting aspects. Firstly, a comparison of chymotrypsin- and subtilisin-catalysed transesterifications of *N*-acetyl-L-phenylalanine ethyl ester by amyl alcohol with the corresponding hydrolysis in water shows that the enzymes are 10^4–10^5 times more efficient in water than in the organic solvent. Secondly, the thermostability of enzymes is greatly enhanced in organic solvent suspension. Chymotrypsin has a half-life of 4.5 h at 100 °C in octane while its lifetime in solution at 55 °C is only minutes. A more relevant comparison would, of course, be with the half-life of crystalline chymotrypsin at constant water content.

The matter of enzyme thermal denaturation in organic solvent suspension has been examined by DSC for ribonuclease [30]. In anhydrous nonane, the enzyme at a water content of 6% exhibits a melting transition at 124 °C. This is to be compared with the T_m of 61 °C observed in water. At water contents of 11%, ribonuclease melts at 111 °C, either in nonane or neat. Moreover, its half-life at 145 °C is identical in both cases. These various data suggest that the essential aspect of these systems is not the nature (or even the presence) of organic solvent but the degree of hydration of the protein.

ROLE OF CALORIMETRIC STUDIES

Reaction calorimeters useful for examining protein–ligand reactions first became available commercially in the mid-1960s. Since then, calorimetric methods have provided important quantitative information on the thermodynamics and kinetics both of enzyme interactions with substrates or inhibitors and of the actual reactions catalysed by enzymes.

The unique property of calorimetry is that it measures directly a thermodynamic property of a process, namely the heat change. The most obvious application of calorimetry is therefore in determining reaction enthalpies ΔH. Moreover, when the reaction can be studied at various temperatures, the heat capacity change ΔC_p becomes accessible. Prior to the advent of adequate calorimetric methods, reaction enthalpies had to be evaluated by van't Hoff methods from the temperature dependence of equilibrium constants. Even under ideal conditions this is a demanding approach; and, for reactions with large equilibrium constants, it was often impossible. The situation for the heat capacity change was even more unfavourable because its evaluation proceeds from the second temperature derivative of the equilibrium constant.

The above factors alone would have allowed calorimetric methods to

expand substantially our knowledge of enzyme thermodynamics. However, as most reactions proceed which some non-zero ΔH, the enthalpy change itself can be used as a measure of reaction progress. Thus reaction equilibria can be examined without the need for some independent, e.g. spectral, indicator of reaction progress and calorimetric measurements can be performed on turbid, heterogenous, or otherwise intractable samples.

The two principal types of reaction calorimeters are flow and batch instruments. Flow instruments are perhaps the most convenient for the rapid measurement of a single heat of reaction from individual samples. This is because the sample flows directly from the room environment into the measurement process. An example of contemporary flow calorimeters is the TAM 2277 multichannel microcalorimetry system from Thermometric AB-Jarfalla (S), (20–40 ml h^{-1} flow rate, limit of detectability 0.5 μW). Batch instruments require the thermal equilibration of each sample with the calorimeter prior to reaction and this greatly reduces convenience, especially when a large number of different samples must be examined. The advantages of batch instruments include sensitivity, the ability to examine slow reactions and the potential for constructing a full thermal titration curve from a single sample. The Omega, Microcal Inc., Northampton, Mass. (USA) is a heat compensation batch and titration calorimeter. (Vessel volume 1.3 ml, limit of detectability 0.8 μW).

With either type of instrument, a single measurement provides a single heat of reaction under specified conditions. When heats of reaction are available at a variety of reactant ratios, i.e. different ligand concentrations for a fixed amount of protein, a thermal titration curve can be constructed. Analysis of such data yields the reaction heat per mole of the limiting reactant, the reaction stoichiometry in moles of ligand per mole of macromolecule and the reaction equilibrium constant. Measurements at multiple temperatures provide not only ΔC_p but also independent van't Hoff estimate of ΔH. Methods for this analysis are straightforward and have been published [31]; moreover, commercial software implementing these procedures is available (Thermometric, Microcal).

Of particular importance to enzyme studies is the ability of calorimetric methods to determine reaction rate parameters. Johnson and Biltonen [32] have demonstrated the applicability of flow calorimetry to measure bimolecular (or higher molecularity) reaction rates with half-times in the range from 5 seconds to several hours. Reaction enthalpies are also obtained. Reaction kinetics can also be studied in batch calorimeters [10], but their frequently slow time-response can necessitate application of the Tian correction [33] for instrument response. Whichever instrumental system is used, calorimetric methods require only that the reaction possess a non-zero ΔH, i.e. no spectral or other measurable changes need accompany the reaction, and they can be performed on heterogeneous samples. This latter attribute makes reaction calorimetric methods highly useful for

the study of enzyme processes taking place in the environments that form the subject of this paper.

An important aspect of enzyme function is the linkage of environmental conditions to reaction properties. Solution species such as hydrogen ions, divalent cations, chloride, etc. will bind differentially to enzymes and enzyme–substrate or enzyme–affector complexes to modulate enzyme-catalysed reaction rates. Calorimetric studies of reactions in which the solution conditions are systematically varied provide a way of elucidating such linkage phenomena. For example, Bolen and Slightom [34] examined the acylation of α-chymotrypsin by 3-(2-furyl)-acryloylimidazole at different pH values and in buffers having different proton ionisation enthalpies. From these data they evaluated the numbers of protons bound or released at each step in the reaction and the pK values for these proton binding sites. Data on the binding of protons, inorganic ions and denaturants to a variety of other proteins are tabulated by Wiesinger and Hinz [35].

Reaction calorimetric methods have permitted evaluation of the thermodynamic parameters for many enzyme-catalysed reactions of biochemical interest. In general, ΔH values are reasonably accessible while the free energy changes, consequently, the associated entropy changes are more difficult to obtain. Rekharsky et al. [36] have recently published a critical compilation of such thermodynamic parameters organised according to the IUB enzyme classification scheme [37,38].

Finally, one needs to consider the contributions of differential scanning calorimetry (DSC) to our understanding of protein thermal stability. Early studies (for a review see refs. 39 and 40) demonstrated the feasibility of measuring the melting temperatures, enthalpies and heat capacity changes for milligram or smaller amounts of proteins. The current commercial availability of highly sensitive differential scanning calorimeters such as the Soviet DASM-4 and the MC-2, Microcal Inc., facilitates measurements on proteins in solution. For studies of solid samples such as proteins at low extents of hydration, pan-type instruments like the Perkin–Elmer DSC-7 remain indispensable.

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