Pure & Appl.Chem., Vol.55, No.3, pp.515-528, 1983.
Printed in Great Britain.

0033-4545/83/030515-14$03.00/0
Pergamon Press Ltd.

BIOTHERMODYNAMICS AND CALORIMETRIC METHODS

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Abstract - Recent calorimetric work in some areas of biothermodynamics is surveyed. Work on simple model compounds largely concentrates on solute-water interactions. A special attention is given to hydrophobic interactions and accompanying heat capacity changes. Current calorimetric work on biochemical compounds and assemblies of compounds mainly involve different kinds of non-covalent binding and association processes and DSC measurements relating to the structural stability of biopolymers and lipid aggregates. There is an increasing amount of work done on living cells where, however, calorimeters largely are used as monitors for biological events rather than as tools for recording thermodynamic quantities.

INTRODUCTION

Biothermodynamics covers a vast area ranging from work on simple model compounds to living organisms and eco-systems, Table 1.

TABLE 1. Different levels for thermodynamic work in biochemistry and biology.

Water, simple model compounds
Low molecular biochemical compounds
Biopolymers, lipid aggregates
Cell organelles
Living cells
Animals, plants
Eco-systems

Different experimental and theoretical approaches will be used in biothermodynamic investigations on such diverse systems and it is hardly possible to cover the entire field in one short paper. I have chosen to concentrate on a few areas of calorimetric work on well-defined systems for which there is some hope of correlating thermodynamic and molecular properties.

The thermodynamic functions which we investigate experimentally in calorimeters are of course not connected with any assumptions concerning the molecular nature of the compounds. In a strict sense, therefore, thermodynamic experiments will not tell us anything about structure or other molecular properties of the systems investigated. Nevertheless, empirical correlations between thermodynamic properties and molecular properties determined by non-thermodynamic methods have proved to play a key role for what we consider as an understanding of biochemical substances and their function. Such correlations are made on different levels of complexity but by necessity they start from observations made on very simple compounds, often called model compounds. A wealth of accurate experimental results on simple model compounds have now accumulated but the need is by no means saturated. To a significant extent such studies are undertaken by investigators who are not involved in biochemical work. It is interesting to note, however, that such work frequently leads to a deeper involvement on the biochemical or biological level and thus serves as a form of recruitment base for biothermodynamicists.

Life cannot exist without water. Studies of the properties of water and the mutual interactions between water and biochemical substances and their models have a central position in today's biothermodynamics. Current work on biochemical compounds concentrates on purified biopolymers, in particular proteins and nucleic acids and to assemblies of such compounds such as nucleic acid protein complexes and membranes. Other substances of

interest include intermediates of metabolic pathways and various key compounds like co-enzymes, vitamins, hormones, messenger substances and energy storage substances. Thermodynamic work on these groups of compounds appears at present not to be of a very systematic nature. Attempts to characterize cell organelles like ribosomes and mitochondria by calorimetric methods exist but are not very frequent. There is an increasing number of investigators who do calorimetric work on the level of living cells and on small and large animals. For microorganisms, blood cells and other animal cells calorimeters are largely used as monitors for biological events rather than as tools recording thermodynamic quantities. Very often such work is conducted with a direction of practical applications in fields like biotechnology and clinical analysis. There is at present a rapid methodological development in this area and I believe that this trend will lead to a deeper thermodynamic analysis of the thermal power values recorded.

For systems like animals the purpose of calorimetric experiments is usually to obtain data useful for discussions of energy balances on a basic physiological level or on a more practical level, e.g. in connection with livestock feeding. In recent times there have been significant developments in human whole-body calorimetry. Such work is primarily connected with clinical investigations.

Several areas of current bio-calorimetric work have recently been treated in two monographs (1), (2). In this paper special attention will be given to recent calorimetric work on simple model compounds, protein ligand binding and thermal transitions of proteins.

WORK ON SIMPLE MODEL COMPOUNDS

In model compound studies certain features of complex biochemical substances are simulated. Work on very simple compounds has the advantage that the influence of well-defined properties of the thermodynamic values can be studied in considerable detail. On the other hand, results from such work should not be applied uncritically on real biochemical and biological systems.

Currently work on simple models concentrates on studies of weak non-covalent interactions, in particular water-solute interactions. The thermodynamic formalism in this field of work, as well as results from studies of many groups of compounds, has recently been reviewed by Franks (3). Model substances include the rare gases, hydrocarbons, various substituted hydrocarbons like alcohols, carboxylic acids, esters, amines, amides, etc. Compounds forming the building blocks for biopolymers (amino acids, peptides, nucleotides and their constituents, sugars) are also important as well as lipids including micelles and liposomes. In order to explore the unique properties of water it is frequently essential to extend the investigations to non-aqueous solvents or to mixtures or organic solvents and water. Such solvents are also used as models for the environment in the interior of a biopolymer molecule or of a lipid phase.

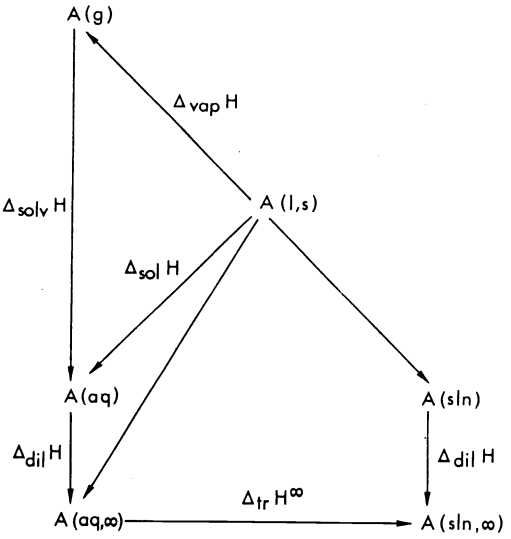


Fig. 1 Scheme of calorimetric model experiments in studies of solute-solvent interactions. A(l, s or g) represents a pure compound and A(aq) and A(sln) its solutions in water and in another solvent, respectively.

The most basic calorimetric model experiments are those where pure compounds A(s, l or q), are transferred to infinitely dilute solutions, Fig. 1. From calorimetric experiments ΔH and ΔC_p-values are obtained. Corresponding changes in Gibbs energy must be obtained from various types of equilibrium measurements. If performed at different temperatures, such non-calorimetric work can of course also lead to values for ΔH, ΔS and ΔC_p. However, if calorimetric enthalpy values are available, the entropy changes are usually best obtained by a combination of equilibrium values (ΔG^o) and calorimetric enthalpy values, eqn. (1).

$$\Delta G^o = \Delta H^o - T\Delta S^o \qquad (1)$$

The propagation of errors in the van't Hoff treatment of equilibrium data is such that it is very rare that non-calorimetric ΔC_p-values can be considered reliable, see Table 2.

TABLE 2. Propagation of errors (standard deviation) in van't Hoff treatment of equilibrium data. From King (4).

Experimental design	20-30 °C in 2 °C intervals		0-50 °C in 10 °C intervals		5-50 °C in 5 °C intervals	
Experimental uncertainty in pK	± 0.02	± 0.001	± 0.02	± 0.001	± 0.02	± 0.001
Uncertainties in derived parameters						
ΔH^o/J mol^{-1}	± 4100	± 210	± 840	± 42	± 800	± 42
ΔS^o/J mol^{-1} K^{-1}	± 14	± 0.67	± 2.7	± 0.14	± 1.3	± 0.07
ΔC_p^o/J mol^{-1} K^{-1}	± 2800	± 140	± 110	± 5	± 120	± 6

Calorimetric work of the type indicated in Fig. 1 is mainly conducted with precise "macro" solution calorimeters. Vaporization studies are very rare although adequate instrumentation has been developed (5). From the difference between values for the dissolution into different solvents, values for the transfer of the solute between the solvents is obtained. Such transfer processes are often looked upon as simple models for ligand biopolymer binding processes. It is sometimes essential to perform dilution experiments leading to values for the infinite dilute solution. Dilution experiments conducted over a suitable concentration range can also lead to values for solute-solute interactions, see e.g. (3), (6), (7).

Heat capacity values obtained from solution calorimetric work are of a major importance in model compound studies. However, ΔC_p values reflect the properties of the initial as well as the final system for the dissolution process. If the interest is focused on the solvated state the partial molar heat capacity for the solute (at infinite dilution), $C^\infty_{p,2}$, is a more appropriate function. This value can be derived from corresponding $\Delta C^\infty_{p,2}$ value and the heat capacity for the pure compound, C^*_p

$$C^\infty_{p,2} = \Delta C^\infty_{p,2} + C^*_p \qquad (2)$$

Good C^*_p data are often lacking, even for very simple compounds at 25 °C. Precise drop microcalorimeters (5) have been shown to be very suitable for such determinations as well as for heat capacity determinations of dilute solutions which can lead directly to $C^\infty_{p,2}$ values. The very precise flow heat capacity calorimeter designed by Picker, see (5), and commercially available through Setaram, Lyon, France, has proved to be particularly suitable for this latter type of measurements.

During recent years biochemical model work has to a large extent involved studies of hydrophobic hydration and the "hydrophobic effect". This latter effect can be defined as the tendency for hydrophobic groups to escape from contacts with water through association with other hydrophobic groups. The hydrophobic effect (in biochemistry often called the formation of "hydrophobic bonds") is believed to play a significant role for the structural stability and specificity of biological systems. Table 3 summarizes some thermodynamic characteristics, cf. (8), for a process of the type

hydrocarbon (l) $\rightleftarrows$ hydrocarbon (aq) (3)

i.e. the hydration of a hydrophobic compound. The reverse process, the "dehydration", can be seen as an example of "hydrophobic interaction" ("hydrophobic bonding") together with the van der Waal's interactions taking place when the hydrocarbon molecules have been brought together.

TABLE 3. Thermodynamic characteristics of hydrophobic hydration processes (eqn. 3) at 25 °C

$\Delta G >> 0$

$\Delta H \approx 0 (\pm 2$ kJ mol^{-1})[a]

$\Delta S << 0$

$\Delta C_p >> 0$

[a] See ref. (9) (10)

From a calorimetric point of view it is the heat capacity change which is of special interest. Hydrophobic hydration is characterized by a very large increase in heat capacity, e.g. for a liquid hydrocarbon $\Delta C_{p,2}(aq)$ is about 3 times higher than the value for the pure liquid. The ΔC_p (and $C_{p,2}$)-values change slowly with temperature and show, in particular for nonionic compounds, a remarkable additivity (11) - (14). These factors have rendered C_p-values a special interest in correlations with structural and solution parameters for biochemical compounds and processes, cf. below. Large negative ΔC_p - values for biochemical processes are sometimes taken as a sign of the formation of "hydrophobic bonds", but great caution should be taken when making such statements, cf. (15), (16). It is of interest in this connection to examine sugars and sugar alcohols which also show remarkably high $C_{p,2}(aq)$ values. An empirical scheme (11) for the estimation of $C_{p,2}(aq)$ values for nonionic compounds was shown to work well for a great variety of substances, including hydroxyl compounds, but not for sucrose. A comparison of available data for polyhydroxy compounds suggest that there is a cooperative effect at work which results in large $C_{p,2}(aq)$ values for sugars and sugar alcohols (17). Ring structures seem to give significant contributions. Table 4 shows $C_{p,2}(aq)$ values for some polyhydroxy compounds. It is seen that the values for mannitol and sorbitol are very different although they only differ in position of one of the hydroxyl groups. Such specific solute-water effects seem to be of special interest in view of the fact that sugar moieties are believed to have a recognition function in e.g. immunological reactions.

TABLE 4. Partial molar heat capacities for some polyols in aqueous solution. Δ is the difference between observed and calculated (11) value.

Compound	$C^{\infty}_{p,2}$/JK^{-1} mol^{-1}		Δ/JK^{-1} mol^{-1}
	Experimental	Calculated	
Ethanol	260 ± 1	256	4
Ethylene glycol	193 ± 2	198	- 5
Glycerol	240 ± 4	230	10
Pentaerythriol	329 ± 1	322	7
D-mannitol	452 ± 4	326	126
D-sorbitol	412 ± 5	326	86
Meso-inositol	340 ± 5	172	168
α-D-glucose	347 ± 3	173	174
Sucrose	650 ± 2	271	379
Raffinose	931 ± 7	369	562

For the development of theoretical concepts about the hydrophobic interaction experimental data for the hydrocarbons and the rare gases are of particular importance. Recently Benson and coworkers (18) described a method for determination of solubility of slightly soluble gases in water which seems to be precise enough to lead to reliable ΔH and ΔC_p-values. In our laboratory we have recently developed a flow microcalorimetric vessel

(19) by which aqueous enthalpies of solution of gaseous hydrocarbons and rare gases can be determined. It can be expected that rather soon "final" values for ΔG^o, ΔH^o, ΔS^o and ΔC_p^o for these solution processes will be available. Flow micro-solution calorimeters specially designed for slightly soluble liquids (20), (21) and solids (22) have also been reported. Solution and heat capacity calorimetry at increased pressure seem to offer interesting possibilities for studies of solute-water interactions, but no such studies with direction of biochemical model systems seem to have been carried out so far.

BIOCHEMICAL SYSTEMS

Current calorimetric thermodynamic work on biochemical compounds and assemblies of compounds mainly involve different kinds of non-covalent binding and association processes and measurements relating to the structural stability of biopolymers and lipid aggregates. Significant amounts of work have also been done on water-protein interactions. It is noteworthy that calorimetric measurements on polysacharides are very sparse (23) despite their immense importance in biology. A special area probably worth more attention is calorimetric investigation of light-induced processes, cf. (24), (25). Table 5 gives a list of recent reviews and discussion papers covering the most active areas in biochemical calorimetry.

TABLE 5. Recent reviews and discussions on biochemical calorimetry.

Subject area	Authors	Reference
General aspects including instrumentation and experimental procedures	Spink and Wadsö	26
	Barisas and Gill	27
	Langerman and Biltonen	28
	Biltonen and Langerman	29
	Jones and Skinner	30
	Krishnan and Brandts (DSC)	31
Protein-ligand binding	Gill	32
	Eftink and Biltonen	33
	Ross and Subramanian	16
	Rialdi	34
Structural stability		
a) Proteins	Privalov	36
	Pfeil and Privalov	37
	Privalov	38
	Pfeil	39
	Mateo	40
b) Nucleic acids	Privalov and Filimonov	41
	Hinz	42
	Privalov	38
	Klump	43
c) Lipids and membranes	Mabrey and Sturtevant	44
	Jones	45
	Bach and Chapman	46
Protein-water interactions (C_p-measurement)	Privalov	38
	Yang and Rupley	47

Specific binding and association processes

Protein ligand binding. Binding of ligands to proteins are of a significant biochemical interest. Example of ligands are enzyme inhibitors of different kinds (often resembling

the substrates), coenzymes, and metal and hydrogen ions. The specificity is often very high, meaning that the binding processes are well-defined. The ligands are usually simple compounds and these processes therefore often appear to be comparatively simple and possible to analyse in some detail. They are also well-suited for micro-calorimetric techniques and in many instances it is possible to determine both equilibrium constants and enthalpy values, and thus also entropy values (26), (29), (33), (49). The calorimetric measurements are typically made by commercially available batch or flow microcalorimeters. Recently a titration attachment to the LKB batch microcalorimeter has become available (48), cf. the earlier work by RÜterjans and coworkers and by Woledge (49). Titration curves can be produced much faster and with less material than with the original batch technique. In addition it appears as if this technique gives significantly more precise results than the original procedure (49).

In a typical continuous flow calorimetric experiment, protein solution of a constant concentration is mixed with ligand solutions of different concentrations. Mountcastle et al. (50) have described a continuous exponential dilution technique by which a complete binding curve can be obtained within 1 h (with some loss in precision).

As part of a new 4-channel microcalorimeter system, a new flow-mixing calorimeter has been designed in our laboratory (51). At low flow rates, ca 10 + 10 ml/h, the useful sensitivity is 0.1 μW, which is an order of magnitude better than our earlier design (52). As part of this calorimeter system there is also a 1 ml titration vessel (an insertion vessel) which can operate on the μW level.

A very sensitive adiabatic shield titration calorimeter has recently been reported by Spokane and Gill (53). Typical heat measurements range from 0.1-1 mJ with a reproducibility of about 0.01 mJ. Typical volume of injected liqud is 10 μl.

A few factors of importance for the design of ligand binding experiments will be pointed out. For more detailed accounts see the review by Eftink and Biltonen (33). It is important to prepare the reacting solutions so that unwanted protonation reactions from differences in buffer composition are avoided. As the measurements normally are conducted on the microcalorimetric level, it is important to watch out for adsorption of biopolymer or buffer components on the walls of the reaction vessels (26). Sometimes enthalpy of dilution of one or both of the reactants is significant and has to be corrected for in separate experiments. Often the binding process will be accompanied by a release of protons which normally will be taken up by the buffer present. In order to explore such effects, it is recommended that the experiments be performed with buffers with different enthalpies of protonation. A related problem is that buffer components and neutral salt present in the reaction mixture tend to bind to the polymer. The degree of binding can change as a result of the ligand-binding process.

The binding of oxygen to haemoglobin has been much studied by thermodynamic methods both because of its physiological significance and because it forms a typical example of a cooperative ligand-binding process. The thermodynamics' formalism together with experimental results have recently been discussed by Gill (32).

Other specific binding reactions. Many proteins tend to associate to form well-defined aggregates which can be studied calorimetrically by mixing or dilution experiments. In a few cases calorimetric measurements as well as X-ray structure determinations have been made, cf. (16). Antigen-antibody binding is an example of a very specific protein-protein association. However, the analysis of thermodynamic results from such binding experiments have been difficult, cf. calorimetric work on hapten-antibody binding (54), because naturally available antibodies form mixtures of different protein molecules. Monoclonal antibody preparations now available ought to be very attractive for use in calorimetric binding studies.

Calorimetric binding experiments involving interactions between protein and lipid micelles (55) and between lipid vesicles (unilamellar liposomes) and surfactants (56) are examples of binding experiments used as models for membrane systems. This is an area which is judged to draw much attention in the near future.

Interpretation of thermodynamic changes. In some cases it is possible to discuss the results of a ligand binding experiment in the light of detailed structural information deduced from X-ray crystallographic work. As an example based on the schematic picture shown in Fig. 2, Eftink and Biltonen (33) proposed several points of specific interaction between an enzyme (ribonuclease A) and a nucleotide inhibitor (c'-cytidine monophosphate). An electrostatic interaction can be envisioned between the phosphate group and the protein residues of His 119, Lys 41 and His 12. The X-ray results suggest also a number of hydrogen bond interactions between the nucleotide moiety and the protein surface. In addition to such specific interactions, several van der Waals contacts can be pointed out. It can be assumed that a certain amount of more-or-less "structured" water has been pushed out from the binding cleft and has thus been transformed into bulk

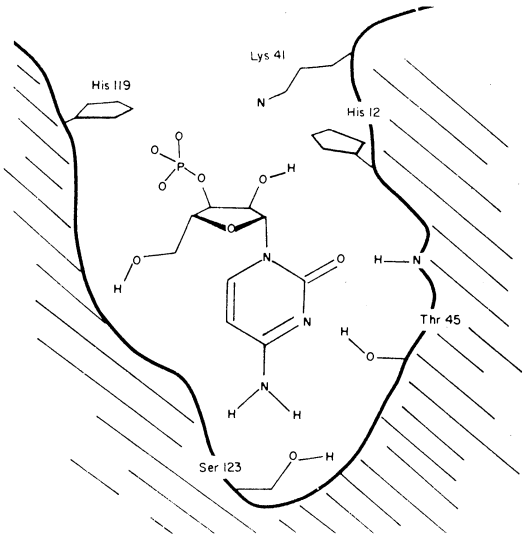


Fig. 2. Proposed structure of the ribonuclease A– 3-CMP complex. From (33).

water. However, there is good reason to proceed very cautiously with such analyses. It is very uncertain if results from simple models can be directly applied on a multi-functional interaction system such as that indicated in Fig. 2. Further, normally we do not have very precise information about changes of the amount and the state of water at the reaction site. It is also difficult to estimate the thermodynamic consequences of a conformational change taking place in the protein as a result of the binding process. We should in this connection also note that the low molecular ligand can significantly change its conformation due to the binding, see e.g. (57). Further, it must be borne in mind that representations like Fig. 2 are arrived at from crystallographic work and do not necessarily represent the situation in solution. At its best, they can show an average situation as proteins are believed to form dynamic fluctuating units in solution, see e.g. (58).

Ross and Subramanian (16) discussed the thermodynamic parameters characterising protein self-association and protein ligand binding reactions, cf. also (15), (33), (59). They concluded that at 25 °C values for ΔG^o, ΔH^o, ΔS^o and ΔC_p^o in most cases all were negative. They could not discern any pattern in ΔG^o relating to corresponding values for ΔH^o and ΔS^o. However, it is very common to find a substantial compensation between the ΔH^o and the $T\Delta S^o$ terms. For many of these association and ligand-binding processes, it has been claimed that hydrophobic interaction is the main driving force which does agree with the observed negative values for ΔG^o and ΔC_p^o. However, as Ross and Subramanian (16) pointed out, the largely negative values for ΔH^o and ΔS^o do not agree with the observations made in model compound work. These workers made an attempt to divide the binding process into two hypothetical steps, cf. Fig. 3. It is assumed that the molecular surfaces which are brought in contact with each other are, at least partially, hydrophobic.

The individually hydrated species (A) are first allowed to interact partially in the sense that non-polar surfaces come in contact with each other and lose their hydration shells. This results in a hydrophobically associated species, B, plus release of "destructured" water.

From model studies it is evident that the hydrophobic binding step should be accompanied by large negative values for ΔG^o and ΔC_p^o and a large positive value for ΔS^o. If the hydrophobic moieties consist of hydrocarbon groups, one might expect that ΔH^o is close to zero at 25 °C but changes rapidly with temperature to significant exothermic or endothermic values at high and low temperatures, respectively (19), (20).

The partial immobilization resulting from this first association step is expected to be unfavorable because of a slight entropy decrease. The corresponding heat capacity change is expected to decrease, i.e. have the same sign as that for the hydrophobic binding. It is believed that the positive entropy change from destructuring of water outweighs any negative contribution to ΔS from the immobilization, cf. (15).

In the step B-C the hydrophobically associated species B is thought to undergo further interactions, which do not involve the solvent, such as specific electrostatic interactions, hydrogen bond formation and ohter interactions which might be summarized as van der Waals forces (dipole-dipole, dipole-induced dipole, London dispersion forces). Starting from

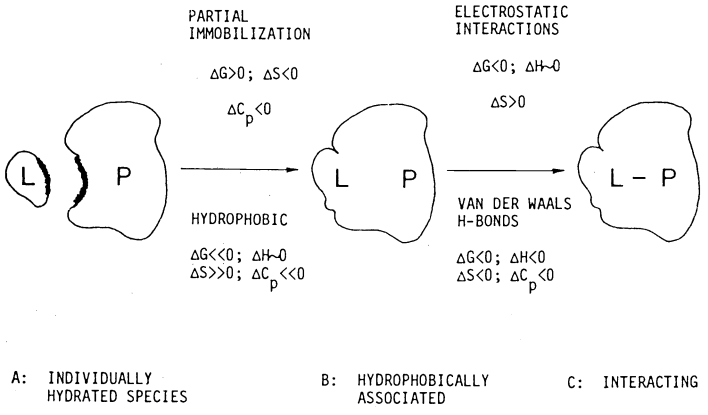


Fig. 3. Schematic representation of a protein binding or association process (after Ross and Subramanian (16)). The fuzzy lines denote domains of water which are more ordered, by hydrophobic hydration, than bulk water. Temperature around 25 °C.

observations made with simple compounds, some qualitative predictions can also be made about their contributions to the overall thermodynamic picture. Only van der Waals forces and hydrogen bonding are expected to be associated with the typically observed gross negative values for ΔH^o and ΔS^o. Ross and Subramanian (16) suggested that the associations of aromatic rings, frequently indicated by X-ray crystallographic work, contribute significantly to the negative ΔH^o and ΔS^o values. Similarly, they predicted also negative contributions from interactions between the highly polarizable sulphur atoms (methionine) with aromatic ring systems. It is in this connection noteworthy that, so far, very little attention has been given to the sulphide and disulphide groups in model compound investigations. There appears to be no experimental calorimetric work (ΔH, ΔC_p) done relating to their hydrophobic properties.

Hydrogen bonds formed in a comparatively low dielectric medium such as the interior of a protein are expected to make substantial negative contributions to ΔH^o and ΔS^o (60), which thus partly compensate each other, resulting in a small change in ΔG^o. If hydrogen bonds made internally replace those earlier made to water they contribute little to the stability of the complex. However, it is highly unfavourable if groups which form hydrogen bonds with water are transferred to the inner part of the complex and do not find any hydrogen bond partners there (61).

Interactions between charged species in aqueous solution are characterized by enthalpy values close to zero and positive entropy changes (16). X-ray work does often indicate formation of salt bridges. Resulting positive entropy changes might contribut significantly to a negative Gibbs energy change. However, model data do not give us much lead to separate such effects into the two hypotehetical reaction steps shown in Fig. 3.

In summary it can be concluded that progress is made in the analysis of thermodynamic data for protein binding reactions. The role of water is very clearly demonstrated. However, we are still far from a stage when discussions concerning various contributions can be held on a truly quantitative level.

Structural changes in biopolymers and in lipid aggregates.
Thermodynamic studies of unfolding processes for proteins and nucleic acids and phase transitions for lipids and membrane material have been of primary importance for the understanding of the forces which stabilize their native structures to an extent which is compatible with their biological function. Below a brief account will be given on current work in these areas with particular reference to proteins.

Proteins. Conformation changes can involve minor structural changes, e.g. induced by the binding of a ligand, partial unfolding or complete unfolding where all non-covalent intermolecular contacts present in the native structure are broken. However, even for the completely unfolded macromolecule, it is not likely that it is completely solvated by the medium, see e.g. (62).

Protein unfolding can be initiated by changes in solvent composition (pH changes, addition of denaturants such as guanidinium hydrochloride, GuHCl) or by temperature changes. Unfolding processes initiated by the addition of denaturants are easy to measure by most micro-reaction calorimeters. However, following the unfolding of the polymer, a large

number of denaturant molecules will adsorb to the newly exposed surfaces. Corresponding heat effects are large and it is necessary to separate them from the unfolding process per se (which is taken to include the hydration of newly exposed surfaces). This can be done by different extrapolation methods (36), (39).

The use of DSC is now a well-developed method for studies of thermal transitions of biochemical compounds and assemblies of compounds. However, there are only a few types of very sensitive instruments available which are able to cope with the dilute solutions (in the order of 1% or less) which normally are requested in order to avoid significant intermolecular interactions. Following the construction of such instruments by Gill (63), Sturtevant (64), Brandts (65), Ross and Goldberg (66), Ackermann (67), Privalov (68) and Suurkuusk (69) and their colleagues, there has been an intense development of working procedures and methods for a strict analysis of the data obtained, cf. Table 5.

In a DSC experiment the differential heat capacity between the sample and a reference is determined as a function of temperature, Fig. 4.

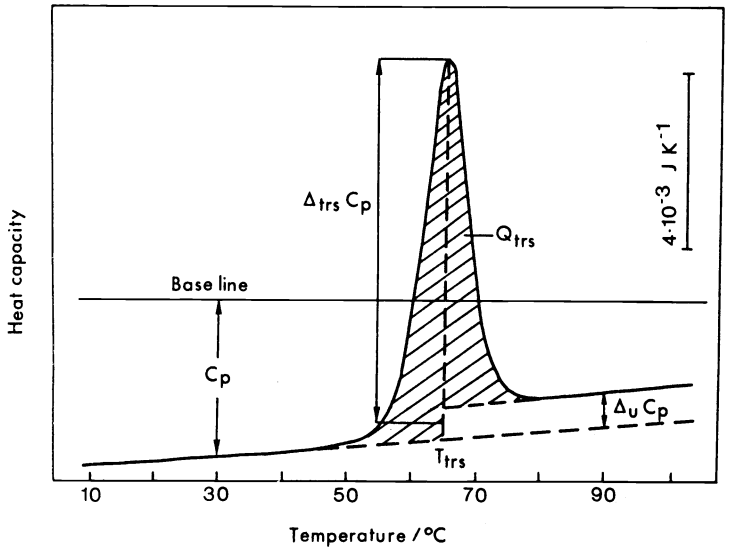


Fig. 4. DSC recording of a dilute lysozyme solution (pH 2.5, 1.8 mg ml^{-1}) by use of the Privalov calorimeter. Adapted from (36).

From the area under the transition peak, Q_{trs}, the calorimetric enthalpy of unfolding is obtained. The strongly positive $\Delta_u C_p$ value indicated in Fig. 4 is typical for unfolding of globular proteins, cf. below.

If a two -state transition is assumed a van't Hoff enthalpy change, ΔH_{vH}, can be calculated from the calorimetric curve, see e.g. (38).

$$\Delta H_{vH} = \frac{4\ RT_{trs}^2}{Q_{trs}} \Delta_{trs} C_p \tag{4}$$

The entropy change for the unfolding process, ΔS_u, equals

$$\Delta_u S = \frac{\Delta_u H}{T_{trs}} \tag{5}$$

By means of the observed value for $\Delta_u C_p$, it is possible to calculate $\Delta_u H$ and $\Delta_u S$ over a broad temperature range assuming $\Delta_u C_p$ to be constant.

$$\Delta_u H(T) = \Delta_u H(T_{trs}) + \int_{T_{trs}}^{T} \Delta_u C_p dT \tag{6}$$

$$\Delta_u S(T) = \Delta_u (T_{trs}) + \int_{T_{trs}}^{T} \frac{\Delta_u C_p}{T} dt \tag{7}$$

Combining the enthalpy and entropy functions (eqn 1) will lead to Gibbs energy changes as a function of temperature.

Values for thermodynamic parameters of unfolding processes vary greatly with the experimental conditions: T, pH, ionic strength, denaturant concentration. In particular Privalov and coworkers have during recent years developed strategies and produced experimental results leading to standard state functions suitable for intercomparison of different types of experimental results. They have also reported graphical representations of $\Delta G^{o} = f(T, pH)$ for several proteins, see e.g. (36) - (39).

For a system consisting of identical and independent units $\Delta H_{cal} = \Delta H_{vH}$ if we are dealing with a two-state process. For small globular proteins this has been shown to be very nearly the case, see e.g. (38). For processes with intermolecular interactions such as phase transitions involving lipid materials $\Delta H_{vH}/\Delta H_{cal} >> 1$. The value for the ratio $\Delta H_{vH}/\Delta H_{cal}$ provides a measure for the number of molecular units which are included in the cooperative unit.

Fig. 4 shows the transition curve for a small globular protein, lysozyme, for which the ratio $\Delta H_{vH}/\Delta H_{cal}$ is close to unity. There are other proteins for which the situation is more complex. For instance, for papain (70) which is an average size globular protein, the unfolding profile looks qualitatively the same but here the corresponding value is 1.8, suggesting that the unfolding process is not a simple two-state transition. In this case it is probable that two parts of the molecule have nearly equal but independent melting behaviour. This interpretation is supported by the known 3-dimensional structure for papain which shows a deep cleft which bisects the molecule into two nearly equal domains.

For several proteins investigated the calorimetric curves show a complex pattern with several peaks. Such profiles have been taken as evidence for independent cooperative units in the molecule. A recent comparatively simple example is provided by the results for pepsin (71), Fig. 5. (Transition corresponding to peak A is not reversible.)

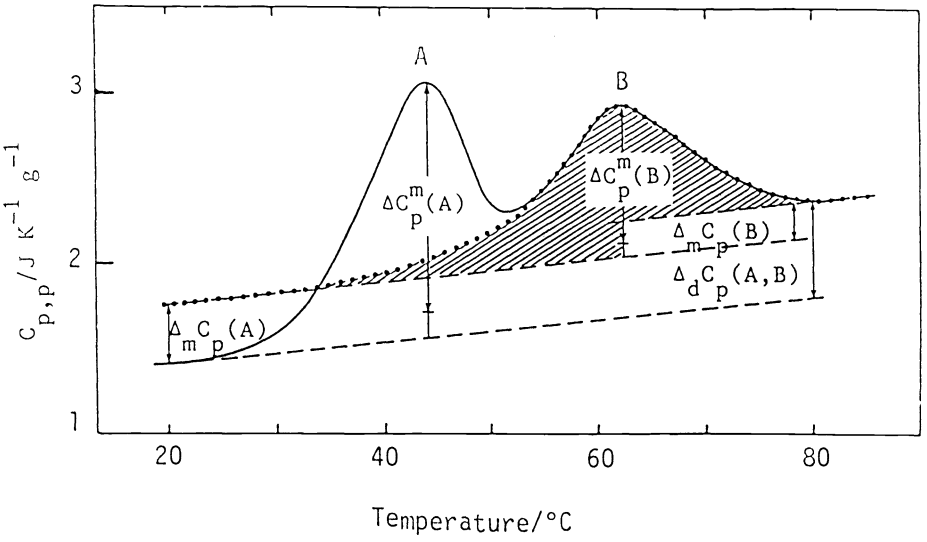


Fig. 5. Partial specific heat capacity function of pepsin solution, pH 6.5, 100 mM NaCl. From reference 71.

Methods for the deconvolution of such experimental curves for proteins and nucleic acids into transition curves for the more-or-less independent cooperative units has been in focus for the last few years among workers in this field. Following the pioneering work by Privalov and coworkers (72), who assumed that the subtransitions are independent two-state processes, Freire and Biltonen (73),(74) developed a method by which it is possible to resolve the experimental curves without making any assumptions about possible interdependencies among the different units. By this procedure the two main peaks for pepsin could be resolved into four transition curves grouped into two independent peaks. The molecular interpretation is that these peaks correspond to the four structural domains which can be distinguished from results of X-ray work. Other much more complex melting curves have been reported for other proteins (38).

It was pointed out earlier that attempts to correlate details of molecular changes with

observed thermodynamic changes for protein ligand binding reactions are still at a qualitative level. Protein unfolding processes are much more complex, not the least due to the very large increase in contact area between the protein material and the solvent. Correlations between changes of structural and thermodynamic parameters can therefore not be very detailed, but a few general conclusions have been drawn. The $\Delta_u G$ for many protein unfolding processes have a maxima around ambient temperature, whereas both ΔH and ΔS typically increase nearly linearly with temperature, Fig. 6. The ΔH and the ΔS term thus show a significant compensation.

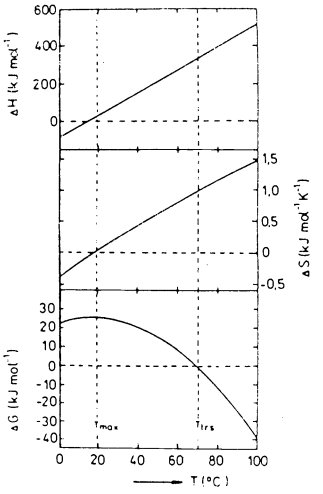
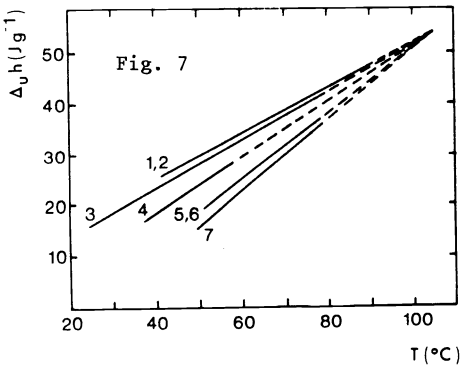


Fig. 6. Unfolding enthalpy, entropy and Gibbs energy change of cytochrome b_5 (pH 7.4) versus temperature. From (39).

Specific enthalpies of unfolding, $\Delta_u h$, for many compact globular proteins show an interesting feature when plotted versus T_{trs}, Fig. 7. Linear relationships are found and the lines converge to a point around 105 °C and 54 J g^{-1} (36), (38), (39). Results for a few other globular proteins, believed to be less compact, show a different pattern. An analysis of available structural parameters shows that for the former group the number of hydrogen bonds per unit of mass is very similar whereas the number of non-polar contacts varies greatly. The number of non-polar contacts correlates well with the observed $\Delta_u C_p$ values, Fig. 8. Hydrophobic hydration effects presumably vanish above 100 °C, which would suggest that $\Delta_u h$ at this temperature mainly is due to the differences in the extent and the strength of hydrogen bonds and van der Waals contacts between the folded and unfolded state. If the value $\Delta_u h \doteq 54$ J·g(105 °C) essentially is due to the hydrogen bond effect, a value of + 7 kJ·mol^{-1} is derived per mol of hydrogen bonds in the native protein. Contributions



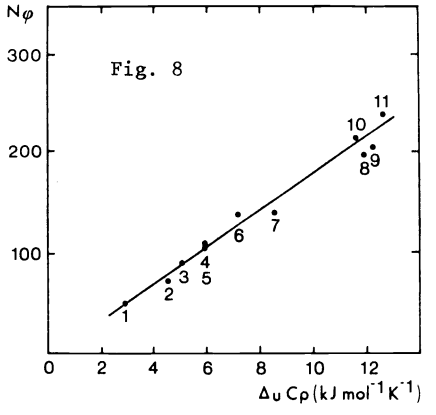


Fig. 7. Specific unfolding enthalpy changes for compact globular proteins versus temperature.
1. ribonuclease; 2. parvalbumin; 3. lysozyme; 4. α-chymotrypsin; 5. cytochrome c; 6. cytochrome b_5; 7. methmyoglobin. From (39).

Fig. 8. Correlation between the number of non-polar contacts, Nϕ, and $\Delta_u C_p$.

1, p. trypsin inhibitor; 2, α-lactalbumin; 3, parvalbumin; 4, ribonuclease; 5, cytochrome b_5; 6, lysozyme (egg white; 7, cytochrome c; 8, lysozyme (phage T4); 9, myoglobin; 10, trypsin; 11, papain; 12, α-chymotrypsin. From (36).

from the hydrogen bond and van der Waals effects are believed to change little with temperature and the large, essentially linear decrease in $\Delta_u h$ is thus ascribed to the hydrophobic effect, in agreement with Kauzmanns hypotheses (75). It has been estimated that at least 80% of the $\Delta_u C_p$ values are due to hydrophobic hydration (36).

For non-globular proteins the thermodynamic changes of unfolding are quite different from that observed for globular proteins (36), (38).

Nucleic acids. A large number of DSC-studies have also been performed on polynucleotides and natural nucleic acids. For the latter substances, very complex melting behaviour has been deduced by deconvolution of DSC-curves, see Fig. 9. Some attempts are being made in correlating the melting behaviour with the complex tertiary structure of these molecules (38), (42), (43). Thermal transitions in nucleic acid-protein complexes have also been studied, for a recent discussion see (43).

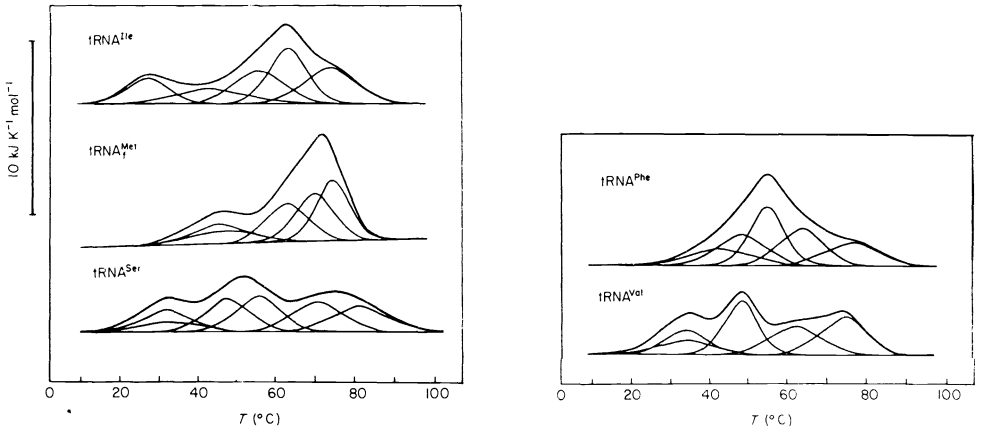


Fig. 9 Sequence of heat sorption of unfolding of various specific transfer RNAs in 150 mM NaCl solutions. From (38).

Lipids and membranes

Lipids form a major constituent of all biological membranes: outer or plasma membranes and membranes of intracellular organelles. The Singer-Nicolson (76) fluid mosaic model is now supported by a wide range of experimental observations. Amphiphilic membrane lipids (phospholipids and glycolipids) exist as bilayers which function as a solvent for membrane proteins and as a permeability barrier for hydrophobic substances.

Addition of water to anhydrous crystalline phospholipids will lead to a variety of phases which can undergo thermal transitions. A large number of DSC studies of such melting phenomena has been performed during recent years (44), (45), (46). Work of special thermodynamic interest is mainly conducted with high sensitivity instruments on aqueous suspensions of pure or mixed phospholipids (44). These suspensions form different kinds of multilamellar structures or unilamellar vesicles (liposomes) which are of particular interest as models for biological membranes. Multilamellar aqueous suspensions of phospholipids are characterized by highly cooperative and reversible thermal transitions. The DSC curves thus show very sharp transitions. Typically the cooperative units are in the range of 100-400 molecules. For single-lamellar vesicles the thermal transitions are broader.

Mixtures of phospholipids and cholesterol (an important lipid in mammalian cells) are also much investigated. Many studies have also been made, mainly by use of low sensitivity instruments, on hydrated lipid systems containing various drugs (46). More-or-less hydrophobic drugs will often cause changes in the fluidity of the lipid. Such characteristics are believed to be connected with the mode of action of the drugs.

Freire and Biltonen (77) have shown that the bilayer partition function can be numerically calculated from DSC data without assuming a particular model for the transition. From the partition function enthalpy, entropy and volume changes can be calculated. Transitions proceed through the formation of clusters which fluctuate in size. The magnitude of the fluctuations is affected by e.g. the addition of small molecular weight compounds to the system.

DSC-work on biological membranes has concentrated on material from E- coli and Acholeplasma laidlawii and on erythrocyte ghosts. Both protein and lipid transitions are observed. Such work has provided us with information about changes in structure and fluidity and about interactions between membrane components and between them and drugs, all factors believed to be of importance with the biological function of membranes, cf. recent papers by Brandts and coworkers, see e.g. (78).

In addition to the high sensitivity DSC instruments of the adiabatic shield type (63)-(65), (67), (68) and the heat conduction type (66), (69), there have recently been promising uses in the lipid field of instruments utilizing the ac principle, see e.g. (79), (80). In ac calorimeters amplitudes of periodic heat pulses are measured. These instruments have a very high resolution of heat capacity differences (0.01%) and transition temperatures (10^{-3} K). However, as yet the accuracy in the C_p determinations appears to be low.

APPENDIX

In the biochemistry section of the IUPAC Conference on Chemical Thermodynamics in London in September, 1982, the following poster presentations were made:

1. G. Barone, P. Cacace, G. Castronuovo, D. Doucas, V. Elia, Naples, Italy:
   Excess thermodynamic properties of aqueous solutions of monosaccarides and derivatives at 298.15 K.

2. A. E. Beezer, London, United Kingdom:
   Thermodynamic parameters for transfer of resorcinol monoethers from water to 1-octanol: Oscillations in values of Gibbs energies for transfer processes.

3. S. R. Caplan, Rehovot, Israel:
   Thermodynamics of active transport.

4. A.-t. Chen and I. Wadsö, Lund, Sweden:
   Simultaneous determination of ΔG, ΔH and ΔS by an automatic microcalorimetric titration method.

5. M. E. Daniels, K. G. Davis, S. H. Gaffney, T. H. Lilley and D. R. Tester, Sheffield, United Kingdom:
   Aqueous solutions containing alkali metal halides and N-acetylglycinamide or glycine - the use of model compounds in rationalising the Hofmeister series.

6. Erich Gnaiger, Plymouth, United Kingdom (Innsbruck, Austria):
   Biochemical composition and calorific value of biological materials: stoichiometric extrapolations from C, H, N analyses.

7. A. G. Haglund, N. V. B. Marsden, Uppsala, Sweden:
   Affinity of alcohol for aqueous sephadex G-15; a quadratic relation between free energy and carbon number.

8. M. Lüscher-Mattli, Bern, Switzerland:
   Thermodynamic functions of biopolymer hydration.

9. J. Skerjanc, M. Petrovic, A. Fabjan, Ljubljana, Yugoslavia:
   Enthalpy of dilution of polystyrensulphonates in N-methylformamide.

REFERENCES

1. J. N. Jones (ed.), Biochemical Thermodynamics, Elsevier, Amsterdam (1979).
2. A. E. Beezer (ed.), Biological Microcalorimetry, Academic Press, London (1980).
3. F. Franks in Ref (1), pp. 15-74.
4. E. J. King, Acid Base Equilibria, Pergamon Press, London (1965).
5. I. Wadsö, Pure & Appl Chem. 52, 465-477 (1980).
6. F. Franks, M. Pedley, and D. S. Reid, J. Chem. Soc. 72, 359-367 (1975).
7. J. J. Savage and R. H. Wood, J. Soln. Chem. 5, 733-750 (1976).
8. F. Franks and D. S. Reid in Water, vol. 2 (F. Franks, ed.) pp 323-380. Plenum Press, London, (1973).
9. S. J. Gill, N. F. Nichols and I. Wadsö, J. Chem. Thermodynamics 8, 445-452 (1976).
10. S. J. Gill and I. Wadsö, Proc. Natl Acad. Sci. USA 73, 2955-2958 (1976).
11. N. Nichols, R. Sköld, C. Spink, J. Suurkuusk and I. Wadsö, J. Chem. Thermodynamics 8, 1081-1093 (1976).
12. J. P. Guthrie, Can. J. Chem. 55, 3700-3706 (1977).
13. G. Perron and J. E. Desnoyers, Fluid Phase Equilibria 2, 239-262 (1979).
14. S. Cabani, S. T. Lobo and E. Matteoli, J. Soln. Chem. 8, 5-9 (1979).
15. J. M. Sturtevant, Proc. Natl Acad. Sci. USA 74, 2236-2240 (1977).
16. P. D. Ross and S. Subramanian, Biochemistry 20, 3096-3102 (1980).
17. Y. N. Lian, A.-t. Chen, J. Suurkuusk and I. Wadsö, Acta Chem. Scand. A36 (1982), in press.
18. B. B. Benson, D. Krause, Jr. and M. A. Peterson, J. Soln. Chem. 8, 655-690 (1979).
19. S. J. Gill and I. Wadsö, J. Chem. Thermodynamics 14 (1982), in press.
20. S. J. Gill, N. F. Nichols and I. Wadsö, J. Chem. Thermodynamics 7, 175-183 (1975).
21. S. O. Nilsson and I. Wadsö. To be published.
22. S. J. Gill and M. L. Seibold, Rev. Sci. Instrum. 47, 1399-1401 (1976).
23. D. S. Reid in Ref. (1), pp. 168-144.

24. A. Cooper and C. A. Converse, Biochemistry 15, 2970-2978 (1976).
25. A. Cooper, FEBS Letters 100, 382-384 (1979).
26. C. Spink and I. Wadsö in Methods of Biochemical Analysis 23, 1-159. Wiley, New York (1976).
27. G. B. Barisas and S. J. Gill in Ann. Rev. Phys. Chem. 29, 141-166 (1978).
28. N. Langerman and R. L. Biltonen in Methods in Enzymology 61, 261-286. Academic Press, New York (1979).
29. R. L. Biltonen and N. Langerman in Methods in Enzymology 61, 287-318. Academic Press, New York (1979).
30. M. N. Jones and H. A. Skinner, Ann. Rep. Progr. Chem. Sect. C. Phys. Chem. Roy. Soc. Chem.76, 253-284 (1979).
31. K. S. Krishnan and J. F. Brandts, Methods in Enzymology 49, 3-14. Academic Press, New York (1978).
32. S. J. Gill in Ref. (1), pp. 224-255.
33. M. Eftink and R. L. Biltonen in Ref. (2), pp. 343-412.
34. G. Rialdi in Ref. (35). In press.
35. M. A. V. Ribeiro da Silva (ed.), Thermochemistry Today and its Role in the Immediate Future, Reidel, Dordrecht, in press.
36. P. L. Privalov, Adv. in Prot. Chem. 33, 167-241 (1979).
37. W. Pfeil and P. L. Privalov in Ref. (1), pp. 75-115.
38. P. L. Privalov in Ref. (2), pp. 413-451.
39. W. Pfeil, Molecular and Cellular Biochem. 40, 3-28 (1981).
40. P. Mateo, in Ref. (35). In press.
41. P. L. Privalov and V. V. Filimonov, J. Mol. Biol. 122, 447-464 (1978).
42. H.-J. Hinz in Ref. (1), pp. 116-167.
43. H. Klump in Ref. (35). In press.
44. S. Mabrey and J. M. Sturtevant, Methods in Membrane Biology 9, 237-274 (1978).
45. M. N. Jones in Ref. (1), pp. 185-223.
46. D. Bach and D. Chapman in Ref. (2), pp. 275-309.
47. P.-H. Yang och J. H. Rupley, Biochemistry 12, 2654-2661 (1979).
48. A. E. Beezer, W. H. Hunter, R. P. Lipscombe, R. D. Newell, and D. E. Storey, Thermochemica Acta 55, 345-349 (1982).
49. A.-t. Chen and I. Wadsö, J. Biochem. Biol. Methods 3, 1982 (in press).
50. D. Mountcastle, E. Freire and R. L. Biltonen, Biopolymers 15, 355-371 (1976).
51. J. Suurkuusk and I. Wadsö, Chemica Scripta (1982), in press.
52. P. Monk and I. Wadsö, Acta Chem. Scand. 22, 1842-1865 (1968).
53. R. B. Spokane and S. J. Gill, Rev. Sci. Instr. 52, 1728-1733 (1981).
54. B. G. Barisas, J. M. Sturtevant and S. J. Singer, Biochemistry 10 2816-2821 (1971).
55. P. Soares de Araujo, M. Y. Rossenau, J. M. H. Kremer, E. J. J. van Zoelen and G. H. de Haas, Biochemistry 18, 580-586 (1979).
56. G. C. Kresheck, K. Kale and M. D. Vallone, J. Colloid Interface Sci. 73, 460-466 (1980).
57. C. D. Barry, D. R. Martin, R. J. P. Williams and A. V. Xavier, J. Mol. Biol. 84, 491-502 (1974).
58. F. R. N. Gurd and T. M. Rothgeb, Adv. Prot. Chem. 33, 73-165 (1979).
59. J. L. Finney, B. J. Gellatly, J. C. Golton and J. Goodfellow, Biophys. J. 32, 17-33 (1980).
60. G. C. Pimentel and A. L. McClellan, Ann. Rev. Phys. Chem. 22, 347-385 (1971).
61. J. Janin and C. Chothia, Proc. FEBS Meeting 52, 227-237 (1979).
62. J. Suurkuusk, Acta Chem. Scand. Ser. B. 28 , 409-417 (1974).
63. S. J. Gill and K. Beck, Rev. Sci. Instr. 36, 374-376 (1965).
64. T. Y. Tsong, R. P. Hearn, D. P. Wrethall and J. M. Sturtevant, Biochemistry 9, 2666-2677 (1970).
65. W. M. Jackson and J. F. Brandts, Biochemistry 9, 2294-2301 (1970).
66. P. D. Ross and R. N. Goldberg, Thermochim. Acta 10, 143-151 (1974).
67. M. Grubert and Th. Ackermann, J. Phys. Chem. 93, 255-264 (1974).
68. P. L. Privalov, V. V. Plotnikov and V. V. Filimonov, J. Chem. Thermodyn. 7, 41-47 (1975).
69. J. Suurkuusk, R. B. Lentz, Y. Barenholz, R. L. Biltonen and T. E. Thompson, Biochemistry 15, 1393-1401 (1976).
70. E. J. Tiktopulo and P. L. Privalov, FEBS Letters 91, 57-58 (1978).
71. P. L. Privalov, P. L. Mateo, N. N. Khechinashvili, V. M. Stepanov and L. P. Revina, J. Mol. Biol. 152, 445-464 (1981).
72. P. Privalov, V. Filimonov, T. Venkstern and A. Bayev, J. Mol. Biol. 97, 279-288 (1975).
73. E. Freire and R. L. Biltonen, Biopolymers 17, 463-479 (1978).
74. R. L. Biltonen and E. Freire, CRC Crit. Rev. Biochem. 5, 85-124 (1978).
75. W. Kauzmann , Adv. Protein Chem. 14, 1-63 (1959).
76. S. J. Singer and G. L. Nic olson, Science 175, 720-731 (1972).
77. E. Freire and R. L. Biltonen, Biochem. Biophys. Acta 514, 54-68 (1978).
78. K. A. Lysko, R. Carlson, R. Taverna, J. Snow, and J. F. Brandts, Biochemistry 20, 5570-5576 (1981).
79. O. S. Tanasijczuk and T. Oja, Rev. Sci. Instr. 49, 1545-1548 (1978).
80. S. G. Block and G. S. Dixon, Biochemistry 20, 6740-6744 (1981).