Microcalorimetric Studies of Macromolecules

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The paper demonstrates the possibilities of modern scanning microcalorimetry technique for thermodynamical studies of the principles of organization and stabilization of the unique three-dimensional structure of biological macromolecules.

Interest in calorimetric methods for studying biological macromolecules has rapidly increased during the last few years. One can assume that this is connected with recent progress in the calorimetric technique resulting in the appearance of micro-methods in calorimetry. But in reality this technical progress in itself was stimulated by the rise of new problems in science which could not be solved without measuring infinitesimal heat effects. In this review one of these problems in the field of molecular biophysics will be considered, namely the problem of organization and stabilization of the unique three-dimensional structures of biopolymers.

There is only one experimental approach to this problem. It consists in the disruption of the native structure of a macromolecule and evaluation of the required energy expense. But for such a complex system as are macromolecules, this evaluation cannot be done by treating equilibrium, since this needs knowledge of the mechanism which is just the problem. It is possible to do this only by direct calorimetric measurements of corresponding heat effects. As for the disruption of the macromolecular structure, this can be done by various extremal actions on this system, but the most efficient for a thermodynamical study is heating. This follows from the fact that temperature is a variable, which is conjugated to enthalpy. The linkage relation between these extensive and intensive parameters includes a partition function

$$\Delta H = RT^2 \frac{\partial}{\partial T} \sum \nu_k \ln Q_k \qquad (1)$$

where ν_k is the mole fraction of molecules of type k and Q_k is the corresponding partition function (see Freire & Biltonen, 1978). For the simplest case of one molecule with only two macroscopic states this relation boils down to the Van't Hoff equation

$$\Delta H = RT^2 \frac{\partial}{\partial T} \ln K \qquad (2)$$

which defines the temperature dependence of the equilibrium constant for these states. Thus, if the functional dependence of the enthalpy on the temperature is known, we can have a complete statistical thermodynamic description of a system.

For the macromolecular systems, which can be studied individually only in dilute solutions, the information on enthalpy-temperature relation can be obtained only by means of scanning microcalorimetry. Therefore in this review we will concentrate attention on the possibilities of this method in studying macromolecules.

The scanning calorimeter measures the relative heat capacity of a system as a function of temperature at some temperature range. The main difference between the scanning microcalorimeter and the usual heat capacity calorimeter is in qualitative differences in their sensitivity and precision. This is achieved by continuous heating in adiabatic conditions and by a differential scheme of measurement with automatic compensation of effects (Privalov, 1974). The instrument DASM-1M which is at present produced by the Academy of Sciences of the USSR, permits to measure the relative heat capacity of 1 cm^3 of liquid with an error of less than 10^{-5} cal K^{-1} in a temperature range of 0 to 120°C while the sensitivity to a heat capacity change is at least of an order higher (Privalov *et al.*, 1975). This opens a possibility to observe not only the heat capacity change of dilute solutions of macromolecules at heating and its difference from the solvent (Fig. 1) but also to

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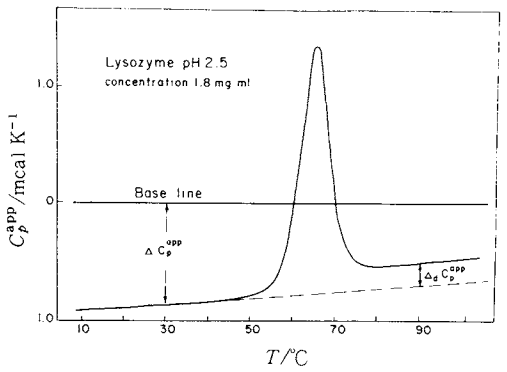

Fig. 1. Scanning microcalorimetric recording of heat capacities of the solvent (base line) and of the dilute solution of protein in this solvent obtained at a heating rate of 1 K min^{-1} on the instrument DASM-1M. Volume of the calorimetric cell – 1.0 cm^3.

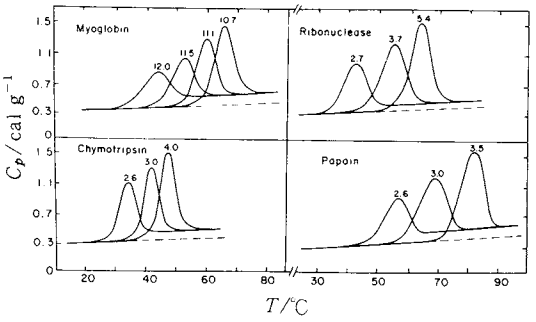

Fig. 2. Temperature dependence of partial specific heat capacity of various globular proteins in solutions with different pH values.

determine at any temperature within this range the partial heat capacity of the macromolecules in dilute solution (Fig. 2).

The presented proteins are typical globular proteins. As is seen, the temperature dependence of their partial heat capacity is not a simple one. Here against the background of a smooth change of heat capacity which can be regarded as trivial, a peak is observed. This intensive heat absorption is connected with the temperature induced conformational transition of protein, their heat denaturation. The denaturation results in a drastic change of many properties of a protein. One of these properties is the heat capacity which is essentially larger for a denatured protein than for a native one. The denaturational changes of the specific heat capacity $\Delta_d C_p$ do not depend on pH and seem to be independent of temperature, but it is very specific for the given protein.

Assuming that all the excess heat capacity ΔC_p, which is above the "trivial" heat capacity, corresponds to the heat effect of a temperature induced process, it is evident that the total heat effect of this process will be equal to the integral of ΔC_p. Thus, for the calorimetrically determined molar enthalpy of denaturation we will have:

$$\Delta_d H^{cal} = MQ_d = M\int_T \Delta c_d \, dT = \int_T \Delta C_p \, dT \quad (3)$$

where c_p and C_p correspond to the specific and molar partial heat capacity.

The evaluation of the expression (3) includes some complications connected with the estimate of the "trivial" heat capacities in a transition zone, especially when they are different for the native and the denatured states (Privalov & Khechinashvili, 1974). But this difference in heat capacities $\Delta_d C_p$ is in itself a very important characteristic of a process, since it is nothing else than the temperature derivative of the enthalpy of denaturation

$$\Delta_d C_p = \frac{\partial \Delta_d H}{\partial T} \quad (4)$$

Thus if $\Delta_d C_p$ is known, the enthalpy of transition can be calculated for any temperature far from the midpoint of transition T_{tr}:

$$\Delta_d H(T) = \Delta_d H(T_{tr}) - \int_T^{T_{tr}} \Delta_d C_p \, dT \quad (5)$$

Having in mind that a temperature induced process is necessarily connected with heat absorption, it is evident that the relative amount of heat, absorbed at a given temperature $\theta(T) = Q(T)/Q_d$ can be regarded as a direct measure of progress of the temperature induced reaction. For a simplest case when there are only two states, we have for the equilibrium constant:

$$K = \frac{\theta(T)}{1-\theta(T)} = \frac{Q(T)}{Q_d - Q(T)} \quad (6)$$

Substituting it in equation (2), we get for the Van't Hoff or the effective enthalpy of the denaturation:

$$\Delta_d H^{eff} = \frac{RT^2}{\theta(1-\theta)} \frac{d\theta}{dT} \quad (7)$$

For the calorimetric curve

$$\frac{d\theta}{dT}=\frac{1}{Q_d}\frac{dQ(T)}{dT}=\frac{\Delta c_p}{Q_d} \qquad (8)$$

i.e. it is nothing else than the normalized intensity of heat absorption or the normalized excess heat capacity at a given temperature. Thus, for the effective enthalpy of transition at a given temperature we have:

$$\Delta_d H^{eff}(T)=\frac{RT^2}{\theta(1-\theta)}\frac{\Delta C_p(T)}{Q_d} \qquad (9)$$

and for the middle of transition, where $\theta=1/2$ and $T=T_{tr}$

$$\Delta_d H^{eff}(T_{tr})=4RT^2\frac{(\Delta C_p)_{tr}}{Q_d} \qquad (10)$$

Here $(\Delta C_p)_{tr}$ is the height of the heat absorbance peak at the midpoint of transition which is close to the temperature of a maximum, but equals it only for the symmetric peak (Privalov & Khechinashvili, 1974).

If the considered process is indeed of a two-state transition type, the effective enthalpy should be equal to the real or calorimetric one, *i.e.* the ratio

$$\frac{\Delta_d H^{cal}}{\Delta_d H^{eff}}=\frac{MQ_d^2}{4RT_{tr}^2(\Delta C_p)_{tr}} \qquad (11)$$

should be equal to 1.

It is noteworthy that the right side of eq. (11) is completely determined by the shape of the heat capacity peak, since Q_d corresponds to its area and $(\Delta C_p)_{tr}$ to its height. Thus, from the analysis of the shape of a heat capacity curve we can unequivocally decide whether the considered process can be regarded as a two-state transition or not. The multi-state transition can be analyzed in detail using the general eq. (1). As has been shown recently by Freire & Biltonen (1978), it permits a numerical evaluation of the thermodynamical characteristics for all the intermediates.

It should be emphasized, to avoid misunderstanding, that this kind of analysis of a temperature induced process is possible only with the enthalpy function of temperature. No other characteristic of a state and no other "melting profile" except that of the heat capacity can be used for this.

An analysis of heat capacity profiles of temperature induced transition of biological macromolecules reveals that the ratio (11) is indeed very close to 1 in the case of heat denaturation of small globular proteins (Fig. 3). The deviation from 1 is about 5% (Privalov & Khechinashvili, 1974). It follows that there are some intermediates at transition of these proteins from the native to the denatured states, but the concentration of these intermediates is small, *i.e.* all intermediates are unstable thermodynamically. Thus, globular proteins are highly cooperative systems which have only two stable macroscopic states – the native and the denatured.

But there are many exceptions from this statement even among globular proteins. It has been shown that the ratio (11) is equal to 2 for such typical globular proteins as is papain (Tiktopulo & Privalov, 1978) and Bence-Jones protein (Zavyalov *et al.*, 1977) and it amounts to 3 for the elongation factor EF–G (Alakhov *et al.*, 1978). This is quite an incomplete list of exceptions from the two-state behaviour. A careful consideration of all the known exceptions in proteins reveals that they always consist of several subunits. Indeed, papain has a deep cleft which bisects its compact structure into two rather equal parts (Drenth *et al.*, 1970). The EF–G includes three nuclei highly stable to proteolysis (Alakhov *et al.*, 1978). As for Bence-Jones protein, it consists of 4 very similar domains (Edleman, 1970) and we can assume that they are arranged into pairs, which present just a cooperative unit.

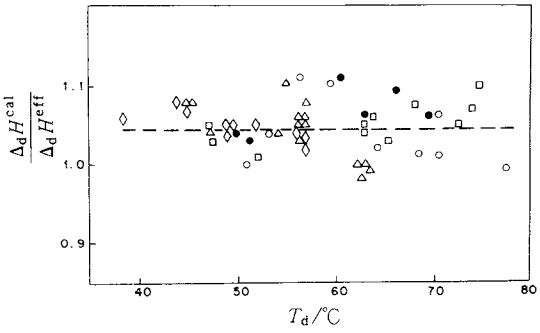

Fig. 3. The ratio of the calorimetric and the effective enthalpies of denaturation of five different globular proteins: metmyoglobin (●), α-chymotrypsinogen (◇), ribonuclease A (△), lysozyme (□), cytochrome c (○). (Privalov & Khechinashvili, 1974).

Since the subunits in each of the considered proteins are thermodynamically very alike, they undergo a conformational transition in the same temperature range and the resulting melting curve is not more complex than it is for the proteins consisting of one subunit. This can be seen by comparing the melting profile for papain (Fig. 2) with that of other proteins which represent a single system. In the case of papain, the subunits manifest themselves only in the inconsistency of the area of a peak (the calorimetric enthalpy) with its sharpness (the effective enthalpy) predicted by eq. (11). The other situation is when protein subunits are thermodynamically different and melt in a different temperature range. In this case the peaks of individual transitions do not coincide and the observed overall heat capacity profile is a complex one. Fig. 4 illustrates just this case. The complex curve of heat absorption at heating troponin C can be easily decomposed into two constituents. For each of them the ratio of the calorimetric and effective enthalpy (*i.e.* eq. (11)) is close to 1. Thus, we conclude that there are two cooperative subunits with different stabilities in troponin C. A more complex case is presented by fibrinogen. It has been shown firstly by Donovan & Mihalyi (1974) that fibrinogen at heating absorbs heat at two different ranges: 55–65°C and 90–100°C. The heat absorption at lower temperatures corresponds to the melting of its D fragment, while that at higher temperatures corresponds to the melting of the E fragment. But a detailed investigation of the melting profiles carried out at our laboratory shows that the ratio (11) for the first transition is equal to 6, while it is 2 for the second. Having in mind that fibrinogen has two D fragments and one E, we come to the conclusion that the E fragment consists of two independent cooperative subunits, while the D fragment consists of three.

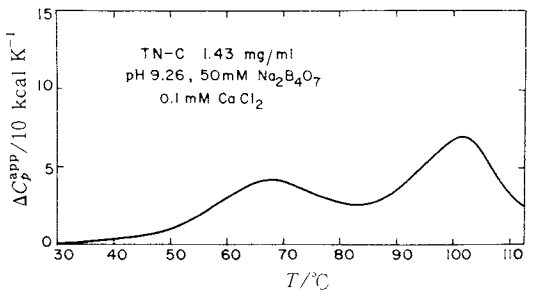

Fig. 4. Scanning calorimetric recording of heat absorption at heating troponin C.

A complex melting profile is typical for fibrillar proteins such as tropomyosin (Potekhin & Privalov, 1978a), L-meromyosin (Potekhin *et al.*, 1978), paramyosin (Potekhin & Privalov, 1978b) (see Fig. 5). It evidences that these long superhelical molecules consist of blocks with different stabilities. Most interesting is that the proteolytic enzymes split the polypeptide just between the blocks, revealing that the boundary is a weak point in the structure. Using proteolytic fragmentation, it is possible to separate individual blocks and even to locate them along the macromolecule (Fig. 6).

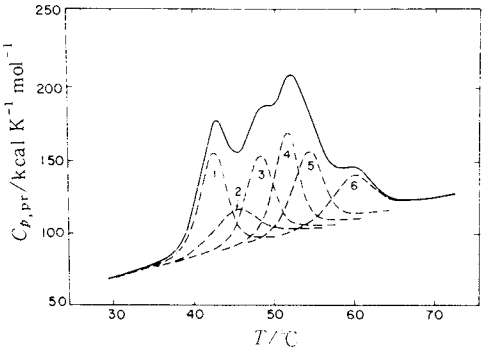

Fig. 5. Temperature dependence of heat capacity of L-meromyosin (Potekhin & Privalov, 1978b).

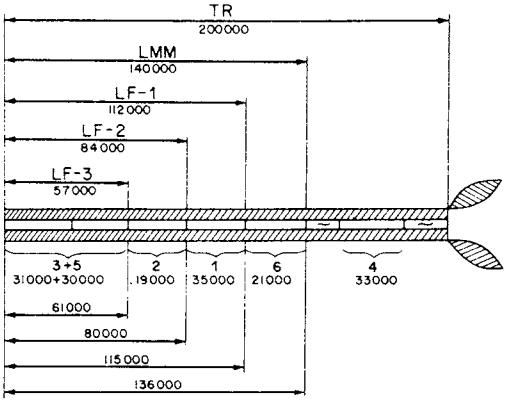

Fig. 6. Proteolytic fragmentation of myosin and the distribution of cooperative blocks along the L-meromyosin. The numbers indicate the sequence of melting in Fig. 6 (Potekhin & Privalov, 1978b).

A complex melting profile is observed also for nucleic acids (Fig. 7). Among them the smallest ribonucleic acids, the transfer RNAs are the best studied thermodynamically. They were studied most intensively since the sequences for many of them are known, while for the phenylalanyl specific transfer RNA we know even the three-dimensional structure which is assumed to be common also for other specific transfer RNAs.

It is very typical for transfer RNAs that their heat capacity profiles can be decomposed into simple constituents for which the ratio (11) is equal to 1 (Fig. 8). At a change of the solvent conditions, these constituents shift independently in the temperature scale, changing the observed overall melting profile. It follows that the unfolding of transfer RNA includes several independent steps, and each of these steps is a two-state transition (Privalov *et al.*, 1975; Privalov & Filimonov, 1978). These steps can be identified analyzing the correlation between their thermodynamic characteristics and the structural characteristics of the elements of tRNA structures. The results of analysis of several transfer RNAs are presented in Fig. 9. It is remarkable that in all cases the first transition corresponds to the unfolding of the tertiary structure of transfer RNA. After this first step, the compact structure of transfer RNA unfolds into a topologically flat

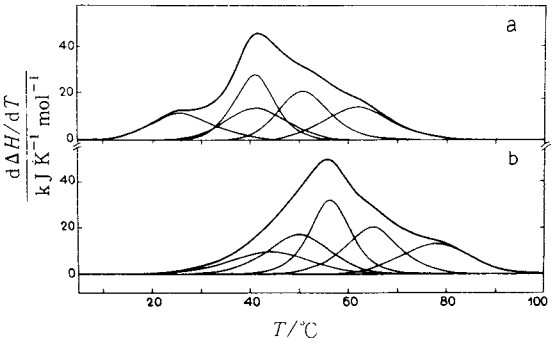

Fig. 8. Excess heat absorption at heating $tRNA^{Phe}$ at two different solvent conditions: (a) – 20 mM NaCl, (b) – 150 mM NaCl in a 5 mM NaH_2PO_4/NaOH buffer. The observed complex curve is decomposed into simple constituents representing two-state transitions (Privalov & Filimonov, 1978).

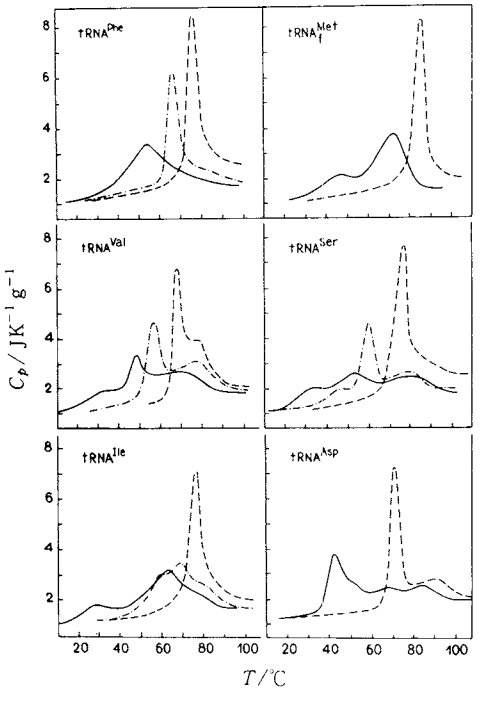

Fig. 7. Partial heat capacities of various specific transfer RNA at different salt content. —— 150 mM NaCl; —·—150 mM NaCl and 1 mM $MgCl_2$; — — — 1 mM $MgCl_2$ (Privalov & Filimonov, 1978).

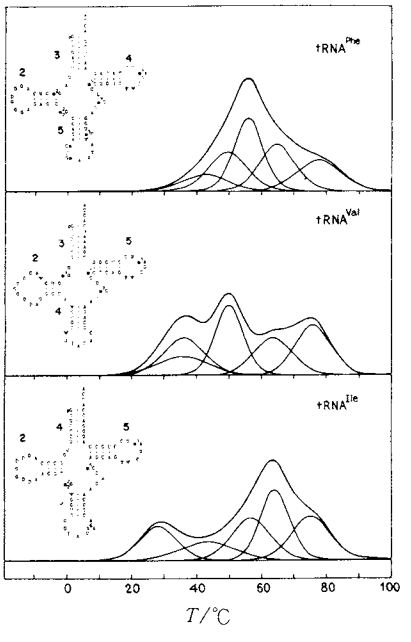

Fig. 9. Melting profiles and nucleotide sequences of various specific tRNAs. Numbers on the branches of the cloverleaf structure indicate the sequence of melting (Privalov & Filimonov, 1978).

cloverleaf structure with separate branches which melt independently of each other. This correlation analysis leads also to the estimation of thermodynamical characteristics for the elementary interactions in tRNA which are responsible for maintaining the native structure, *i.e.* to the estimation of base-base interactions. The data obtained by the analysis of six specific transfer RNAs are presented in Table I.

It is very attractive to do a similar analysis for proteins and to obtain characteristics of the interactions responsible for maintaining their three-dimensional structure. On the one hand, this is favoured by the fact that we know more structures of proteins than structures of nucleic acids. But on the other hand, in studying proteins we meet several serious difficulties. The first is that the cooperative regions for proteins are much larger than those in small nucleic acids. Usually they consist of about 100–300 residues, while each cooperative region in transfer RNA includes less than 20 bases. The second is that there are many more variations in intermolecular interactions in proteins than in nucleic acids. In nucleic acids the interaction is mostly between two different pairs of bases, while in the proteins there are about 20 different residues which interact in various combinations. The last and most serious is that the conformation of the denatured state, in contrast to the native structure, is much less certain for proteins than for nucleic acids. At present there is no doubt that nucleic acids have a random coil conformation at a sufficiently high temperature. But there are still many debates about the denatured state of proteins. According to many authors, protein unfolds completely only in concentrated GuHCl or urea solutions, but heat denatured protein has a residual structure (Tanford, 1968; Tanford & Aune, 1970). It is evident that in this case it is impossible to define intramolecular interactions in the native structures by measuring the enthalpy of heat denaturation.

But in spite of many indirect evidences of the existence of the residual structure in the denatured protein, calorimetrically it has not been found. It has been shown by Pfeil & Privalov (1976) that the enthalpy of protein denaturation is quite the same function of temperature for any kind of denaturation (being the heat, the pH or the GuHCl denaturation) if the ionization and the solvation effects are correctly taken into account (Fig. 10). It follows that if there are residual structures, their enthalpic contribution should be the same for any kind of denaturation. But since there is forcible evidence that a polypeptide in concentrated GuHCl is in a random coil conformation (Tanford, 1968), we had to conclude that the contribution of the residual structure into a heat

Table I Hydrogen Bonds and Contacts between Non-Polar Groups in Proteins

Protein	Molecular weight M (dalton)	Number of internal hydrogen bonds, N_H	$\frac{N_H}{M}=n_H$ ($\times 10^3$)	Number of non-polar contacts N_φ	$\frac{N_\varphi}{M}=n_\varphi$ ($\times 10^3$)	Observed change of heat capacity at denaturation $\Delta_d c_p$ (cal K^{-1} g^{-1})
Ribonuclease	13,600	81	6.0	90	6.6	0.090
Parvalbumin	11,500	71	6.2	71	6.2	0.090
Lysozyme egg white	14,300	89	6.2	108	7.5	0.100
Lysozyme T4	18,600	98	5.3	138	7.4	0.110
Papain	23,400	139	5.9	204	8.7	0.120
α-chymotrypsin	25,200	173	6.9	238	9.4	0.120
Trypsin	23,800	165	6.9	215	9.0	0.125
Cytochrome b_5 fragment 85 res.	10,200	59	5.8	105	10.3	0.130
Cytochrome C	12,400	70	5.6	136	11.0	0.135
Myoglobin	17,900	133	7.4	213	11.9	0.155
Pancreatic trypsin inhibitor	6,500	28	4.3	49	7.5	0.110

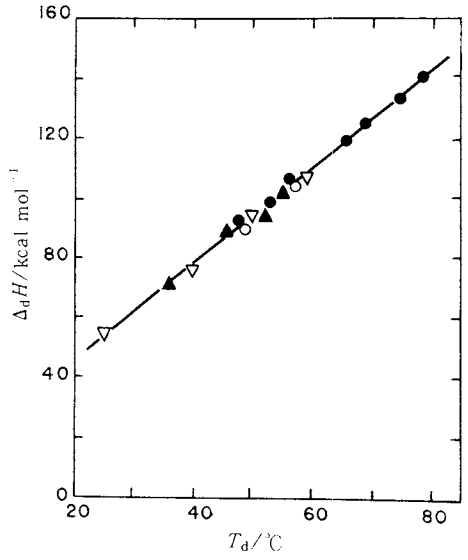

Fig. 10. Enthalpies of lysozyme denaturation obtained by various methods and at different conditions plotted against the temperature of denaturation. In solutions without GuHCl: ●–denaturation by temperature at fixed pH, ○–denaturation by pH at fixed temperatures. In solutions with GuHCl: △–denaturation by temperature at fixed concentration of GuHCl, ▽–denaturation by GuHCl at fixed temperatures and pH (Pfeil & Privalov, 1976).

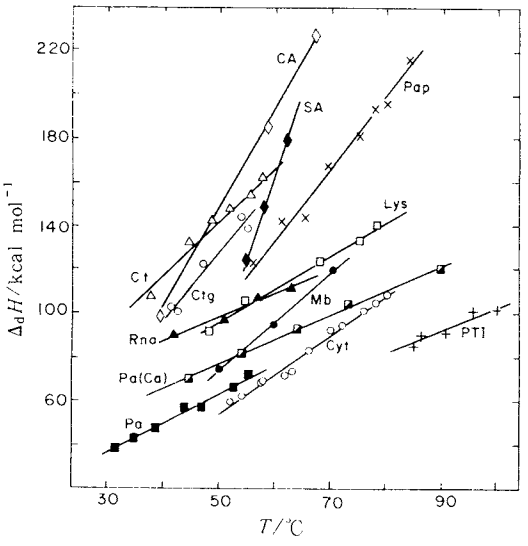

Fig. 11. Temperature dependences of the molar enthalpies of denaturation for proteins: ribonuclease A (Rna), parvalbumin (Pa), lysozyme (Lys), α-chymotrypsin (Ct), carbonic anhydrase B (CA), cytochrome C (Cyt), metmyoglobin (MB), papain (Pap), serum albumin (SA), pancreatic trypsin inhibitor (PTI).

denatured protein is unnoticeable. This means that the enthalpy of denaturation, and particularly the enthalpy measured at heat denaturation, can be used as a measure of intramolecular interactions in the native state.

A remarkable feature of the enthalpy of protein denaturation is that it is strongly dependent on temperature (Fig. 11). This means, according to eq. (2), that denaturation of protein is accompanied by an essential increase of heat capacity in contrast to nucleic acids, where the denaturational increase of heat capacity is very small and practically no dependence of the enthalpy on temperature can be observed (Filimonov & Privalov, 1978).

As seen in Fig. 11, no regularity can be noticed for the enthalpy functions of globular proteins. Quite a different situation appears if we will consider the specific and not the molar quantities, *i.e.* the enthalpy values calculated per unit of protein mass. The specific enthalpies of all compact globular proteins incline to a definite value at an

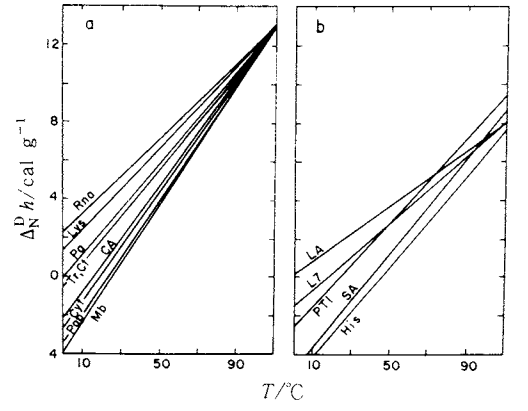

Fig. 12. Temperature dependences of the specific enthalpies of protein unfolding. Designation is the same as in Fig. 11. The other proteins are: trypsin (Tr), chymotrypsinogen (Ctg), lactalbumin (LA), ribosomal protein L7, histone H1 (His). (a) compact proteins; (b) incompact proteins.

increase of temperature up to 110°C; at this temperature they reach 13 cal g^{-1} (Fig. 12a). For the globular proteins with a non-compact structure such as histones, ribosomal proteins, *etc.*, the

specific enthalpy values are lower (Fig. 12b). It seems very likely that the observed regularity is connected with packing of the residues in the protein interior. The correlation analysis of structural and thermodynamic characteristics has been done in our laboratory on several proteins with well-known structures. The obtained results are collected in Table II. Its consideration leads us to the following two conclusions:

(1) The native structures of all proteins for which the enthalpy at 110°C is equal to 13 cal g^{-1} are equally saturated by hydrogen bonds, *i.e.*, the amount of internal hydrogen bonds per unit of mass of these proteins n_H is essentially the same.

(2) Saturation of the structures of these proteins by non-polar contacts, *i.e.*, the number of pairs of non-polar groups located at a distance of up to 0.4 nm, calculated per unit of mass of protein, n_φ, is different for different proteins and correlates with the observed change of specific heat capacity of protein at unfolding (Privalov & Khechinashvili, 1974).

The last fact was not unexpected. Following Kauzmann (1959), it was generally believed that the unfolding of a compact protein structure has to be accompanied by a heat capacity increase as a result of the interaction of non-polar groups with water. If we assume that this influence of non-polar groups on water decreases to zero at 110°C, we can explain the physical meaning of the point where the specific enthalpies of globular proteins coincide. Here the unfolding enthalpy of a compact structure should correspond to the enthalpy of disruption of all the other bonds except the hydrophobic ones involved in maintaining the compact structure. The bonds responsible for this enthalpy might be the hydrogen bonds and the van der Waals' interactions between groups packed in the compact structure. The amount of hydrogen bonds and of van der Waals' contacts are much the same per unit mass of the considered compact proteins. If we assume that the van der Waals' contacts are much less important contributors to the enthalpy of unfolding and attribute all the enthalpy to the disruption of intramolecular hydrogen bonds, we will get 1.7 kcal per mole of hydrogen bonds at 110°C. This value is surprizingly close to the values expected for the interpeptide hydrogen disruption in the water medium (see *e.g.* Kauzmann, 1959). But the real situation does not seem to be so simple. Studies of the denaturation of fibrillar proteins reveal that the enthalpy of unfolding calculated per mole of residues in this linear system, is 30% less than that found in globular proteins (Potekhin & Privalov, 1978a). This means that globular proteins have some additional energy resource which is absent in fibrillar proteins. This might be the van der Waals' interactions which are present in a greater amount in globular structures than in linear ones.

Table II Thermodynamic Parameters of the Base Interaction according to Privalov & Filimonov (1978)

Base Pair	ΔH kJ mol^{-1}	ΔS J K^{-1} mol^{-1}	ΔG^{298} kJ mol^{-1}
G–C	60	150	15
A–U	40	120	4
A–ψ	40	120	4
G–U	30	90	3
G–A	30	90	3

Let us consider briefly the other important thermodynamical characteristics of macromolecules which can be obtained calorimetrically.

The entropy of conformational transition can be estimated at any given temperature by the equation:

$$\Delta_d S(T) = \frac{\Delta_d H(T_{tr})}{T_{tr}} - \int_T^{T_{tr}} \frac{\Delta_d C_p}{T} dT \qquad (12)$$

Having the enthalpy and the entropy functions, we can evaluate the Gibbs energy of transition at any temperature (Privalov, 1974):

$$\Delta_d G(T) = \Delta_d H(T) - T\Delta_d S(T) = \Delta_d H(T_{tr}) \left(\frac{T_{tr}-T}{T_{tr}}\right) - \int_T^{T_{tr}} \Delta_d C_p dT + T\int_T^{T_{tr}} \Delta_d C_p d\ln T \qquad (13)$$

It is evident that just this difference in Gibbs energy corresponds to the work required for the disruption of the native structure at the temperature T, *i.e.* it can be considered as a measure of the stability of this structure.

As can be seen in Fig. 13, there is a great analogy between the enthalpy and the entropy functions, while the Gibbs function is quite different. In contrast to the enthalpy and entropy which increase linearly or practically linearly with the

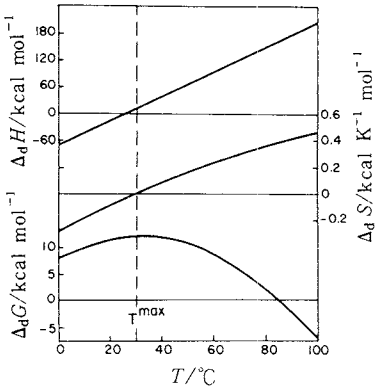

Fig. 13. Enthalpy, entropy and Gibbs energy functions for myoglobin unfolding.

increase of temperature, the Gibbs function has a maximum at room temperatures. At these temperatures of maximum stability the entropy of protein unfolding is zero and the stabilization of the native structure is achieved only by the enthalpy factor. At a lower temperature the enthalpy approaches zero, but here the entropy of unfolding plays already a stabilizing role. Thus, the stabilization of a protein structure is achieved by a small shift of the enthalpy and entropy factors on the temperature scale. But it is remarkable that the stability of very different proteins does not differ greatly (Fig. 14) being (12±5) kcal mol^{-1}. The stabilities of subunits of the large proteins and

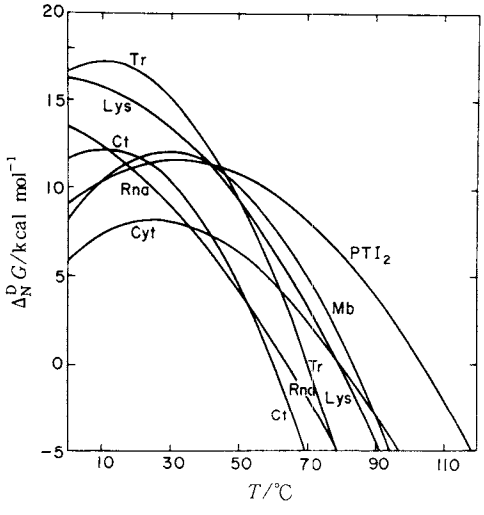

Fig. 14. Temperature dependence of Gibbs energies of stabilization of the native structure of globular proteins. Designation is the same as in Fig. 11.

the nucleic acids are of the same order. For such large systems as are macromolecules this value is not large at all. Indeed, in dividing it by the number of residues or bases constituting the cooperative unit, we will obtain a value which is much less than that of the energy of thermal motion. Thus it becomes evident that integration of elements into a cooperative unit is the most important property of these systems. But it is noteworthy that integration never exceeds the limit which is necessary to achieve some distinct level of stability of the entire system. If the number of elements is larger, they are assembled into separate cooperative units. The reason for such a construction of biological molecules is evident: it is technologically simpler and the whole construction is much more flexible, allowing relative displacements of rigid parts. This might be important for the efficient functioning of these molecular machines against the background of thermal agitation which is extremely intensive at the molecular level.

The presented examples of course do not cover all that has been done with macromolecules by scanning microcalorimetry, but I hope that they demonstrate the principal possibilities of this technique in biophysical studies.

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