THERMAL INVESTIGATIONS OF BIOPOLYMER SOLUTIONS AND SCANNING MICROCALORIMETRY

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1. Introduction

The interest in thermal investigations of biopolymers in solution has greatly increased recently. This is connected firstly with the rise of a number of problems in the physico-chemistry of macromolecular solutions, the solution of which requires direct data on thermal effects of temperature variation, and, secondly, by the increase of technical possibilities in this field and the appearance of a new measuring technique – scanning microcalorimetry.

Though the possibilities of modern scanning microcalorimetry are still insufficiently known, since only a limited number of laboratories have had the equipment at their disposal and the published reviews [1–8] were too laconic, the possibility of direct measurement of the most important thermodynamic parameters – heat capacity, enthalpy and entropy as a function of temperature, the most fundamental physical variable, is, naturally, attractive. Primarily it is of interest to those dealing directly or indirectly with the problems of intramolecular cooperative transformations of biopolymers, the stability of their space structures and the intramolecular interactions, and interactions with the solvent which ultimately determine the structures and structural transformations of biopolymers in solution. The aim of this review is to explain the principle of scanning microcalorimetry and to outline its practical possibilities. To this end we have summarized all the main results obtained up to now in thermal studies of individual macromolecules in solution. Therefore, a whole range of other studies such as thermal investigations of dry preparations [9,10], thermal investigations of the solvent state in solution [11–13] are not included.

The difficulty is that the study of thermal properties of individual macromolecules in solution and of the processes related to their intramolecular transformations must be carried out on diluted solutions (<1%) in which the effects of intermacromolecular interactions are negligible. However, in such solutions the measured heat effects themselves are very small, especially against the background of the heat continuously introduced into the system. Thus, the heat capacity of a biopolymer in 0.3% solution is only a one thousandth part of the total heat capacity of the solution. Therefore, superprecise methods of heat capacity determination over a wide temperature range are necessary. The difficulty is, however, aggravated by the fact that even very dilute solutions of biopolymers have a high viscosity change with temperature. This completely excluded the use of the precise calorimeters usually used for measuring the heat capacity of liquids (see [14]), since stirring was obligatory in such equipment. With solutions of high and inconstant viscosity this led to undeterminable thermal effects which considerably exceed the thermal effect to be determined.

The first measurement of heat connected with macromolecular transformations in solutions – gelatin gel melting – was made in 1962 [15] with a specially elaborated vacuum adiabatic ab-

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solute calorimeter [16]. The apparatus was used in 1963 to measure the heat connected with intramolecular transition – heat denaturation of ovalbumin [17] and serum albumin [18]. In 1964 an analogous apparatus – the precise absolute calorimeter – was used to measure the melting heat of DNA [19] and the heat of conformational transitions in poly-γ-benzyl-glutamate [20]. Later, the absolute calorimeter was also used for investigating the melting of polynucleotides [21] and lysozyme [22].

Though these papers showed the possibility of such measurements in principle, it was obvious that for a detailed study of intramolecular processes a different measuring technique was necessary since the operational volume of absolute calorimeters was too large (from 50 ml [16] to 200 ml [23]) and the minimum operational concentration at which it was still possible to measure heat effects in solutions was too high (up to 2%). Investigations of this kind required heroic preparative work (a gram of preparation per measurement!) while the results obtained did not guarantee their reliability due to the very high concentration of the solutions.

A detailed study of intramolecular processes became possible only after the creation of an apparatus based on a new principle – scanning microcalorimeters. The first model was described in 1964 [24], its sensitivity exceeded that of previous calorimeters by as much as three orders. This undoubtedly was a qualitative step which opened new perspectives in studying macromolecular solutions. During the last ten years a series of communications on such instruments has been published [25–32] forming a special field of thermophysical measurements.

2. Scanning microcalorimetry technique

The distinguishing feature of scanning microcalorimeters which determines their extreme sensitivity is a combination of three factors: a differential scheme of measuring, continuous heating and complete adiabatization of the measuring cell from the outside (see fig. 1). The thermal changes are here reduced either to recording the temperature difference between two identical cells with the solution and solvent heated at the same power [26,32] or the difference in power to equally heat the cells [25,27]. Continuous heating at a constant rate permitted the effective application of compensation methods and servosystems and to pass to an intensive automatization of the process. As for adiabatization, it is necessary not only to remove the disturbing thermal influence of the outside on the measuring cell but also to preserve the internal conditions in the cell itself, such as the temperature and its gradients. If the latter is neglected, the instrument sensitivity decreases considerably. The Perkin–Elmer DSC-1 and the Dupont 900 non-adiabatic scanning calorimeters in which heating at a constant rate is effected by increasing the power with temperature [33] can serve as an example. Though this somewhat simplifies the construction, the sensitivity and precision per unit volume of these non-adiabatic calorimeters is considerably lower than those achieved with adiabatic ones. Attempts to use these instruments for thermal studies of biopolymer solutions have not given completely satisfactory results – the data obtained, even at high concentrations (up to 20%), are rather indistinct and of little use for qualitative analysis [34–37].

The precision of calorimetric measurements is directly connected with the precision of sample filling. For precision determination of the relative heat capacity, the task proved to be far from easy, since it is obvious that the accuracy of chamber filling must be no less precise. Its complexity was aggravated because removal of the calorimetric cell from the adiabatization system considerably worsened reproducibility, while external filling through tubes led to a series of complicated problems due to thermal expansion, vaporization and degassing during the heating process. Before these problems were settled, the precision and the reliability of microcalorimetric measurements remained low in spite of high sensitivity. Therefore in earlier studies the first scanning microcalorimeters were used for registering the heat effects of cooperative transitions, but they proved unsuitable for determining partial heat capacity. Considerable progress in this field

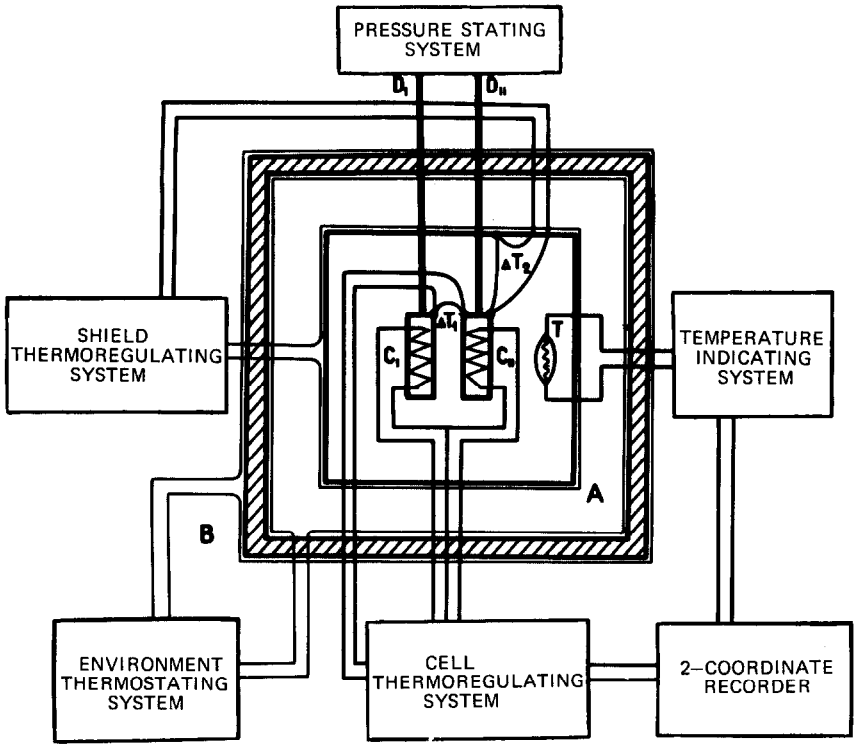

Fig. 1. Block-scheme of the microcalorimeter: A – adiabatizing shield; B – thermostat; C_I and C_{II} – calorimetric cells; D – capillary input; ΔT – thermal sensor.

was achieved only after the elaboration of a non-dismountable calorimetric block with an adiabatization system of thermal shields [30,31]. Utilization of external filling (see fig. 1) with input capillaries and measuring at a constant increased pressure applied by the same tubes allowed greatly increased reliability of the measurements, reproducibility of the results and simplicity of the measurement procedure. As a result elaboration of standard laboratory instruments became possible.

One of the most sophisticated models of the automatic scanning microcalorimeter was recently designed by the Special Bureau of Biological Instrumentation of the Academy of Sciences of the USSR.

The main operating parameters of this instruments are as follows:

Operational temperature range:	0–100°C
Rate of heating:	0.1:2.0 deg/min
Operational volume:	1.0 ml
Sensitivity of heat capacity:	$4 \times 10^{-6} \frac{\text{cal}}{\text{deg·ml}}$
Precision of heat capacity determination of solvent relative to solution:	$2 \times 10^{-5} \frac{\text{cal}}{\text{deg·ml}}$
Precision of temperature recording:	0.1°C

The relative heat capacity is recorded on a two-coordinate recorder in temperature coordinates. The apparatus is equipped with a digital temperature indicator with a programmer cutting off heating at any set temperature.

This instrument is the first commercial preci-

sion scanning microcalorimeter and there is every reason to believe that it will be widely used in physico-chemical studies of solutions, and biopolymer solutions in particular.

3. Calorimetric measurements

A typical microcalorimetric record is given in fig. 2. Curve I is the baseline with a calibration mark obtained by filling of the two chambers with solvent. Curve II was obtained by filling of one of the chambers with a protein solution.

It is easy to see that the heat capacity of the protein solution is considerably lower than that of the solvent and an intensive heat absorption connected with protein denaturation occurs in the region of 70°C. It is possible to determine the denaturation heat of the protein and hence the specific and molar enthalpies of denaturation (Δh_d and ΔH_d) from the area of the denaturation peak above the extrapolated heat capacity values of the native and denaturated protein. It is possible to calculate the partial specific heat of the protein at any temperature by the lowering of the recording line from the baseline Δ_T having in mind that:

$$-\Delta_T = [c]^p_p \cdot m_p - [c]^s_p \cdot \Delta m_s \qquad (1)$$

where $[c]^p_p$ and $[c]^s_p$ are the partial specific heats of the protein and the solvent, m_p is the mass of the protein in the cell and Δm_s is the mass of displaced solvent. Since

$$\Delta m_s = m_p \cdot \frac{[V]^p}{[V]^s} \qquad (2)$$

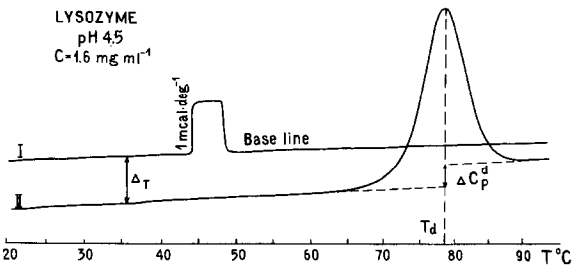

Fig. 2. Microcalorimetric record of heated lysozyme solution.

where $[V]^p$ and $[V]^s$ are the partial volumes of the protein and the solvent, then

$$[c]^p_p = [c]^s_p \cdot \frac{[V]^p}{[V]^s} - \frac{\Delta_T}{m_p} \qquad (3)$$

Knowing the temperature dependence of the partial specific heat we can determine the difference of enthalpy, entropy and free energy of the native and denaturated state at temperatures differing from the temperature of transition T_d since

$$\Delta H(T_0) = \int_{T_0}^{T_d} [c]^n_p \, dT + \Delta H_d + \int_{T_d}^{T_0} [c]^d_p \, dT$$

$$= \Delta H_d - \int_{T_0}^{T_d} \Delta S_p dT \qquad (4)$$

$$\Delta S(T_0) = \int_{T_0}^{T_d} \frac{[c]^n_p}{T} \, dT + \frac{\Delta H_d}{T_d} + \int_{T_d}^{T_0} \frac{[c]^d_p}{T} \, dT$$

$$= \frac{\Delta H_d}{T_d} - \int_{T_0}^{T_d} \frac{\Delta c_p}{T} \, dT \ldots\ldots \qquad (5)$$

$$\Delta G(T_0) \mp \Delta H(T) - T_0 \Delta S(T) = \Delta H_d - T_0 \frac{\Delta H_d}{T_d}$$

$$- \int_{T_0}^{T_d} \Delta c_p dT + T \int_{T_0}^{T_d} \frac{\Delta c_p}{T} \, dT = \Delta H_d \frac{T_d - T_0}{T_d}$$

$$- \int_{T}^{T_d} \frac{T - T_0}{T} \Delta c_p dT \ldots\ldots \qquad (6)$$

where $\Delta c_p = [c]^d_p - [c]^n_p$ is the difference of partial specific heats of the denaturated and the native state. A linear extrapolation of one of the components into the region of temperatures where it cannot be measured experimentally is usually used for its determination.

It is seen from the equations (4), (5) and (6) that not only the thermal effect of the transition but also the partial specific heat of the biopolymer in solution is of great importance in studying its thermodynamic properties.

4. Resutlts

Up to the present the method of scanning microcalorimetry has been used in studying practically all the main classes of biopolymers: globular proteins, fibrillar proteins, nucleic acids and phospholipids. Since the objects, problems and results differ greatly we shall consider them separately.

4.1. *Globular proteins*

As has been mentioned, globular proteins were the first native biopolymers to undergo calorimetric investigation, and the greatest number of calorimetric investigations has been made on them. This was because of the crucial importance of the information obtained by the calorimetric method in elucidating the mechanism of their heat denaturation, and hence the principles of stabilization of their space structure.

Heat denaturation of globular proteins connected with an abrupt change of all the physicochemical properties takes place in a rather narrow temperature range determined by some internal properties of protein and conditions of the external medium. Thermodynamic analysis of this process was usually based on the assumption that here we were dealing with a simple monomolecular chemical reaction when the protein passed from one state to another without intermediate thermodynamically stable states. Using any parameter sensitive to the state of protein to determine the equilibrium constant $k = \frac{\nu}{1-\nu}$ of the native and denaturated form we can calculate the effective enthalpy of transition from the Van 't Hoff equation:

$$\Delta H^{\text{eff}} = -R \frac{\mathrm{d}\ln \frac{\nu}{1-\nu}}{\mathrm{d}(1/T)}$$

The simplicity of such an approach naturally, contributed to its popularity though nobody proved the validity of the Van 't Hoff equation in the process of denaturation, in other words, nobody proved the assumption that denaturation can really be considered as a transition between two states. It was possible to demonstrate this unambiguously only by comparing effective thermodynamic parameters obtained from the sharpness of the transition with the real ones determined by direct calorimetric measurements [38].

The enthalpy of denaturation of globular proteins was calorimetrically determined for the first time in 1963 on ovalbumin [17] and serum albumin [18]. In 1965 the enthalpy of denaturation of ribonuclease was measured [39]. Heat denaturation of ribonuclease was investigated calorimetrically in more detail in 1970 [40]. In the same year the denaturation heats of chymotrypsinogen [41] and lysozyme [22] were determined. In 1971 the results of a detailed microcalorimetric investigation of three globular proteins – chymotrypsinogen, ribonuclease and myoglobin were published [42–44]. These data were later refined with a new precise model of the scanning microcalorimeter which allowed the determination of partial specific heats of globular proteins over a wide range of temperatures [45,46] and to supplement the data on lysozyme [47], chymotrypsin [48], cytochrome [49] and serum albumin [50]. A detailed analysis of these results has been published [51].

Thus, at present there is information on thermal properties of two large globular proteins with an unknown space structure – ovalbumin [17] and serum albumin [18,50] (see also [52]) and very detailed data on six globular proteins with well-known space structures: ribonuclease [39,40,46,51], chymotrypsinogen [41,42], chymotrypsin [48,51], myoglobin [42,43,51], lysozyme [29,47] and cytochrome [49,51]. The distinctive feature of the last is that these proteins are not large (molecular weights from 10000 to 25000), compact and which behave almost completely reversibly to thermal influence. The latter fact is extremely important for a thermodynamic analysis.

It is very noteworthy that despite a considerable difference in size and structure of the globular proteins studied, their thermal properties proved to have much in common.

Fig. 3 represents a typical picture, for globular proteins, of the dependence of partial specific heat on temperature at different pH's, determining thermostability. This picture is for lysozyme [47], but as it is easy to see (fig. 4) it is analogous for other proteins [45]. It follows

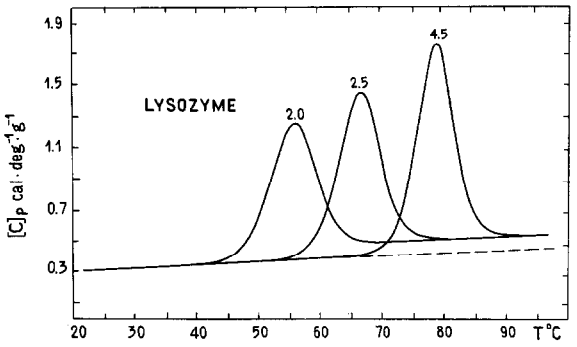

Fig. 3. Temperature dependence of partial specific heats of lysozyme at different pH values.

from the figures that:

1. The initial partial specific heats of native globular proteins are very similar [51].
2. With increasing temperature, the specific heats of all the proteins increase similarly and linearly up to the temperature region at which denaturation heat absorption starts [41,42,51].
3. Specific heat of the denaturated protein is higher than that of the native one. The difference of specific heats Δc_d is different for different proteins [41,42,51].
4. With the growth of thermostability, the enthalpy of denaturation increases and the transition becomes sharper [41,42,51].
5. Effective enthalpies of the transition, determined by the sharpness of the transition according to the Van 't Hoff formula, coincide within the error of determination with the calorimetric enthalpy determined by the peak area [41–49,50].

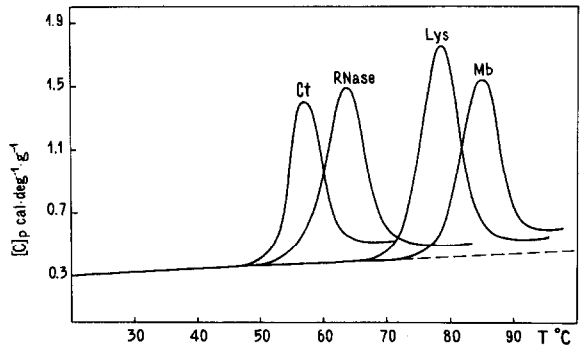

Fig. 4. Temperature dependence of partial specific heats for chymotrypsin, RNase, lysozyme, myoglobin.

It follows from the latter that heat denaturation of globular proteins can be considered to a good enough approximation as a transition between two states – the native and denaturated, while all the intermediate states are thermodynamically unstable [41,42,51]. In other words, heat denaturation is a sharply cooperative process involving the whole globule [11].

The calorimetric data also show that before the process of denaturation a gradual change of the state of the protein molecule occurs with increase of temperature, accompanied by an increase of its specific heat. However, these changes do not have the character of cooperative transitions between macroscopic states with different enthalpies [41,51]. It is more likely that we are dealing here with a redistribution between microstates of the same native macrostate or local fluctuation of structure that can be also interpreted in terms of anisotropic heat expansion of the globule [51,53]. This conclusion is extremely important since lately a great number of papers have appeared where it has been noted that protein molecules undergo transitions of the phase type in the region of physiological temperatures (see e.g. [54,55]).

Thermodynamic functions, characterizing the native state of the protein globule – the difference of enthalpy, entropy and free energy of the denaturated and native states, were determined directly from scanning microcalorimetry data on the temperature dependence of protein partial specific heats using equations (2), (3) and (4) [51]. Temperature dependence of these functions calculated per gram of protein for five globular proteins is given in figs. 5, 6, 7. It is seen from the figures that the differences of enthalpy and entropy increase with increasing temperature. It is noteworthy that these functions intersect in a rather narrow temperature range near 100°C. The dependences of the difference of free energy on temperature have a more complicated shape with a maximum near 20–30°C.

Analysis of the structural parameters of the proteins considered showed [51] that they are somewhat unlike in the saturation of hydrogen bonds and differ greatly in the number of hydrophobic contacts. The number of contacts in pro-

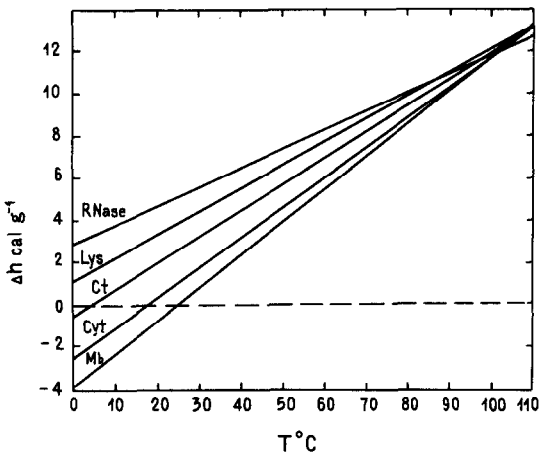

Fig. 5. Temperature dependence of the enthalpy difference of the denaturated and native states of globular proteins: RNase, lysozyme (Lys), chymotrypsin (Ct), cytochrome (Cyt), myoglobin (Mb).

tein correlates directly with the temperature dependence of the enthalpy of transition or the difference of specific heats of the denaturated and native states. Hence it was concluded that the thermal effect of denaturation is mainly determined by two contributions: 1) by the positive contribution of the enthalpy of hydrogen bond disruption, depending little on temperature and being 1.7 kcal/mole at 100°C; 2) by the negative contribution of hydrophobic bond disruption increasing considerably with a decrease of temperature.

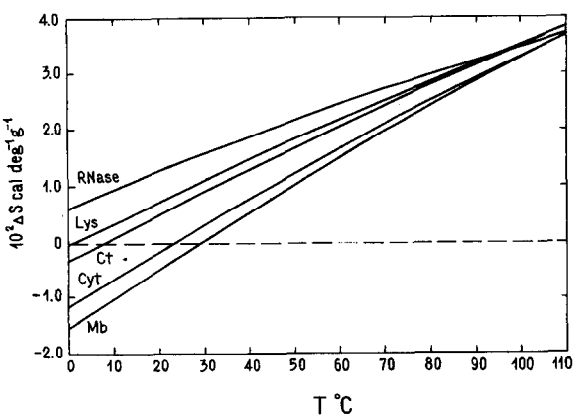

Fig. 6. Temperature dependence of the entropy difference of the denaturated and native states of globular proteins: RNase, lysozyme (Lys), chymotrypsin (Ct), cytochrome (Cyt), myoglobin (Mb).

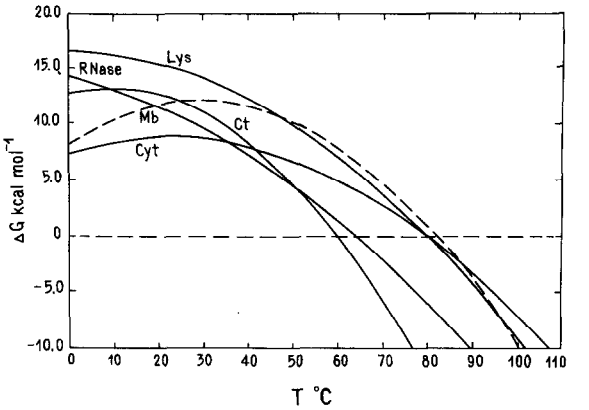

Fig. 7. Temperature dependence of the free energy of stabilization of the native structure of globular proteins: RNase, lysozyme (Lys), chymotrypsin (Ct), cytochrome (Cyt), myoglobin (Mb).

It is rather curious that the denaturational thermal effect of serum albumin proved to be much weaker than the thermal effect observed in proteins with a compact structure [50]. Hence, it could be concluded that not nearly all the H-bonds of this protein form a cooperative region.

4.2. *Fibrillar proteins*

Among the fibrillar proteins studied by the calorimetric method were myosin and tropocollagen.

4.2.1. Myosin

Only one paper [56] was devoted to myosin in which it was shown qualitatively that denaturation of its heavy and light subparticles (H and L meromyosins) proceeds separately in different temperature ranges.

4.2.2. Tropocollagen

Tropocollagen, however, has been very intensively studied. This is partly because it is a convenient object for thermal studies due to the exceptional sharpness of the process of its heat denaturation (see fig. 8). But the main reason was the possibility to clearly formulate the experimental task and to carry out a thermodynamic analysis of existing models. This was determined in the first place by the fact that the final state of tropocollagen, into which it passes

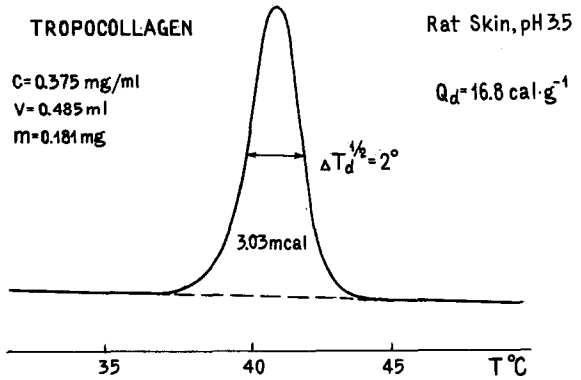

Fig. 8. Microcalorimetric record of heat adsorption on heat denaturation of tropocollagen.

as a result of heat transformations (denaturation), is that of a random coil or, in other words, the standard state for macromolecular systems which could be taken as zero in thermodynamic analysis of space structures. Secondly, there were sufficiently elaborated models for native tropocollagen based on X-ray diffraction analysis which could be described in terms of thermodynamics. Thus, proceeding from model reasons, it was possible to determine changes of thermodynamic parameters for this protein during the transition from the initial (native) into the final (denaturated) state and, comparing them with the experimental data of thermal measurements, to test the existing concepts on the structure and the mechanism of its stabilization.

At present only two models of collagen structure are current: the Rich and Crick model and Ramachandran's [57,58]. In both models collagen is considered as a complex helix of three polypeptide chains in a poly-L-proline helical conformation. According to the Rich–Crick model, three polypeptide chains are linked by one H-bond per triplet in its first position, while in Ramachandran's model besides this bond there also exists a bond in the second position of the triplet. The first position in triplets of all the tropocollagens is occupied mainly by glycine, the second position can be occupied by an imino acid (proline or oxyproline) not forming an H-bond with neighbouring chains. Thus, according to Ramachandran's model, the more imino acids there are in the tropocollagen structure, the less H-bonds there must be, while according to Crick's model their number does not depend on the imino acid content. At the same time, with increasing imino acid content the rigidity of the polypeptide chain increases, determined by the rigidity of the pyrrolidine link. Consequently, the entropy of denaturation must decrease with the growth of imino acids and hence, the thermostability must increase. Thus, the enthalpy of denaturation must either decrease (according to Ramachandran) or be constant (according to Crick) with the growth of thermostability. From this, it is clear that with the available calorimetric data on denaturation enthalpy and entropy of collagens with different imino acid contents a choice can be made between the existing models [59].

The first results of calorimetric investigations of rat skin tropocollagen were published in 1964 [24,60]. A detailed study of tropocollagens with different imino acid contents was carried out in 1968 [61,62]. The very unexpected results were also corroborated later in papers [63,64] and partially in [37] though the precision of measurements in the latter was too low for a quantitative conclusion.

The main results of these studies are given in fig. 9. Despite the theoretical prediction, the enthalpy and entropy of denaturation increases sharply with the increase in thermostability.

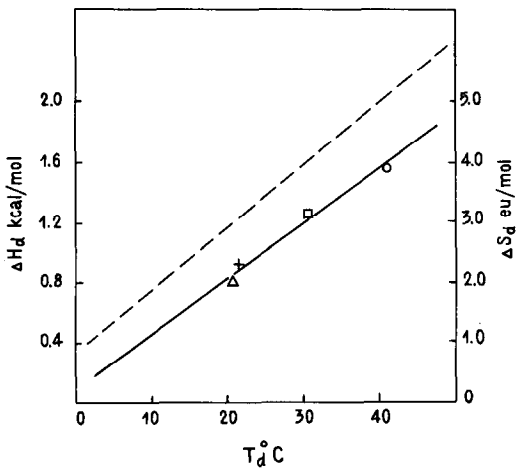

Fig. 9. Dependence of enthalpy and entropy of denaturation of tropocollagens on their thermostability.

Moreover, its value is too great to be explained by a disruption of intramacromolecular hydrogen bonds only. This result emphasized the necessity of revising our concepts on the mechanism of stabilization of the collagen structure at the expense of the internal hydrogen bonds [62,65]. Analysis of the existing possibilities led to the conviction that we are dealing here with some cooperative system including not only the collagen molecule itself but also the adjacent layer of water forming a supporting framework [61,62].

Proceeding from calorimetric data it was also shown there that the tropocollagen molecule is heterogeneous and includes no less than 20 separate cooperative regions [61].

A very interesting fact was established calorimetrically [66–68], on addition of neutral salts the peak of heat absorption in the heated tropocollagen solution doubles while the total area remains the same (see fig. 10).

When the ionic strength and pH approach the physiological, the temperature region of the first peak is also close to the physiological temperature. It was shown [66,69] that the first peak is not connected with denaturation of the elongated tropocollagen molecule but with a certain rearrangement, as a result of which it becomes more compact and its capacity for intramolecular interactions changes – it loses the ability to form liquid crystal structures but acquires a new, especially important, ability to form crystal fibrillar structures of the native type [70,71].

Several papers [72–74] have been devoted to clarification of the details of this biologically extremely important process.

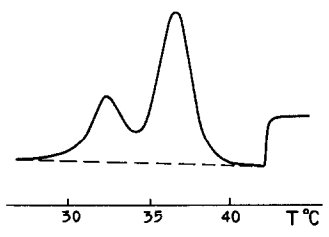

Fig. 10. Heat absorption in salt solution of tropocollagen.

4.3. *Nucleic acids*

The bulk of nucleic acid calorimetric studies has been devoted to the melting of the double helical DNA structure and its synthetic models, while investigations of melting of the tRNA space structure were begun only recently.

4.3.1. DNA

DNA dissociates under the influence of increasing temperature into two polynucleotide chains folded into separate random coils. Since the separation of the complementary chains appeared to be one of the most important events in the process of reduplication of genetic information and the stability of the double-helical conformation is evidently connected with its reliable maintenance, it was natural that both the mechanism of stabilization of the double-helix and the mechanism of its breakdown attracted scientific effort immediately after Watson and Crick discovered the double helical structure of DNA and its biological meaning was realized. Therefore it is not surprising that a great number of studies, experimental and theoretical, was carried out in this field during a very short period of time. Thermodynamic investigations formed an essential part of these studies. Many authors determined the thermodynamic parameters of the transition from the analysis of the process of disruption of the native DNA structure observed by different indices and the change of its stability under the influence of different agents using various approaches and concepts about the mechanism of this phenomenon [75–78]. Naturally, to test these concepts information on the real thermodynamic parameters, obtained by direct calorimetric, measurements, was needed.

The enthalpy of the DNA denaturation was first determined calorimetrically from data on the heats of interaction of the DNA solution with acid at a fixed temperature by Sturtevant and Geidushek in 1958 [79,80]. Later in 1965 the enthalpy was determined with greater accuracy by the same authors [81] with a correction for the protonization heat on acid denaturation. The heat of transition at 25°C and pH 6.0 was evaluated as 8.2 kcal/mole whatever the GC content of DNA. The enthalpy of poly (A+U) com-

plex formation was determined by the same method of isothermal heat measurements and equalled 6 kcal/mole pairs [82], while from the same data with a correction for the degree of helicity it was 8.7 kcal/mole [83]. On urea denaturation of DNA the enthalpy value obtained was 9.5 kcal/mole pairs [84].

The heat of melting of the DNA double helix with increasing temperature was measured for the first time in 1964 in an absolute calorimeter [19] and on a scanning microcalorimeter [24,85]. Measurements of heats of denaturation of T_2 phage DNA was the first work carried out on this precision instrument. Later, with the improvement of the scanning microcalorimetry technique the results became more exact [86]. Our detailed studies of the denaturation of T_2 phage DNA practically ended in 1969 [87,88].

The advantage of studying denaturation of phage DNA was its sharp transition temperature which is essential for measurements of dilute solutions. The operational concentration of solutions can be reduced to 0.05% with an expenditure of only a part of a milligram per experiment [88] (see fig. 11).

Calorimetric measurements of the enthalpy of melting were carried out on DNA of other origins [89] and the influence of the GC composition on the enthalpy was studied. Here the measurements are complicated by the great width of the temperature range of transition (up to 20°). Nevertheless a weak dependence of the melting enthalpy on the GC composition has been shown.

Investigations of melting of model polynucleotide complexes were much simpler experimentally. Their melting temperatures are very sharp due to their homogeneity and at the same time there is no such rigid limitation here in the quantity of the preparation as there is with DNA of natural origin, especially phage DNA. Up to the present very detailed studies of the following polynucleotide complexes have been carried out: poly (A+U) [90–92], poly (I+C) [93,94], poly C and poly I [92], poly A [89].

Fig. 12 represents the dependence of the melting heat on the ionic strength and pH for T_2 phage DNA [88].

It is seen from the results that the heat of DNA denaturation strongly depends on pH, almost does not depend on the ionic strength and, according to the data [89], depends insignificantly on the GC content. The effect of pH is to be expected since denaturation at acidic and basic pH's is accompanied by ionization of the corresponding groups of purine and pyrimidine bases so that the denaturation heat also includes the ionization heats of these groups. At the same time, a change in the ionic strength of the medium at neutral pH does not lead to a change of the denaturation heat, though the temperatures at which denaturation takes place at different ionic strengths are different. Hence it follows that the heat of DNA denaturation, i.e. the difference of enthalpy of the denaturated and native states cannot essentially depend on temperature. This conclusion is also corroborated by the fact that no change of the specific heat of the system on denaturation was observed experi-

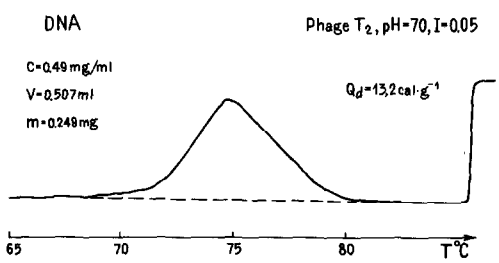

Fig. 11. Heat absorption in heated solution of T_2 phage DNA.

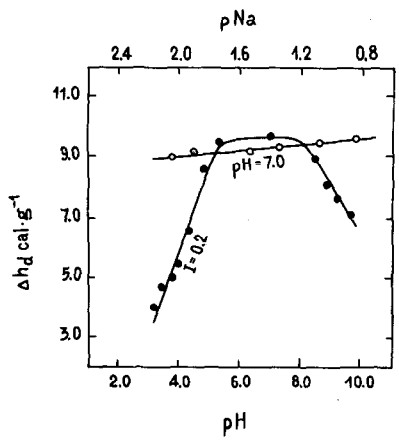

Fig. 12. Dependence of the enthalpy of melting of the T_2 phage DNA double helix on pH and ionic strength.

mentally. Judging by the microcalorimetric data, the specific heats, of native and completely denaturated DNA are practically the same and, hence, the enthalpy of transition must also depend little on temperature.

The equality of the specific heats of native and denaturated DNA greatly simplifies the determination of the free energy of stabilization of its native structure according to equation (4).

ΔG_i values at 37°C determined when $\Delta c_p^d = 0$ as a function of pH and ionic strength are given in fig. 13. These results allowed an analysis of the mechanism of the effect of ionic strength on stabilization of the DNA native structure to be made [88]. It was shown that a change of ionic strength of the solution does not lead to a change of the electrostatic interaction energy of the charged phosphate groups of the DNA molecule, since the number of counter-ions forming a 'coat' around the macromolecule does not change [93,94]. Therefore, the dependence of the free energy difference of the denaturated and the native states on the ionic strength is not determined by electrostatic interaction, but by the free energy of mixing of counter-ions which have passed on denaturation from the 'coat' into the solution

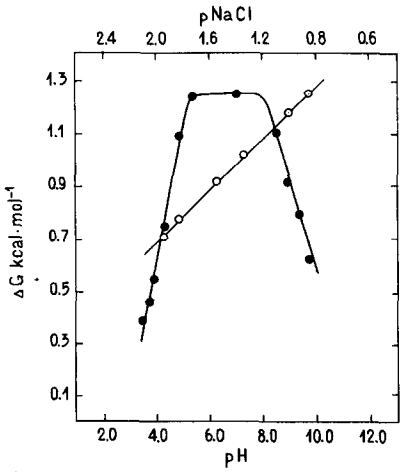

Fig. 13. Dependence of the free energy of stabilization of the T_2 phage DNA double helix on pH and ionic strength.

and hence denaturation must be less favorable [95].

4.3.2. RNA

Calorimetric studies have only been carried out on tRNA despite the fact that it is one of the least accessible RNAs. This is because it has been the most intensively studied by different methods. The primary structure of many of its types was known and there were definite indications of its spatial organization. Therefore, the thermal data could be used with great benefit here for a quantitative analysis of the tRNA space structure and the factors stabilizing it.

The first investigations of tRNA melting carried out on a scanning microcalorimeter [96,97] showed that the melting curve of mixed tRNA shows a rather complicated character. Enriching by valine-specific tRNA led to an even more complicated melting profile. According to the authors, the complicated curve can be separated into 5 component peaks, the first of which is attributed by them to the melting of the tertiary structure (20% of the total thermal effect, equal to 9.8 cal/g) while the others are attributed to the melting of four helical regions in the tRNA structure. However, this division gives rise to great doubts since the areas denoted of the separate peaks do not accord in any way with their widths.

According to the data [98] where melting of tyrosine and phenylalanine tRNA at high magnesium content was studied, the melting of the space structure proceeds as a single process. Moreover, judging by the coincidence of the effective and calorimetric enthalpy it can be presumed that it proceeds as a two state transition.

4.4. *Lipids*

The investigation of lipids and biomembranes by the method of scanning microcalorimetry seems very promising. Though only qualitative results have been obtained on these systems [99] due to their complexity. There is, however, every reason to believe that the thermal effect of cooperative temperature transitions characteristic of these systems will yield much information about their physical properties. A review of the

results obtained in this field has recently been published [100].

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