"INTRAMOLECULAR" HEAT OF FUSION OF MACROMOLECULES

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Many macromolecules are characterized by a high degree of intramolecular ordering. Various treatments lead to sharp changes in their structure, involving disordering. A microcalorimetric method is described which can be employed to measure the amount of heat absorbed during intramolecular structure changes, i.e., the heat of cooperative transconformation of macromolecules. Results are presented of measurement of the heat effect of co-operative transconformation of DNA and procollagen.

As shown recently, many macromolecules (biopolymers and their synthetic models) have a very high degree of intramolecular ordering—a definite arrangement of groups and distribution of secondary bonds[1]. Under certain influences, especially heat, the macromolecule can go over from one ordered state into another with a lower degree of order[2]. This phenomenon of cooperative transconformation in macromolecules is highly reminiscent of phase transitions in macroscopic systems and, in analogy, it is frequently called "intramolecular melting," while the midpoint transition temperature is called the "melting temperature"[3,4]1). However, if we take into consideration the colossal molecular weight of ordinary biopolymers (from 10^4 to 10^8), which brings these giant molecules close to macroscopic systems, this analogy is no longer so far reaching.

As a result of transconformation, an abrupt change takes place in all the physical and chemical properties of the macromolecules, properties connected with their shape, internal symmetry, reactivity of the groups, and biological activity. Thus, under thermal transformation of DNA—the macromolecule of desoxyribonucleic acid, which bears the basic genetic information—its characteristic viscosity decreases to $\frac{1}{20}$th, the absorption of ultraviolet at 260 mμ increases by 40%, and the transforming activity almost completely disappears. All these changes are the result of decay, upon reaching of a definite temperature, of the rigid double helix of DNA into component polynucleotide chains, which are rolled up into random balls[5].

1)Unlike the melting of solids, the thermal transconformation of molecules occurs not at a point but extends through some temperature range and is thus reminiscent of the melting of microcrystals with defects.

Starting from general thermodynamic considerations, one could assume that heat is absorbed in the transconformation of macromolecules, as in the melting of solids. From the absorbed heat one could determine directly the number and energy of the broken bonds, and also the change of entropy in the conformation transitions. However, in spite of the exceeding importance of information of this type, the experimental data on the heats of transconformation of bio-macromolecules were lacking until recently. The main cause was the great difficulty of recording weak thermal effects in dilute solutions of macromolecules having very high and furthermore variable viscosity, and which in addition are available only in very limited amounts (the dilution is to reduce the interaction). If we take further account of the fact that the transconformation of the macromolecules takes place in a sufficiently broad temperature interval and, consequently, at a low heating rate the thermal absorption is strongly stretched out in time, it becomes obvious that such research encounters technical difficulties.

The first measurement of the heat absorbed in transconformation of nucleic acids and protanes was carried out in the Biophysics Laboratory of the Physics Institute of the Georgian Academy of Sciences, using a specially developed automatic adiabatic differential microcalorimeter.

PROCEDURE

The microcalorimeter used for the registration of weak thermal effects in solutions heated at a fixed rate employs photoelectric amplification, with negative feedback based on heat balance.

When heat is absorbed in one of the two identical ampoules of the differential microcalorimeter,

a thermal emf is produced across a thermopile that registers the temperature difference between the ampoules. The thermal current is amplified by a photoelectric amplifier and is fed to the ampoule heater in such a way as to compensate for the absorbed heat. The compensation current, from which it is possible to judge directly the magnitude of the heat absorption, is automatically recorded.

To reduce the thermal noise in the medium and to produce a uniform temperature field, the double ampoule of the differential microcalorimeter is placed in high vacuum and is surrounded by a system of thermal screens, the temperatures of which follow automatically the temperature of the ampoule that is heated at a constant rate.

The construction of the double ampoule and the system of thermal screens for it are illustrated in Fig. 1.

The double ampoule 5 of the microcalorimeter was made of gold. The volume of each ampoule was 0.7 ml and the wall thickness was 0.1 mm. The ampoules were connected by a thin steel rod. The distance between ampoules was 3 mm. In the gap between them is a thermopile of 30 chromel-constantan thermocouples, which serves as a transducer for the temperature difference between the ampoules. Inside the ampoules are cylindrical electric-heating elements with resistance of 200 ohms each.

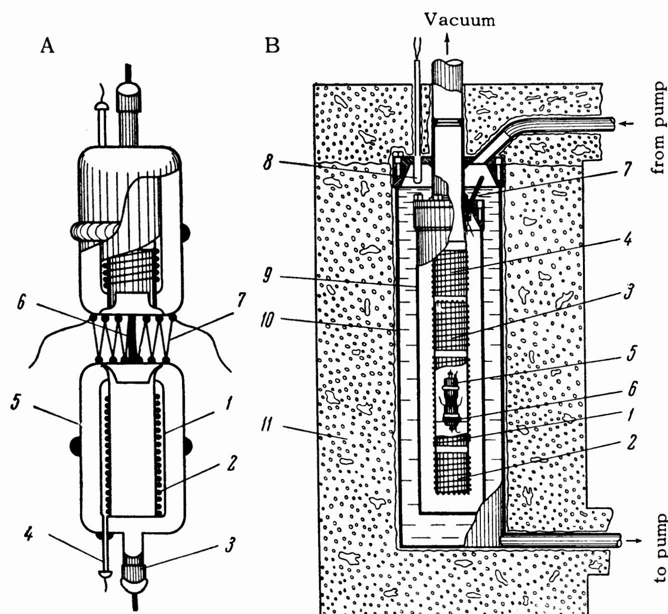

FIG. 1. A – Double ampoule of microcalorimeter: 1 – jacket of gold-foil heating elements, 2 – manganin electric heaters, 3 – neck with sealed in stopper, 4 – heater lead, 5 – ampoule body, 6 – connecting steel rod, 7 – thermopiles; B – diagram of thermal screening of macrocalorimeter: 1 – central heat screen, 2 – lower end heat screen, 3 – upper end heat screen, 4 – upper heat screen, 6 – silver rim, 7 – heat transducer of water jacket, 8 – platinum resistance thermometer, 9 – vacuum jacket, 10 – water jacket, 11 – foamed plastic.

As can be seen from Fig. 1B, the double ampoule 5 is placed inside the middle heat screen 1 and is covered on both sides by the end screens 2 and 3. To reduce heat dissipation, all the leads feeding the ampoule are glued to the side surfaces of the screens.

The vacuum jacket 9 of the calorimeter is surrounded by a water sheath 10, the temperature of which also follows automatically the temperature of the heat screens. The photoelectronic servomechanism of the calorimeter ensures the following automatic control accuracy: the temperature of the water sheath deviates from the temperature of the heat screens by not more than $\pm 0.005°$. The temperature of the screens follows the temperature of the ampoule to within $\pm 0.001°$ for the central screen and $\pm 0.005°$ for the end screens. The temperatures of the ampoules differ in the absence of heat absorption by not more than $\pm 10^{-6}$ deg.

The sensitivity of the microcalorimeter, as will be seen from the sample records shown below, reaches $\pm 3 \times 10^{-7}$ W over a very wide range of temperatures and heating rates.

For reliable operation of the instrument, the room must be thermostatically controlled ($\pm 0.1°$) and special measures must be adopted to reduce vibration.

RESULTS AND DISCUSSION

Figure 2 shows a typical record of the heat absorption observed in thermal transconformation of DNA (DNA of the bacteriophage T_2 in a phosphate buffer 0.007 M, pH = 7.0, and 0.1 M NaCl). The ampoule contained 640 mg of 0.107% DNA solution, i.e., altogether 0.685 mg of compound. The ampoule was heated at a rate of 1° in 10 minutes. The absorption of heat started at 76°C and ended at

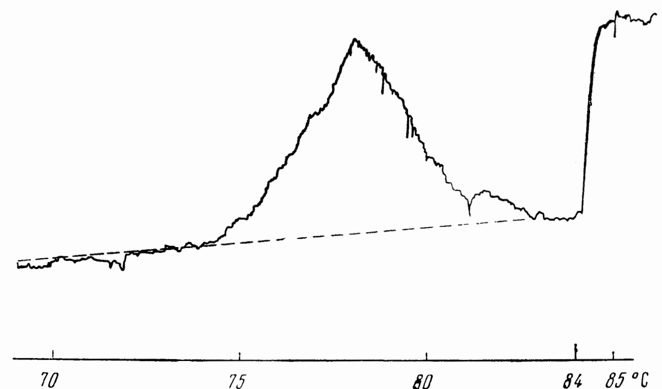

FIG. 2. Typical record of heat absorption in DNA solution during the course of thermal transconformation.

83°C. A fiducial marker of height 2.10×10^{-5} W is shown at 84°.

From the area of the heat-absorption curve and the size of the fiducial marker it is possible to calculate the heat absorbed during the process of transconformation. It amounts to $(8.15 \pm 0.35) \times 10^{-3}$ cal. By recalculating to grams of dry weight we obtain from this the "heat of melting" of the DNA double helix: $Q_m = 11.9 \pm 0.6$ cal/g at the given ion strength and pH of the solution.

It is more convenient, however, and more common practice in the investigations of the transconformation mechanism, to use the transconformational change in enthalpy ΔH_m per pair of nucleotides in the double helix. Since the average molecular weight of the nucleotide of DNA of the phage T_2 is 357, we obtain from the value of Q_m given above $\Delta H_m = 8500$ cal/mole-vapor per pair of nucleotides. This is considerably in excess of what can be expected by assuming that the helical structure of DNA is made up of hydrogen bonds only. We see that the exceedingly high thermal stability of DNA is due to the presence of other bonds besides the hydrogen bonds.

It is known that the thermal stabilization of DNA depends on the external conditions, particularly the pH and ionic strength of the solution[6,7]. Since cooperative transconformation of macromolecules occurs under the condition

$$\Delta F_m = \Delta H_m - T_m \Delta S_m = 0,$$

we get

$$T_m = \Delta H_m / \Delta S_m.$$

It is customarily assumed that ΔS_m depends little on the external conditions and that the thermal stability of DNA is determined essentially by the enthalpy term[8].

An experimental determination of the thermal effect of the transconformation of DNA, made for different pH and ion strengths of the solution, has shown that simultaneously with the thermal stability, i.e., the temperature of the transconformation, a change takes place also in the heat of transconformation (see the plot in Fig. 3). However, this connection between the heat and the temperature of the transconformation turned out to be much stronger than would be expected from the assumption that the entropy term is constant. Indeed, judging from the obtained data, which are summarized in Fig. 3, the change in entropy of DNA per pair of nucleotides in transconformation at 55 and 85°C amounts to 12.4 and 25.2 entropy units, i.e., a difference by approximately a factor of 2. This circumstance must be taken into account in any

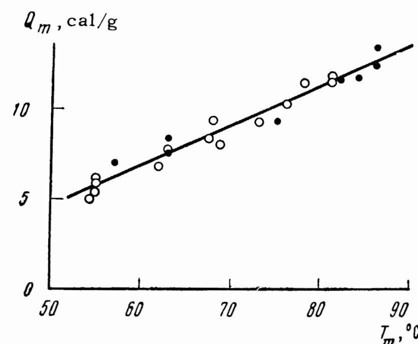

FIG. 3. Dependence of the specific "melting heat" of DNA on the "melting temperature." Full circles – solutions with different pH and constant ion strength, equal to 0.15 M of NaCl; light circles – solutions with constant pH, equal to 7.0 and different NaCl concentration – from 0.001 to 0.15.

future theoretical analysis of the question.

The thermal absorption curve has a very remarkable shape that differs appreciably from the random-distribution curve. If we take into consideration the fact that the magnitude of the bonds in guanine-cytosine and adenine-thymine pairs is different, it follows directly from the shape of the thermal absorption curve that these pairs of bases are distributed along the polynucleotide chain in far from random fashion. This deduction is in full agreement with the role of the material information carrier, which is ascribed to DNA.

The intramolecular heterogeneity of DNA, which is connected with its role as information carrier, is apparently one of the causes of the smearing of its conformational transition. The transconformation of procollagen—a protein with more regular and homogeneous structure—is much sharper.

A plot of thermal absorption observed in thermal transconformation of procollagen is shown in Fig. 4. (Citrate-dissolved procollagen, obtained by the Orekhovich method[9] in a citrate buffer with pH = 4.0 and ion strength 0.1.)

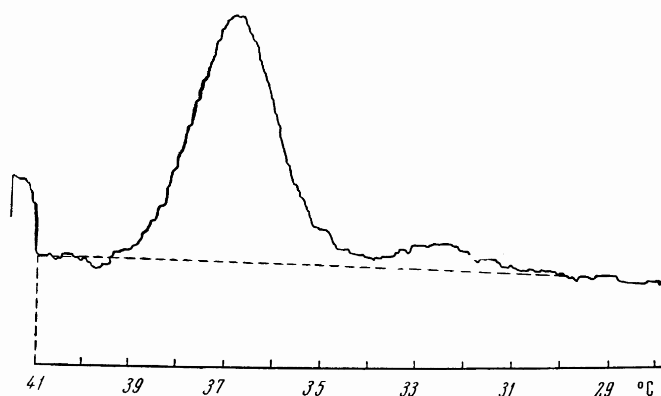

FIG. 4. Heat absorption observed during thermal transconformation of procollagen.

In its usual state, procollagen is a rigid triple helix[10], but under thermal action the polypeptide chains which constitute the macromolecule diverge and unravel into random balls[11]. The structure of the procollagen "melts" at relatively low temperature, from 35 to 39°C. As can be seen from the curve of Fig. 4, this is accompanied by absorption of an appreciable amount of heat.

In this case the ampoule contains 630 mg 0.15% solution of procollagen, i.e., the total albumen content of the ampoule was 0.975 mg. Judging from the area under the curve, some $(6.95 \pm 0.20) \times 10^{-3}$ cal were absorbed in the transconformation process equivalent to 7.15 ± 0.25 cal/g when recalculated to dry weight in grams.

If we assume that the molecular weight of procollagen is 360,000[12], then the molar "heat of melting" of procollagen is found to be of the order of 3,000 cal/mole. On the other hand, for the transconformation change in enthalpy ΔH_m per peptide unit (unit link of the polymer with average molecular weight on the order of 93[13]), we get 680 cal/mole. This is much lower than the estimate ΔH_m for procollagen, based on different indirect considerations. Thus, some authors have assumed that ΔH_m in an aqueous medium can reach an order of 1500 cal/mole[14].

Starting from the obtained value of ΔH_m, we can calculate the parameter σ, which takes into account the degree of correlation of the neighbors in the polymer chain in the theory of cooperative transconformation of polymers, developed by Zimm and Bragg[15].

According to Flory[4], for linear polymer systems there is the following connection between the quadrulent width of the temperature transition interval T_m (i.e., the width of the temperature interval within which from ½ to ¾ macromolecule goes over into a new state) and the transconformation change in enthalpy ΔH_m per link of the chain:

$$\Delta T_m = 2RT^2{}_m\sigma^{1/2} / \Delta H_m. \quad (1)$$

Judging from the presented plot of heat absorption in procollagen (see Fig. 4), ΔT is of the order of 2°, while T_n is equal to 310°K. Substituting the determined value of $\Delta H_m = 680$ cal/mole into (1) we obtain $\sigma = 1.2 \times 10^{-5}$. The smallness of the parameter σ is evidence of the strong correlation between the states of the neighboring links in procollagen. Indeed, since $RT \ln \sigma$ determines the energy necessary to initiate the disordered phase[4,15], for the case of procollagen, with the obtained value of σ, it is necessary to expend about 7,000 cal/mole to initiate the unraveling of the triple helix, i.e., to break simultaneously approximately 10 bonds, if we assume that the breaking of each bond (or more accurately the freeing of each link) consumes 680 cal/mole. Since this is more than triple the number of bonds necessary to initiate the melting of an α-helical conformation of strong interdependence between the states of the individual links of the macromolecule and the same interdependence or cooperation causes such an abrupt realignment of the conformation of the procollagen—"melting."

It must be noted that when a solution of procollagen is heated, a weak absorption of heat is observed, besides the heat-absorption peak near 37°C, also at lower temperatures, 31—34°C. This pre-denaturation stage of the transconformation of procollagen was first disclosed calorimetrically by determining the absorbed energy. At the present time it is not clear with which structural changes of the procollagen this stage is connected.

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