CALORIMETRIC INVESTIGATION OF THE THERMAL TRANSFORMATION OF COLLAGEN

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The clarification of the nature of the transconformation of long polymer chains (both synthetic and natural) has been attracting lately great interest among physicists, and phase transformations of this type are presently considered in theoretical investigations [1].

The class of natural linear polymers includes, in particular, also the collagen molecule, which consists of two left-twisted polypeptide chains connected by hydrogen bonds. It was of interest to investigate the process of thermal transconformation of this protein. As a result of the experiments carried out by us by the method of differential adiabatic microcalorimetric analysis (the sensitivity of the microcalorimetric setup was 10^{-7} W), it was found that collagen macromolecules extracted from tissue by two different methods can experience conformation transformations of two types. As seen from Fig. 1a, which shows the temperature dependence of the heat absorption of collagen solutions (plotted with an EPP-09 electronic potentiometer), the process of thermal transconformation of salt-extracted

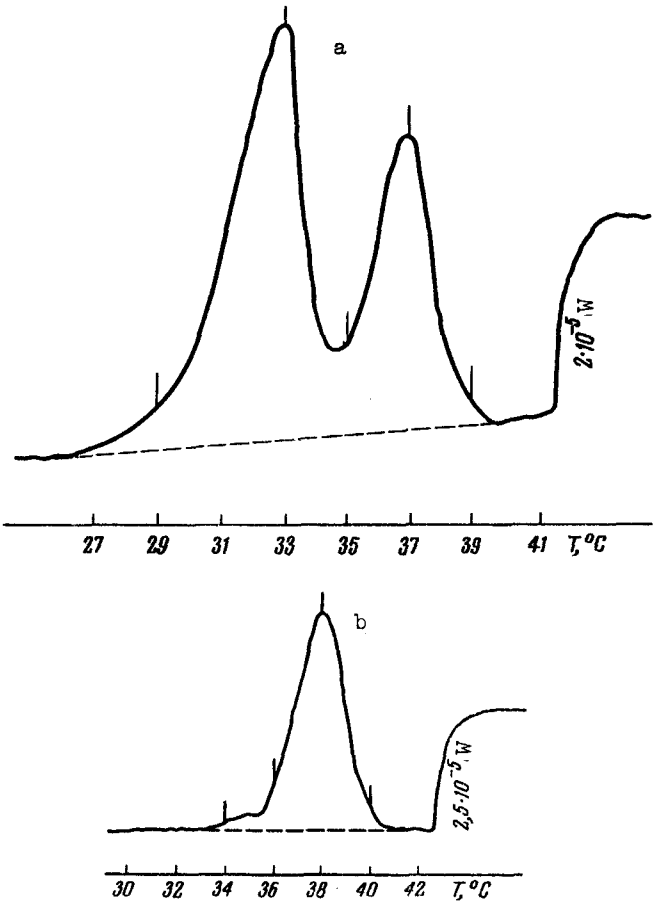

Fig. 1. Calorimetric plot of heat absorption of solutions of collagen extracted from normal connective tissue, in 0.1 M acetate buffer (PH 4.1): a - salt-extracted collagen, b - acid-extracted collagen.

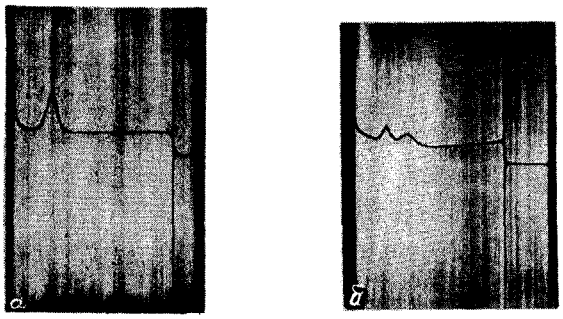

Fig. 2. Sedimentation patterns of solutions of denatured collagen extracted from normal connective tissue in 0.1 M acetate buffer (PH 4.1, 40°C): a - salt-extracted, concentration 0.42%, 56 100 rpm; 90 min after reaching maximum speed, inclination angle 70°; b - acid extracted, concentration 0.47%, 56 100 rpm, 90 min after reaching maximum, angle 75°.

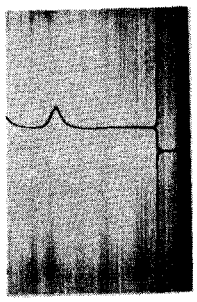

Fig. 3. Sedimentation pattern of solutions of denatured collagen separated by acid extraction from tumorous tissue; 0.1 M acetate buffer, PH = 4.1, 40°C, concentration 0.32%, 56 100 rpm, 130 min after reaching maximum speed; angle of inclination 70°

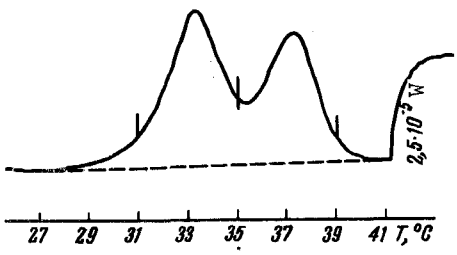

Fig. 4. Calorimetric plot of heat absorption of solutions of acid-extracted collagen separated from tumorous tissue, 0.1 M acetate buffer, PH = 4.1.

collagen occurs in two stages, with disintegration of the triple helix into three individual polypeptide chains. On the other hand, in the conformation transformation of the acid-extracted collagen (Fig. 16), only one stage of the intramolecular melting process is observed, with disintegration of the molecules into single and double chains that twist up into random bunches. In the double chain, all the main bonds turn out to be broken, with the exception of the small number of transverse additional inter-chain cross links, which make no measurable contribution to the heat of the transconformation process. The different character of the disintegration of the collagen molecules was confirmed by us by a sedimentation analysis (Figs. 2a, b), in a manner similar to that used in [2]. We call the reader's attention to the fact that two-stage diagrams of thermal absorption correspond to sedimentation diagrams with one peak, and vice-versa.

It was of interest to ascertain whether the character of the intramolecular phase transformation can differ, depending on whether the tissue from which the investigated protein was separated is normal or tumorous. It should be noted that we established a difference in the character of the crystallization of the water in normal and tumorous tissues cooled to - 130° C. Indeed, whereas 73.1 cal/g is absorbed in the process of heating normal

muscle and connective tissue from - 130 to 0° C upon melting of the intratissue ice, only 66.4 cal/g is absorbed when tumorous tissue is heated. Thus, the amount of "bound" (i.e., not frozen-out) water per gram of substance is much larger in tumorous tissue than in normal tissue. From our point of view, this means that in destruction of the tissue increases the effective area of contact between the surfaces of the macromolecules and the water molecules [3]. This, in turn, means the release of chemically active groups uniting the protein molecules into supermolecular structures of higher order.

The collagen molecules separated from a malignant tumor[1] (sarcoma M-1), compared with collagen molecules extracted from normal connective tissue, revealed the following properties:

a) In "cancerous" molecules of acid-extracted collagen, in spite of the norm, there are no additional chain cross links, and consequently the sedimentation of all the transconformation products occurs at a single rate (Fig. 3)[2].

b) The single-stage process of thermal transconformation characteristic of molecules of acid-extracted collagen gives way in this case to two stages, although the total heat of melting is 18 ± 1 cal/g of protein (10.5 ± 0.5 cal/g during the first stage and 7.4 ± 0.6 cal/g in the second) remains unchanged in both cases, within the limits of experimental error (Fig. 4).

c) The peak of heat absorption at 38° C, observed in the process of thermal transconformation of the collagen extracted from the normal tissue, breaks up into peaks of maxima at 33.5 and 37.5° C.

Thus, we not only investigated the character of the transconformation of protein macromolecules but, starting from purely physical concepts of intramolecular phase transformations we demonstrated clearly, by purely physical methods and with collagen as an example, that the process of transconformation of macromolecules separated from a tumorous tissue differs considerably from the analogous process occurring in "normal" molecules. This offers evidence that the structural properties of the collagen proteins of a tumorous tissue, whose thermal stability turns out to be considerably altered, are altered.

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[1] I. M. Lifshitz, Zh. Eksp. Teor. Fiz. 55, 2408 (1968) [Sov. Phys.-JETP 28 (1969)].
[2] K. A. Piez, M. S. Lewis, G. K. Martin, and J. Gross, Bich. Bioph. Acta 53, 596 (1961).
[3] E. L. Andronikashvili, G. M. Mrevlishvili, and P. L. Privalov, Dokl. Akad. Nauk SSSR 171, 1198 (1966).

[1] This tissue is the polymorphic-cell sarcoma obtained in 1943 by L. Shabad and M. Bloch by grafting a tumor induced with 3,4-benzpyerene.

[2] It should be noted that the acid-extracted fraction predominated quantitatively in the tumors investigated by us, and there was practically no salt-extracted fraction during this period of tumor development (15 days after the grafting). It also turned out that the amount of "free" water decreased continuously with increasing growth of the tumor.