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PHASE DIAGRAM OF COLLAGEN AND INTERPRETATION OF THE HEAT OF THE ORDER-DISORDER TRANSITION IN WATER

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As is known, fibrillar proteins undergo macroscopic phase transitions (often defined as “supercontraction”), caused by an intramolecular cooperative transition of the helix-coil type. The peculiarities of such transformations consist in the fact that the transition temperature depends on the applied stress. Indeed, whereas for an unloaded system the melting temperature is

$$T_{\mathrm{m}} = \Delta H / \Delta S \tag{1}$$

where ΔH is the molar heat and ΔS the entropy of transition, for an oriented system subjected to the action of an external force f, relation (1) should be replaced by

$$T_{\mathrm{m}} = \frac{\Delta H \pm |f \Delta L|}{\Delta S}, \tag{2}$$

where ΔL is the change in length as a result of melting (^{1,2}). If a chain possesses conformational polymorphism, i.e., can exist in different ordered conformations or in the conformation of a statistical coil, and the applied external force is not very large, it should stabilize the helical conformation, which is more extended in comparison with the equilibrium (after melting) coil conformation; in this case $dT_{\mathrm{m}}/df > 0$, and melting is accompanied by contraction of the system. In the opposite case (the external force stabilizes the coil conformation, more extended than the helix), melting should be accompanied by elongation of the system, $f\Delta L < 0$, and the load lowers the melting temperature (^{3}).

Thus, the theory predicts the existence of a certain critical load at which the sign of the deformation and of the temperature increment of the transition changes to the opposite.

In works (^{4,5}) we were able to demonstrate the existence of such a critical load for collagen fibers isolated from rat-tail tendons. All measurements were

Fig. 1. Dependence of melting temperature on applied stress (phase diagram) for collagen fibers in pure water. The hatched region corresponds to a set of experimental points at stresses greater than the critical value.

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carried out in an aqueous medium, under conditions of equilibrium between the swollen amorphous phase and the stable helically ordered crystalline phase. Thermomechanical curves were recorded directly in the coordinates deformation –temperature of contraction, with stress as a parameter, as well as curves of isometric heating (for all details see work (^{4})).

The results of these measurements are presented in Fig. 1. This curve is nothing other than the phase diagram of collagen, of course "in pure water." All points on the stress-temperature plane lying under the curve correspond to linear-crystalline order in the collagen fiber, while beyond this curve–to complete amorphization. To our knowledge, such a complete phase diagram of a one-dimensionally ordered system is given here for the first time. The critical parameters are: $T_\text{k} = 106°$; $\sigma_\text{k} = 165$ kg/cm^2.

Although obtaining such a diagram confirms the general considerations given above, additional discussion is required in view of the fact that the structure of collagen does not fully correspond to the model adopted for the calculations in the works (^{5}). Indeed, the individual strands of the triple-helical collagen molecules have the conformation of the polyproline II helix, which is practically incapable of further stretching. Thus, even when the triple helix breaks down into independent molecular strands, the load here cannot stabilize a disordered conformation that is more extended than the helix.

Fig. 1. Dependence of the melting temperature on the applied stress (phase diagram) for collagen fibers in pure water. The hatched region corresponds to the set of experimental points at stresses greater than the critical value.

Returning to relation (2), one may abandon its interpretation as an identity following from the heat-exchange equation (^{2}), and instead regard the expression

$$\Delta S = \frac{\Delta H + f\Delta L}{T} \tag{2a}$$

as an equation for determining the entropy of melting. In doing so, it is accepted that both ΔH and ΔS themselves depend on f, or on the stress σ. Then it becomes immaterial in what terms the effect of the load, characterized by the term $f\Delta L$ in (2), is interpreted. Of course, one may speak of external work: it can compensate for a heat deficit (stretching disorganizes the helical order) or, conversely, cause an additional expenditure of heat (stretching strengthens the order). But this effect may also be interpreted from a purely entropic standpoint, taking the heat of melting as unchanged or almost unchanged (there are some experimental grounds for this (2)), and assigning the term $f\Delta L$ to the change in entropy, according to equation (2a). In that case we no longer specify the morphological changes during melting, but merely estimate the change in the conformational ensemble during melting under load. In all cases where application of a load is analogous to increasing the rigidity of individual chains (2), the conformational ensemble of the system is impoverished upon melting and

$$\partial \Delta S / \partial \sigma < 0 \tag{3}$$

$$\frac{\partial T_{\mathrm{m}}}{\partial \sigma} > 0. \tag{3a}$$

In our case, at small loads the usual mechanism operates: upon melting, less extended coils are formed. If, however, stretching leads to a decrease in rigidity, i.e., to an increase in the conformational ensemble upon melting, then

$$\frac{\partial \Delta S}{\partial \sigma} > 0; \qquad \frac{\partial T_{\mathrm{m}}}{\partial \sigma} < 0. \tag{4}$$

We would now like to emphasize that, in the case of multispiral systems, such a decrease in rigidity is not necessarily associated with the destruction of the helical conformation of individual strands, but may be due to a small reversible sliding of the monospirals relative to one another. Such allowance for interchain interactions in multispiral

systems makes the use of relations of the form (3) and (4) entirely legitimate; of course, in this case the value of ΔS found in solving equation (2a) is substituted into formula (1), i.e., one that explicitly takes into account the term $f\Delta L$.

It is not difficult to see that both thermodynamic approaches correspond to a one-dimensional equation of state, which is an analogue of the Clapeyron equation (2):

$$\left\{\frac{\partial (f/T)}{\partial (1/T)}\right\}_{p=\text{const, equilib.}} = \Delta H / \Delta L. \tag{5}$$

Let us now turn our attention to the ascending branch of the curve in Fig. 1. It exactly reproduces the course of the left branch of the diagram of isometric heating of a collagen fiber in water, although here external loads are plotted (Fig. 2b).

Fig. 2. Double extrapolation for determining the critical parameters T_{cr} and σ_{cr}.

Fig. 2 and Fig. 3

Figure 2: Fig. 2 and Fig. 3

a—dependence of the relative deformation on load; *b*—dependence of the temperature of hydrothermal contraction on load. The straight line is the change in stress during isometric heating of the fiber. The points are the melting temperatures of the fibers under the corresponding external loads.

Fig. 3. For the calculation of the specific and molar heats of melting by formula (3) (explanations in the text)

Such equivalence of external stresses and stresses arising in an immovably fixed fiber indicates that we are indeed dealing with equilibrium systems and therefore have the right to carry out thermodynamic calculations. Using the data of work ([4]), we calculated the heat of transition per one monomeric unit, taking the average molecular weight of a unit to be 93 ([6]). The graph for the calculation by formula (5) is shown in Fig. 3. The initial portion of the curve, corresponding to small stresses, is taken from Flory' s work ([6]). The calculation gives $\Delta H_{\mathrm{m}} = 2.19 \pm 0.25$ kcal/mol—approximately 30% higher than in Flory. Most likely, this discrepancy is due to a different method of preparing the samples ([4]).

Let us now turn to the ascending branch of the isometric-heating diagram extrapolated to the "critical" temperature 106° (Fig. 2). Since this curve characterizes phase equilibrium, we can, by analogy with how this was done for globular proteins ([7,8]), introduce the "relative degree of denaturation"

$$y = \sigma/\sigma_{\mathrm{max}}. \tag{6}$$

Of course, in this case the plotting of the curve must begin with zero load—

load. The equilibrium constant between the crystalline and amorphous phases is equal to

$$K' = y(1-y). \tag{7}$$

Determining K' at different temperatures, we find the true molar heat of transition

$$\Delta H = -Rd\ln K'/d(T^{-1}), \tag{8}$$

equal to 50 ± 6.5 kcal/mole (Fig. 4). As is known(9), the melting of fibrillar proteins is a typical cooperative process, in the elementary act of which a certain multitude of links participates. This effective multitude can be characterized by a certain cooperativity parameter, which depends on the nature of the interactions of neighboring groups of the chain, but in its meaning approximately

Fig. 4. Arrhenius plot for the equilibrium constant K'

Figure 3: Fig. 4. Arrhenius plot for the equilibrium constant K'

corresponds to the number of links forming the effective statistical unit of the process. It is precisely such an effective unit that the quantity ΔH corresponds to.

Fig. 4. Arrhenius plot for the equilibrium constant K'.

Thus, in the system under consideration, the correlation of interactions determining the cooperativity of the collagen-melting process encompasses approximately 20–25 links. If this correlation took place only along the triple-helical molecule, this would correspond approximately to one turn of the triple-helical chain.

In conclusion we note that determining the heat of the order–disorder transition in proteins and nucleic acids, calculated per one monomeric link on the basis of calorimetric or thermomechanical data, is of a somewhat formal character, for it does not characterize the cooperative nature of the process. Indeed, we are deprived of the possibility of saying *a priori* how many adjacent links must simultaneously pass into the amorphous state; it is clear only that the transition of "one link" into the amorphous phase is impossible, since it interacts with its neighbors(36). The Arrhenius equation automatically gives the energy or heat referred to a mole of effective units, which represent a certain correlation of monomeric links.

Thus, the combination of two types of measurements of the molar heat of melting makes it possible to determine the effective cooperativity parameter $\Delta H/\Delta H_{\mathrm{m}}$, which is already of great importance for understanding the nature of the process. What has been said applies equally to fibrillar and globular proteins and to polynucleotides. Apparently, with the known limitations, it also applies to the melting processes of ordinary oriented polymers.

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