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Temperature-induced phase transitions in proteins and lipids

Volume and heat capacity effects

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The partial specific heat capacity and volume of globular proteins and dispersions of phosphatidylcholines in aqueous solutions have been determined over a broad temperature range using a precise scanning microcalorimeter and a vibrational densimeter. It is shown that the temperature-induced, gel-to-liquid crystalline phase transition in phosphatidylcholines proceeds without a noticeable change in heat capacity but with a significant increase in the specific volume, whereas heat denaturation in proteins takes place without a noticeable change in the volume but with a significant increase in heat capacity. This principal difference between temperature-induced conformational phase transitions in proteins and lipids demonstrates clearly that heat denaturation of proteins, in contrast to the gel-to-liquid crystalline phase transition in lipids, cannot be regarded as a process similar to melting. Consequently, the 'molten globule' does not appear to be a suitable model for a heat-denatured protein.

1. Introduction

Notwithstanding the great difference between proteins and lipid dispersions in water, they have one very important feature in common; the order in relative disposition of their constituent elements (amino acid residues, hydrocarbon chains) at sufficiently low temperatures and the ability to lose this order in a highly cooperative way with increasing temperature. Temperature-induced gross conformational transitions in proteins (heat denaturation) and lipids (gel-to-liquid crystalline transition in bilayers) are usually considered as first-order phase transitions, since they proceed with discontinuity of the first derivatives of thermodynamic potentials – the enthalpy and entropy functions (see, e.g., refs. 1–3). Therefore, these phase transitions are accompanied by a significant heat effect which has been extensively studied calorimetrically (for lipids, see refs. 4–9; for proteins, see refs. 10 and 11).

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However, to compare the phase transitions observed in various proteins and lipids (which usually take place at different temperatures), one requires knowledge of the temperature dependence of the changes in enthalpy and entropy of the transition:

$$\frac{\delta \Delta_t H}{\delta T} = \Delta_t C_p, \quad \frac{\delta \Delta_t S}{\delta T} = \frac{\Delta_t C_p}{T} \tag{1}$$

Thus, for a thermodynamic analysis of a conformational transition, one should know its accompanying heat capacity change, i.e., one must determine the partial heat capacities of both phases of the system considered.

This experimental task requires much more advanced instrumentation than that used for measurements of the relatively intensive heat absorption effects observed upon sharp, temperature-induced phase transitions in proteins and lipids. Its practical realization became possible only after the appearance of precise scanning microcalorimeters with a fixed operational volume [12,13]. Using this technique, it was shown by various authors that the denaturation of proteins is always accompanied by a significant increase in heat capacity [10,14,15]. As for lipids, many authors have reported a decrease in heat capacity for the phase transition (see, e.g., refs. 16 and 17). This result was somewhat disappointing, since it contradicted the commonly accepted point of view that phase transitions in lipids proceed with an increase of the rotational freedom of hydrocarbon chains, which should result in an increase in heat capacity.

In this paper we will show that a decrease of the apparent heat capacity observed calorimetrically for a phase transition in lipids does not indicate in any way at all an actual decrease in partial specific heat capacity. This erroneous conclusion resulted from neglecting of the volume effects which should be taken into account when using a scanning microcalorimeter with a fixed operational volume. We will demonstrate also that there is a principal difference in heat capacity and volume effects between proteins and lipids which reveals a quantitative difference in the nature of the phase transitions occurring in these two objects.

2. Materials and methods

In the experiments we used L-α-dimyristoylphosphatidylcholine (DMPC), L-α-dipalmitoylphosphatidylcholine (DPPC) and L-α-distearoylphosphatidylcholine (DSPC). The first two were purchased from Calbiochem and used without further purification, the third being kindly provided by Dr. Schäfer, G.D.R. Academy of Sciences. The lipids were checked by calorimetrically observed melting profiles according to Albon and Sturtevant [2].

Lipid dispersions for densitometric and calorimetric experiments were prepared by heating the lipid in water to a temperature well above the main transition and vortex-mixing. Deionized, glass-distilled water was used throughout. The pH varied from 6.5 to 7.0.

The globular proteins used in the experiments were hen egg white lysozyme, pancreatic ribonuclease A (RNase) and sperm whale metmyoglobin (Mb). The first two were purchased from Serva, the latter being obtained as described elsewhere [18]. The purity of all preparations was checked electrophoretically and was found to be higher than 97%. All experiments described here were performed on aqueous solutions in the presence of 10 mM glycine buffer with pH ranging from 2.5 to 4.0. Prior to experiments, all solutions were carefully dialyzed against the corresponding solvent to achieve complete equilibrium in low-molecular-weight components.

For calorimetric measurements, differential scanning microcalorimeters DASM-1M and DASM-4 (with a special cell for viscous solutions) were employed [12,13]. The scanning rate was 0.25 or 0.5 K min^{-1}. The concentration, either of the protein solution or of the aqueous dispersion, varied from 1 to 10 mg/ml. A relatively high concentration was required to increase the accuracy in determining the partial heat capacity. However, these concentrations are still sufficiently low for the concentration dependence of the partial heat capacity to be neglected. Therefore, the latter was determined without extrapolation to zero concentration, assuming that $C_{p,2} = C^{\infty}_{p,2}$ at all concentrations used [13].

Density measurements were performed using a DMA 602 vibrational digital densimeter (Anton Paar, Austria) [19]. Partial specific volumes were obtained from solvent and solution densities [20]. The concentration of lipids and proteins used in these experiments varied from 0.5 to 5 mg/ml.

3. Results

In fig. 1, normalized scanning calorimetric curves are shown for 1 mg/ml solutions of either a protein (lysozyme) or a lipid (DPPC) or KCl.

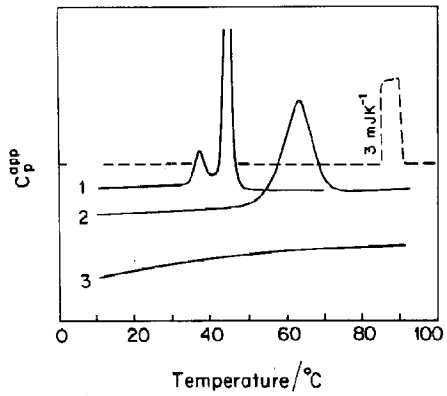

Fig. 1. Normalized scanning calorimetric recordings of dipalmitoylglycerophosphocholine (curve 1), lysozyme at pH 2.4 (curve 2) and KCl (curve 3), each at 1 mg/ml. Broken line: baseline with calibration marker.

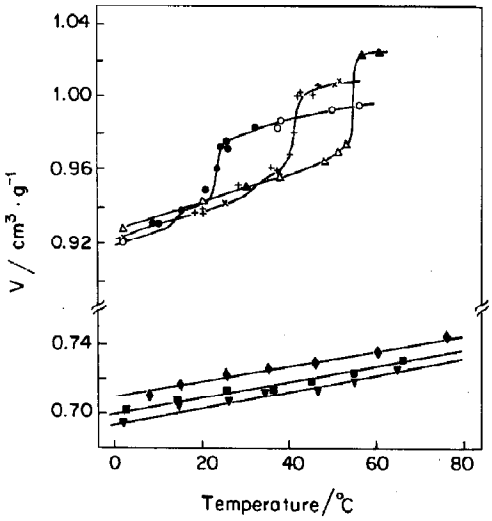

Fig. 2. Partial specific volume vs. temperature for DMPC, DPPC and DSPC as well as for lysozyme and ribonuclease. DMPC: (○) from ref. 23, (●) this work. DPPC: (×) from ref. 23, (+) this work. DSPC: (△) from ref. 23, (▲) this work. Lysozyme (pH 1.9) in 10 mM glycine buffer: (■). Ribonuclease (pH 2.5) in 10 mM glycine buffer: (▼). Metmyoglobin (pH 3.9) in 10 mM sodium acetate buffer (◆).

The apparent heat capacity of the protein in solution increases, as is observed generally, with increasing temperature below and above the transition zone (here from 40 to 80 °C), and the transition is accompanied by a positive heat capacity change. The apparent heat capacity of the lipid dispersion is considerably higher below the pre-transition and main transition and is less temperature dependent than that of the protein. The lipid phase transition seems to be accompanied by a negative heat capacity change, which has been recently reported for a variety of lipids [17].

A further difference between protein and lipid is revealed in fig. 2 by the temperature dependences of the partial specific volumes of the proteins and lipids. The partial specific volume of the phosphatidylcholines amounts to about 0.92 cm^3 g^{-1} at 0 °C. The value is temperature dependent and marked changes at the pre-transition and especially at the main transition are observed, as described before [16,21–24]. In contrast, the partial specific volume of the proteins is about 0.70–0.75 cm^3 g^{-1} [20]. It is much less temperature dependent and does not show any clear transitional changes, as shown in earlier studies [25–27].

The temperature dependence of the partial specific volume of the solute has a strong influence on the calorimetrically observed, apparent heat capacity of the solution and should be taken into account when determining the partial specific heat capacity of the solute. This results from the principle on which the operation of modern, precise scanning calorimeters is based – that of a fixed operational volume of the calorimetric cell [12,13]. When these instruments are used, the partial specific heat capacity of the solute (subscript 2) can be calculated from the apparent heat capacity difference between the solvent (subscript 1) and solution (superscript s) $\Delta_1^s C_p^{app}(T)$ by the equation:

$$C_{p,2}(T) = V_2(T)C_{p,1}(T)/V_1(T) - \Delta_1^s C_p^{app}(T)/m_2(T) \quad (2)$$

where $V_1(T)$ and $V_2(T)$ are temperature-dependent partial specific volumes of the solvent and solute, respectively, $C_{p,1}(T)$ the specific heat capacity of the solvent and $m_2(T)$ the mass of the solute in the calorimetric cell at temperature T.

The heat capacity of the solvent is determined by the same scanning calorimeter, using as a standard a liquid of known heat capacity. For

dilute aqueous solutions, water is usually used as a standard, since its specific heat capacity is known with the highest accuracy over a broad temperature range. In precise determinations of the partial heat capacity of a solute, one should also take into account the temperature dependence of the mass of the solute in the calorimetric cell with a fixed volume (for details, see ref. 13).

For proteins in aqueous solutions the first term in eq. 1 remains nearly constant over the entire temperature range from 5 to 80 °C, whereas for lipids its temperature dependence cannot be neglected, especially since the phase transition is accompanied by a rather significant volume effect.

The partial specific heat capacity of three phosphatidylcholines (DMPC, DOOC and DSPC), determined by taking into account all the above corrections, is plotted in fig. 3 as a function of temperature. As seen on the enlarged scale in this figure, no heat capacity change is associated with the phase transition: the heat capacity of all lipids before the pre-transition and after the main transition is actually the same. At the same time, one can observe a definite dependence of the partial specific heat capacity of lipids on chain length, as mentioned previously by Wilkinson and Nagle [16] and Blume [17].

The partial specific heat capacity functions for three globular proteins (lysozyme, RNase and Mb) are presented in fig. 4 as functions of temperature.

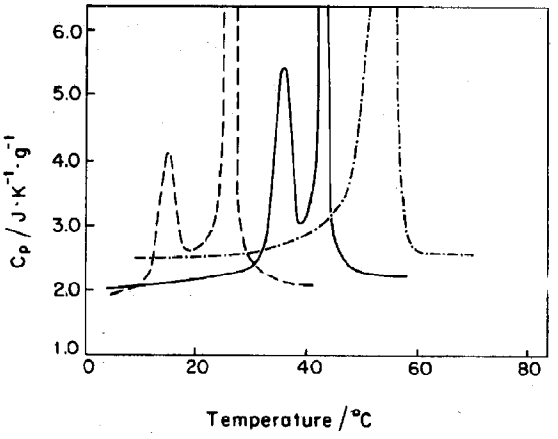

Fig. 3. Partial specific heat capacity vs. temperature for aqueous dispersions of DMPC (— — —), DPPC (———) and DSPC (·–·–·).

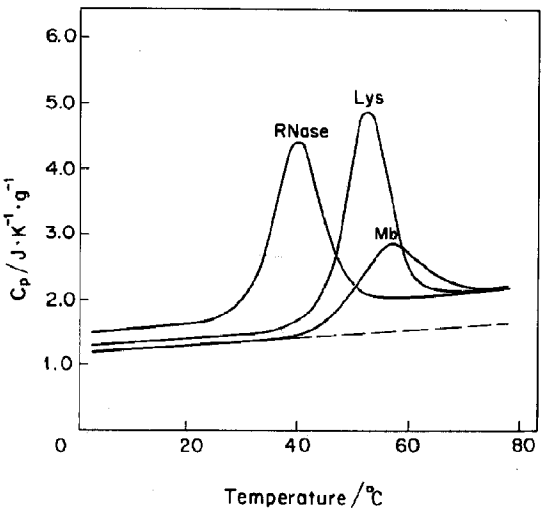

Fig. 4. Partial specific heat capacity vs. temperature for ribonuclease (RNase) (pH 2.5), lysozyme (Lys) (pH 1.9) in 10 mM glycine buffer and metmyoglobin (Mb) (pH 3.9) in 10 mM sodium acetate buffer.

The denaturational phase transition in this case results in a quite significant heat capacity increment. All three proteins have rather different heat capacities in the native state, but in denatured states their heat capacities are almost identical. Therefore, the specific heat capacity increment for denaturation of these proteins is different and does not show any correlation with their molecular weight.

4. Discussion

The heat capacity functions of proteins and lipid dispersions show some common and some divergent properties.

The partial specific heat capacity of native globular proteins varies between 1.20 and 1.50 J K^{-1} g^{-1} at 25 °C and does not show any correlation with molecular weight [10]. The partial specific heat capacity of lipids is considerably higher: for DPPC it amounts to 2.18 ± 0.08 J K^{-1} g^{-1} at 25 °C and increases with chain length. Thus, it appears as if the main contribution to the heat capacity of lipids is from the hydrocarbon chain, which has a higher specific heat capacity than that

of the polar head group. The experimentally estimated partial heat capacities of proteins and lipid dispersions are greater than the heat capacity values calculated neglecting water contributions [15,17,28], which indicates an essential role of 'hydrophobic hydration' even in a lipid bilayer. Judging by the greater heat capacity of lipids as compared to that of proteins, one can also suppose that non-polar groups are more accessible to water in lipids than in compact, native globular proteins. However, it is also possible that the rotational freedom of these groups is greater in lipid bilayers than in proteins.

Proteins show a marked temperature dependence of the heat capacity and a significant positive heat capacity change upon denaturation. When there is no heat effect from a pre-transition (which is believed [1,29] to be connected with the cooperative reorientation of chains), the heat capacity of lipids in a gel phase increases slightly with temperature and does not show any change in the heat capacity upon transition (fig. 3). The decrease in apparent heat capacity observed in original scanning calorimetric recordings for a great variety of lipids [17] (see also fig. 1) is due completely to the volume effect of the transition considered in section 3.

In contrast to the heat capacity, the temperature dependence of the partial specific volume is much more pronounced for lipids than for proteins (fig. 2). The gel-to-liquid phase transition in lipids is accompanied by a substantial increase in volume, whereas the volume change upon protein denaturation is imperceptible and cannot be registered by direct volumetric methods. One can ascertain the existence of this effect only by studying the influence of high pressure on protein stability, which shows that protein denaturation occurs with a marginal decrease in volume [30–32].

In considering the gel-to-liquid crystalline phase transition in lipids, it is generally assumed that this proceeds without penetration of water inside the bilayer and that it is caused by the disruption of van der Waals contacts between the hydrocarbon chains with an increase in the average distance between them and in the freedom of rotation of chains (see, e.g., refs. 1, 23 and 33). The expansion of the volume of a bilayer upon the gel-to-liquid crystalline phase transition is indeed observed experimentally (fig. 2). Concerning the increase in rotational freedom of a chain, which should manifest itself by an increase of heat capacity, this has not been demonstrated calorimetrically. Therefore, one can conclude that a heat capacity increase caused by an increase in rotational freedom of polymer chains is too small to be observed calorimetrically.

Up to the present, the consideration of protein heat denaturation has led to vigorous debates. It is more or less clear that a polypeptide chain of a heat-denatured protein is not in a random-coil conformation, as occurs in the presence of a high concentration of denaturant: the hydrodynamic volume of a heat-denatured protein is much smaller that that of a urea- or guanidine hydrochloride (GdnHCl)-denatured protein [34]. In some cases it was found that the hydrodynamic volume of a heat-denatured protein exceeds the value for a densely packed globule by only 30–50% [35,36]. Therefore, it was suggested that upon heat denaturation water does not penetrate inside the globule and that a heat-denatured protein represents a slightly expanded, but still compact, molten globule with disrupted van der Waals contacts and increased rotational freedom of side chains [37].

The resemblance between the molten globule model for a heat-denatured protein and the liquid crystalline phase in lipids is evident. Therefore, one can expect to find a great similarity between the temperature-induced transitions in proteins and lipids. However, as we have seen, expansion of the hydrodynamic volume of a globular protein upon heat denaturation does not signify in any way at all that its thermodynamic (real) volume increases. Since the latter does not increase upon heat denaturation, the observed expansion of hydrodynamic volume can be explained only by the penetration of water into the interior of the protein molecule. This is confirmed by the heat capacity effect of protein denaturation.

The significant heat capacity increment observed upon protein denaturation cannot be explained by an increase in rotational freedom of the polypeptide chain, since the increase of rotational freedom in proteins cannot be much greater than that in lipids, in which it does not lead to a

noticeable change in heat capacity. According to the calculations, the heat capacity increment caused by an increase in rotational freedom of amino acid residues upon denaturation can be only a small part of the calorimetrically observed effect [38]. The only reasonable explanation for the observed denaturational increment in the heat capacity of proteins is the assumption that it is caused mainly by the hydration of internal non-polar groups which become exposed upon denaturation [18,39,40]. It has been shown that the heat capacity increment is almost the same upon heat and GdnHCl denaturation of proteins [41]. This leads to the conclusion that the contacts of non-polar groups with water are practically the same in the heat-denatured protein and in the GdnHCl-denatured protein, where the polypeptide chain is actually in a random-coil conformation.

Thus, the heat capacity and volume effects observed upon phase transitions in proteins and lipids differ fundamentally in their high-temperature phases: while in lipids this phase can be regarded as liquid-like, in proteins it is far from being so and the term molten globule is not a suitable metaphor for its description.

Knowledge of the heat capacity change for a temperature-induced phase transition has permitted comparison of the enthalpy and entropy of transition in various lipids and proteins.

If $\Delta_t C_p$ for lipids is zero, this means, according to eq. 1, that the transition enthalpy and entropy do not depend on temperature and we can compare directly the values found at different temperatures, namely, for various phosphatidylcholines in which the main transition occurs at 288.5 K (DMPC), 308.7 K (DPPC) and 324.2 K (DSPC). According to Blume [17], the total enthalpy of the transitions in these phosphatidylcholines to 32.6, 43.0 and 53.1 kJ mol^{-1}, respectively. Thus, for the total transition entropy of these lipids we have 113, 139 and 164 J K^{-1} mol^{-1}. Since these phosphatidylcholines have the same head groups and differ only in chain length, one can easily calculate the enthalpy and entropy contributions from a single CH_2 group, knowing that there are 14 CH_2 groups in each chain of DMPC, 16 in DPPC and 18 in DSPC. It is only necessary to divide the difference in observed thermodynamic parameters by the difference in CH_2 groups in the lipid considered. This gives 2.6 kJ mol^{-1} for the enthalpy of transition per CH_2 group and 6.2 J K^{-1} mol^{-1} for the entropy of transition. These values are somewhat lower that those found for the melting of polyethylene, in which the melting enthalpy is 4.0 kJ mol^{-1} and the entropy 9.7 J K^{-1} mol^{-1} [42].

A comparison of the transition parameters of lipids with those of proteins is more complicated, because for the latter they are temperature-dependent (since $\Delta_t C_p \neq 0$) and differ for various proteins. However, the specific denaturation enthalpy and entropy values extrapolated to 110 °C are universal for compact globular proteins [10] and are equal to 6.2 kJ mol^{-1} and 17.6 J K^{-1} mol^{-1} when calculated per amino acid residue. It is supposed that they do not include the hydration effects which are small at that temperature. Therefore, comparison of these values extrapolated to 110 °C with those found for the phase transition in lipids, in which hydration is supposed to be absent, is realistic. The denaturation enthalpy and entropy values calculated per mol amino acid residues are almost 3-fold greater than the enthalpy and entropy of the phase transition in lipids calculated per mol CH_2 groups. However, if one takes into account the great difference between the size and flexibility of amino acid residues and of CH_2 groups and the possible contribution of hydrogen bonding in proteins, the observed difference will appear quite reasonable.

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