Melting temperature and a precursor phase change in bacteriorhodopsin as function of pH

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Letter

Melting temperature and a precursor phase change in bacteriorhodopsin as function of pH

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In a recent review in this Journal, one of us has considered melting and precursor cooperative phenomena in chemically bonded assemblies. Here, we move from the phase diagram of simple molecular solids (e.g. nitrogen), as a function, say, of applied pressure, p, to that of bacteriorhodopsin (bR) which is one of the most frequently studied membrane proteins whose X-ray structures have been determined. However, instead of the physical variable, p, Heyes and El-Sayed have measured the melting and pre-melting transition temperatures as a function of pH. In the present study, we make proposals concerning the statistical-mechanical interpretation of their measurements in terms of additional degrees of freedom coming into play above the pre-melting temperature.

1. Background

In a recent review in this Journal, one of us [1] has been concerned with melting and precursor phase transitions in chemically bonded assemblies. In [1], the focus was on d-electron directionally bonded transition metals, on networks of chemical bonds such as graphite or solid silicon [2], and finally on relatively simple molecular solids, with the phase diagrams being considered as a function mainly of an externally applied pressure, p.

Subsequently, we have become aware of the experimental study of Heyes and El-Sayed [3] on the melting and pre-melting transition temperatures of bacteriorhodopsin (bR). Earlier calorimetric and spectroscopic investigations show that there are two main phase transitions as the temperature of bR is increased [4–6]. Thus there is a modest reversible pre-melting transition in the temperature range

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78–80°C and a prominent transition at 96°C, which Jackson and Sturtevant [7] conclude to be *irreversible*. In the study of Heyes and El-Sayed (HES) [3], the temperature dependence of the Fourier transform infrared spectra of bR has been measured as a function of the pH and of the divalent cation regeneration with Ca^{2+} and Mg^{2+}.

As the focus of the present Letter, we have redrawn in figure 1 the transition temperature found in [3] for pre-melting and for melting, as a function of pH for native bR. As HES [3] stress, the melting temperature varies strongly with pH below and above physiological conditions. They emphasise the contrast between this behaviour and that of the pre-melting transition temperature. The latter varies only weakly for those pH samples that exhibit a pre-melting transition.

2. On the physical nature of the reversible precursor transition in relation to melting

HES note specifically that, at the time of writing ($\sim$2000), the origin of the reversible transition was *not yet unambiguous*. Earlier, Jackson and Sturtevant (JS) using difference scanning calorimetry and visible absorbance experiments [7] suggested a conformational transition affecting the chromophase. JS also suggested that the pre-melting transition at 78°C could arise from a change in the crystalline packing of
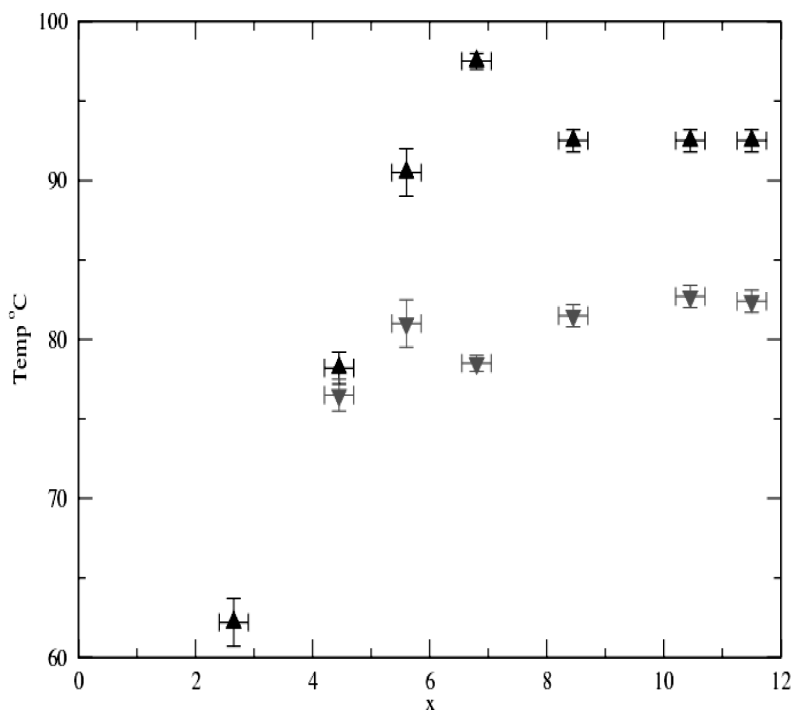

Figure 1.   Melting (triangles) and pre-melting (inverted triangles) transition temperatures as a function of pH for native bR, as determined experimentally by Heyes and El-Sayed [3] (Redrawn after figure 4 of [3]).

the trimers in the lattice. Their idea was investigated using X-ray diffraction and circular dichroism (CD) spectroscopy by Hiraki *et al*. [8]. These authors found that the diffraction pattern of the purple membrane above $78°C$ indicated a disordered structure from disruption of a hexagonal crystal lattice with some local order from the trimer structure in the Bragg peaks and CD spectrum.

2.1. *Additional degrees of freedom brought into play above the pre-melting transition*

An order–disorder theory of the melting transition goes back to the early statistical-mechanical model of Lennard-Jones and Devonshire [9]. This model had a single parameter, U, which in turn was related to a defect energy. Evidently, then, the thermal energy associated with the melting temperature, T_m, $k_B T_m$, has its scale fixed by the parameter U in the above model. Returning briefly to a simple diatomic molecular solid built of say N_2 molecules, experiment shows that at sufficiently low temperatures, the N_2 molecules attached to the sites of a perfect translational crystal lattice are orientationally ordered; an orientationally disordering percursor transition occurs in this molecular solid. A phenomenological extension of the Lennard-Jones and Devonshire model of melting [9] was therefore proposed in the work of Pople and Karasz [10], which is usefully summarised in the book on melting by Ubbelohde [11]. The new feature of the Popl–Karasz phenomenology was a barrier height, B, which had to be overcome, to reveal the additional degrees of freedoom contributing when disorder in the originally orientationally ordered molecules set in. What we wish to emphasise here is that the generalised statistical-mechanical model involving now the *coupling* of the melting and the precursor 'orientational disorder' transition is somewhat reminiscent of figure 1 redrawn from the experimental study of HES. What we stress is that there is no assurance presently that the second parameter B, which must certainly be introduced because of pre-melting, is directly associated with additional degrees of freedom embracing orientational disorder in bR. What seems clear however, from the original linear increase of the melting temperature with pH is that, once additional degrees of freedom enter the statistical model, the melting curve is intimately correlated with the transition temperature of the precursor phase change, leading to melting and pre-melting temperatures becoming essentially independent of pH. There is evidently a characteristic thermal energy $\sim 12°C$ emerging from figure 1 for pH > 8. This should aid later attempts to generalise the Lennard-Jones and Devonshire model to embrace additional degrees of freedom revealed above the premelting transition beyond those considered in the phenomenology of Pople and Karasz.

Figure 1 shows systematically regions we shall delineate as a result of the measurements of HES. The first region of pH, from 2.5 to ~ 4.5, has a single phase boundary. But, near pH ≈ 4.5, a pre-melting transition occurs, though the linear melting curve is continued out to pH ≈ 7. There we have indicated a further region, after which the melting curve rather rapidly becomes parallel to the pre-melting line, the separation, as noted, being around $12°C$.

If we adopt a order–disorder viewpoint regarding the melting line, we propose a parameter which should eventually be subsumed into any realistic statistical mechanical model, which we take to be an interaction energy between protein building blocks, say $\epsilon_J(x)$, where x is the value of the pH and varies between 0 and 7.

The observed linearity of the measured melting curve then allows us to write, with $T_m(x)$ denoting the melting temperature curve:

$$T_m(x) = \epsilon_J^{(0)} + \epsilon_J^{(1)}x.$$

We estimate from the HES measurements that $\epsilon_J^{(0)} = 40.0°C$ and $\epsilon_J^{(1)} = 8.5°C$. $\epsilon_J^{(0)}$ is then the energy required to overcome the coil–coil interaction of the crystal phase. In order to understand the increase in melting temperature with pH, we propose that as the pH is increased, the resulting partial denaturing of the protein coils leads to an *increased* bonding between the protein units of the crystal phase. However, before the protein is fully denatured, the crystal melts. This is reflected in the linear increase in T_m with pH.

By contrast, at pH ~ 7 the denatured protein conformation becomes energetically favourable before melting and results in the crystal undergoing a transition to the observed pre-melting reversible phase. In terms of the ions H and OH, we could alternatively assume that these modify the protein structure in a way that eventually leads to conformations resembling linear polymeric chains. Increasing the pH further does not effect any more changes to the protein conformation and so the phase line remains relatively constant with pH. By contrast, increasing the temperature leads to a disordering of these linear polyermic chains. In particular, this results in the introduction of additional degrees of freedom in this phase. We must then postulate a liquid–liquid phase transition driven by pH, in which a local coordination number change occurs across a further phase boundary. Returning to the requirements for a future statistical model, a further parameter which must be subsumed is the difference in energy (corresponding to $\sim 12°C$) between the parallel melting and premelting curves at large pH > 7. It may be worth adding that the data displayed in figure 1 suggests that the *interaction* between melting and pre-melting lines (due to the presence of at least two parameters in the relevant Hamiltonian) is a maximum at pH $= 7$, after which the parallel line separation $\sim 12°C$ takes over.

3. Discussion

A study of the phase diagram of bR suggests that it can be understood by appealing to a 3-phase model. In the low temperature crystal phase, bR forms a lattice with units comprising the molecules in their natured state. However, the protein can be denatured either by increasing the pH or by increasing the temperature. In the first scenario, the increased pH effects a decrease in the relative intra-molecule bond strengths and uncoils the molecule. By contrast, increasing temperature has the effect of increasing the entropic energy thereby allowing the molecule to overcome the coiling–uncoiling energy barrier. When the increased entropic energy is above the order-disorder barrier energy, this will result in the melt-phase. Then the idea is that, in the region below $x = 7$, coiled protein building blocks interact with energy $\epsilon_J(x)$ until eventually, at $x = 7$, the effect of the pair interactions between the protein building blocks is sufficient to cause the protein building blocks to uncoil. Our further proposal is that until $x = 7$, the coiled conformation remains largely intact. Beyond that, and between melting and pre-melting curves, we anticipate linear polymeric-like conformations, which we expect to persist as a polymer melt above the melting line.

Acknowledgements

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