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Short Note

DYNAMIC PROPERTIES OF BACTERIORHODOPSIN IN PURPLE MEMBRANES UPON HEAT TREATMENT

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Reversible temperature-dependent conformational changes in bacteriorhodopsin of the purple membranes from *Halobacterium halobium* have been studied by the method of deuterium exchange. A noticeable increase in the mobility of structured peptide groups in bacteriorhodopsin was revealed upon reorganization of the supermolecular structure at about 60°C. In the supermolecular structure formed, bacteriorhodopsin molecules have no contacts with external medium at 75–80°C. Membrane destruction results in a drastic increase in molecular mobility within the narrow temperature range 100–110°C. The effects observed are induced by predenaturation changes in the bacteriorhodopsin structure and rearrangements in the structure of a protein-lipid complex. The temperature dependence of the number of peptide groups involved in reversible conformational rearrangements is in good agreement with the microcalorimetry data.

1. Introduction

Purple membranes from *Halobacterium halobium* provide a good model for the study of mechanisms of ion transfer through membranes [1]. Modern concepts ascribe the transmembrane ion transfer to conformational changes in membrane proteins or to local rearrangements of lipids. In this respect, it is important to study pre-denaturational events in membranes conserving the total structure of molecular complexes, since they prepare the molecular structure for transformation into a qualitatively new state. In the present work the method for detection of pre-denaturational temperature-dependent changes in polypeptides and proteins that we developed [2] has been used for studying the dynamic behaviour of the purple membrane structure.

2. Materials and methods

Purple membranes were isolated according to the previously described method [3]. The concentration of bacteriorhodopsin was evaluated spectrophotometrically using $\epsilon_{570} = 63000\ M^{-1}\ cm^{-1}$ [4]. Heat sorption curves were recorded by means of a DASM-1M differencial scanning microcalorimeter [5]. Infrared absorption spectra were recorded with a UR-20 spectrophotometer (Karl Zeiss, Jena, G.D.R.). Samples for measurement in infrared absorption spectra were prepared as films on a fluorite support. The films were obtained from a purple membrane suspension aerated at 20°C. The film studied was placed in an air-tight chamber under an atmosphere of saturated D_2O vapour at 20°C. The chamber was equipped with a check valve to ensure that the internal pressure did not exceed 1.3 atm. The chamber was thermostatted for 30 min at a temperature chosen within the range 40–160°C. The period of thermostatting was chosen experimentally: i.e., it should be sufficiently long to exchange hydrogen for deuterium in the flexible peptide groups while being as short as possible to minimize possible irreversible temperature-induced changes. After this procedure the temperature in the chamber was decreased gradu-

ally to 20°C. The chamber was then allowed access to air and the film on the fluorite support placed in an atmosphere of saturated H_2O vapour at 20°C. Only changes induced by reverse exchange of deuterium for hydrogen were completed within 2–8 h. After the reverse D → H exchange a complex absorption band can be observed in the region of the ND stretching vibrations of the peptide bond in the infrared absorption spectrum. The intensity of this band can be taken as a measure of the number of COND peptide groups resistant to reverse exchange at room temperature. The intensities of the bands of the CH stretching vibrations (~ 2800–3000 cm^{-1}) were used as an internal standard for control of the film thickness.

3. Results and discussion

Fig. 1 shows the infrared spectra of the purple membrane films as a function of temperature of exposure of the films to D_2O vapour. The spectra were recorded at 20°C after reverse D → H exchange. An increase in temperature results in the appearance of a band in the region of ND stretching vibrations. The band consists of two almost equal components (2476 and 2420 cm^{-1}) which is due to the Fermi resonance between the ND vibrations and the amide II and amide III vibrations of the deuterated forms [6,7]. Fig. 2 depicts the temperature dependence of the number of bacteriorhodopsin peptide groups n_0^{ND}, involved in reversible conformational rearrangements. n_0^{ND} is related to the integral intensities of the stretching vibrations of the ND and NH groups (D^{ND} and D^{NH}, respectively) and to the number of peptide groups inaccessible to water molecules (n_0^{NH}) by the relationship:

$$n_0^{ND} = 1.87 n_0^{NH} D^{ND} / D^{NH}.$$

Fig. 2 also shows the curve of heat sorption of the purple membrane suspension.

In order to interpret the results obtained it is natural to assume that the appearance of the peptide groups resistant to reverse D → H exchange is due to reversible, fluctuating changes in the molecular conformation, which bring sterically inaccessible regions of the polypeptide chains into contact with water molecules. It can be seen from fig. 2 (curve 1) that up to 50°C, buried inaccessible

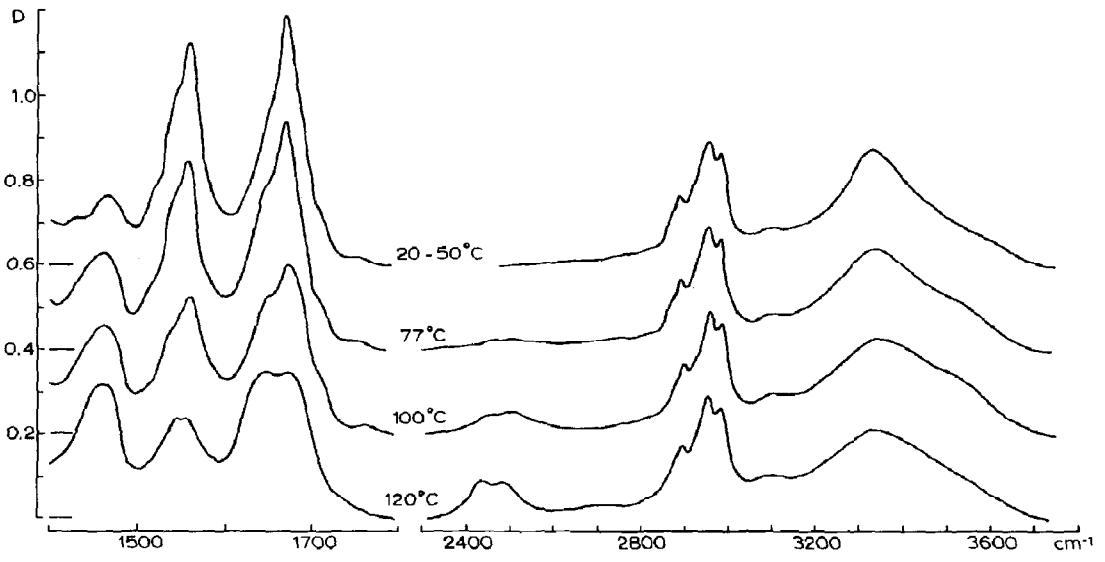

Fig. 1. Infrared absorption spectrum of a purple membrane film as a function of exposure to D_2O vapour at different temperatures.

peptide groups in the bacteriorhodopsin molecule do not change their environment. Elevation of temperature leads to the transfer of some buried peptide groups to the protein surface in contact with water molecules. The number of such peptide groups is maximal at 60°C and constitutes one-third of the total number. Subsequently, the number of bacteriorhodopsin peptide groups in contact with water molecules begins to decrease within the temperature range 60–80°C, reaching a minimum at 80°C. At this temperature nearly all the peptide groups of bacteriorhodopsin are not exposed to water molecules. It is of interest that circular dichroism demonstrates no destruction of α-helix in bacteriorhodopsin in purple membranes up to 70°C [8] while reverse D → H exchange occurs at 70–80°C almost without obstacle. We believe that this becomes possible only in the case when the molecular arrangement of the membrane is altered in such a way that the packing of the protein and lipid molecules in the membrane becomes less compact in comparison with that of the native structure. The membrane adopts a new arrangement in such a manner that the peptide groups directed outside due to a reversible conformational change are fixed in a new state at those positions where the NH group is in contact with molecules of the external medium. In terms of this model the endothermic transition shown in fig. 2 (curve 2) with a maximum at 70°C arises due to the heat expenditure for such a rearrangement.

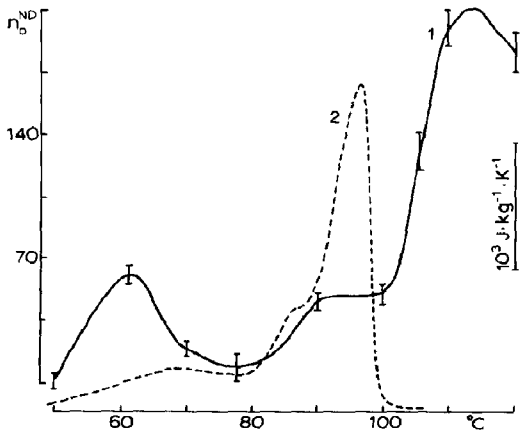

Fig. 2. Temperature dependence of the number of peptide groups involved in reversible conformational rearrangements (curve 1) and of the specific heat capacity of the purple membrane suspension (curve 2).

Heating of the purple membrane suspension above 80°C results in two peaks of heat sorption (fig. 2, curve 2) with maxima at 85 and 98°C. The first peak is due to formation of vesicles from the initially flat purple membranes, the second being caused by denaturation of bacteriorhodopsin [9]. The number of peptide groups involved in reversible conformational rearrangements increases within the temperature range 80–90°C, being practically the same at 90°C as at 60°C. The complex part of curve 1 in fig. 2 in the temperature range 80–100°C appears to be due to a change in the membrane ultrastructure and/or to the denaturation of bacteriorhodopsin which causes destruction of about one-third of the structural α-helix [8].

Above 100°C the number of peptide groups accessible to water molecules increases. At 115°C all peptide groups of the α-helix are involved in conformational rearrangements. This temperature region is characterized by the beginning of the process of destruction of the membranes [9] which leads to some solubilization of bacteriorhodopsin molecules in water (the character of curve 1 in fig. 2 is similar to that for the water-soluble proteins).

It is worth noting that the denaturation processes at high temperatures are clearly demonstrated by the infrared absorption spectra (fig. 1). Above 100°C the width of the amide I band increases, due mainly to the appearance and increase in intensity of the absorption band with a maximum at about 1640 cm^{-1} which is characteristic of the formation of the β-structure.

Hence, monitoring of the temperature dependence of reversible conformational changes in proteins applied to membrane complexes allows one to study the influence of membrane rearrangements on the exposure of a protein molecule to the external medium. According to the data obtained, heat treatment of the purple membrane up to 60°C does not destroy the native structure of the protein-lipid complex, despite a considerable increase in mobility of the peptide groups and their exposure to molecules of the external medium. This phenomenon can be used for selective modifi-

cation of peptide groups of the protein of purple membranes at elevated temperatures.

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