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Biochimica et Biophysica Acta 1234 (1995) 221–224

BBA Biochimica et Biophysica Acta

Lipid–protein interactions in the purple membrane: structural specificity within the hydrophobic domain

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Received 7 June 1994; revised 21 October 1994; accepted 24 November 1994

Abstract

In the absence of native-like interactions between bacteriorhodopsin and its neighbouring lipids, the pigment chromophore is reversibly titrated from its purple 570 nm form to a blue-shifted 480 nm form in the moderately alkaline pH range. Quantitation of this acid-base chromophore equilibrium in vesicles prepared from modified lipid mixtures shows that it is absent under conditions where bacteriorhodopsin is allowed to interact with methyl-substituted alkyl chains. The peculiar homogeneous structure of purple membrane alkyl chain lipids is thus likely to be an essential requirement for maintenance of the native bacteriorhodopsin structure over a wide pH range.

Keywords: Bacteriorhodopsin; Purple membrane; Lipid–protein interaction; Methyl-substituted alkyl chain; Protein structure

1. Introduction

The purple membrane (PM) of the halophilic organism *Halobacterium salinarium* contains a sole protein: the light-activated proton pump, bacteriorhodopsin (bR). Its single polypeptidic chain is organised in seven transmembrane α-helices oriented approximately perpendicular to the membrane plane [1]. In purple membranes, bacteriorhodopsin exists as a two-dimensional (2-D) hexagonal array of protein trimers [2]. This rigid structure is a lipid-poor membrane as those which fill spaces between and within the trimers amount to less than 25% of the membrane dry weight [3]. In addition, purple membrane lipids are particular as they contain only tetramethylhexadecyl alkyl chains attached by an ether-type link to phosphatidylglycerol, phosphatidylglycerophosphate and phosphatidylglycerosulfate polar head groups [4] (Fig. 1).

High protein content and particular lipid composition suggest that strong, specific or functionally significant lipid–protein interactions may take place in purple membranes. For instance, the requirement of native phosphatidyl glycerophosphate or -sulfate for proteinic 2-D lattice formation and the existence of interfacial electrostatic lipid–bR interactions have recently been reported [5,6]. These latter interactions might well require a high degree of specificity [7]. On the other hand, in native PM, glycerolipids exist essentially in a bilayer configuration while, in aqueous suspensions, they do not tend to form bilayers [8]. Such non-bilayer forming lipids containing bulky hydrocarbon chains are thought to effectively seal and package the irregular hydrophobic surface of integral proteins [9], suggesting that interactions taking place at the hydrophobic lipid/protein interface may also be significant.

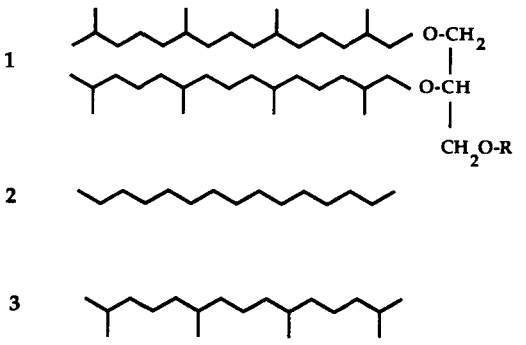

Fig. 1. Structure of the purple membrane lipidic alkyl chains (**1**) where **R** stands for the different native polar head groups. The structures **2** and **3** are those of pentadecane and tetramethylpentadecane, respectively, used in some vesicle reconstitution mixtures.

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SSDI 0005-2736(94)00296-7

The most striking difference between the membraneous and purified forms of bacteriorhodopsin is probably the color of their chromophore. Indeed, purified lipid- and detergent-free bacteriorhodopsin is a red pigment and upon its solubilisation in most detergents, it exists, near pH neutrality, as an acid-base equilibrium between two spectrally distinct species absorbing maximally at 540 and 480 nm [10]. More recently, we have found that the same pH indicator behavior of the bR chromophore can be observed in purple membranes in the presence of a wide variety of solvents and general anesthetics [11]. Observation of this equilibrium is allowed by a relaxed tertiary structure of bacteriorhodopsin which is mostly characterised by more tilted helices with respect to membrane plane [12]. In addition, the direct relationship existing between the lipid solubility of the different solvents and their ability to induce the bR_{570}/bR_{480} chromophores equilibrium suggested that their effect takes place in the membrane hydrophobic core, in agreement with the general hypothesis of xenobiotic-induced lipid-protein mismatch [13]. This suggestion received further support by the recent identification of the lipid/protein interface as the binding site of general anesthetics in purple membrane [14].

In the native patches, the absorption spectrum of bacteriorhodopsin is independent of pH in the range of 4 to 11. When monomeric bacteriorhodopsin is reconstituted in vesicles where native lipids are substituted for more simple ones, its spectral and functional integrity is fully maintained [15] but, within a narrower pH range. Indeed, under mildly alkaline pH conditions, the chromophore of bacteriorhodopsin contained in lecithin vesicles also exists, like in purified bacteriorhodopsin or solvent-treated purple membrane, as an acid-base equilibrium (apparent pK_a = 7.5) between two spectrally distinct purple and red forms [16,17]. In order to see whether the unique alkyl chain composition of purple membrane lipids do play some role in shifting the bR_{570}/bR_{480} chromophores equilibrium towards the native purple form, we have reconstituted the pigment into phosphatidyl choline vesicles added with linear and methyl substituted alkanes. We find that in the presence of the latter, the bR_{570}/bR_{480} chromophore equilibrium is not observable; the pigment is then stabilised in the bR_{570} form, indicating that structurally specific lipid–protein interactions within the hydrophobic membrane core significantly contribute to the maintenance of the native pigment structure over a wide pH range.

2. Materials and methods

Purple membranes were isolated and purified from cultured cells of *Halobacterium salinarium* (formerly *H. halobium*) strain S_9 according to the standard procedure [18]. Bacteriorhodopsin was reconstituted in soybean phosphatidyl choline (Sigma, St. Louis, MO) according to either two procedures: 'instant' vesicles containing all original purple membrane lipids prepared by sonication (for 20 min at 2° C with a 225R Ultrasonics cell disruptor, 70% full power) following the procedure described by Racker [19] and vesicles prepared by cholate dialysis of partially (< 25%) delipidated purple membranes in the presence of solubilised phosphatidylcholine according to the method described by Hwang and Stoeckenius [20]. Both methods gave essentially identical results with respect to chromophore spectral properties. Vesicles were prepared and kept in unbuffered 150 mM KCl. After formation, they were purified by sedimentation through a 1.4–0.7 M linear sucrose gradient. After equilibrium was reached, vesicles located at a buoyant density of 1.163 ± 0.003 g/ml by comparison with a value of 1.187 ± 0.002 g/ml for purple membranes. The reconstitution mixture essentially contained 50 μmol of lipids and 200 nmol of bacteriorhodopsin in 5 ml (PC/bR ratio = 250:1). In many cases, pentadecane or 2,6,10,14,-tetramethyl pentadecane (both from Sigma) partly replaced phosphatidyl choline up to an alkane mol fraction of 0.33 (0.33 pentadecane/0.66 PC on a molar basis or alternatively 0.33 pentadecane/1.32 acyl chain on a fatty chain basis) without noticeable change in the vesicle formation. At higher alkane concentration, vesicle looked differently and their sedimentation pattern was changed; those samples were discarded.

Absorption spectra were recorded with a Pye Unicam SP8-100 spectrophotometer equipped with a diffuse sample holder. The pH of the vesicle suspension, monitored with a microelectrode, was adjusted by injection of minute amounts of dilute HCl or NaOH in a stirred spectrophotometer cell.

3. Results and discussion

The absorption spectrum of bacteriorhodopsin-containing vesicles is given in Fig. 2. As stated earlier, in the neutral through moderately alkaline pH range, their chromophore exists as an acid-base equilibrium between the native purple form (bR_{570}) and the blue-shifted bR_{480} form. Upon progressive alkalinization of the vesicle suspension, the purple bacteriorhodopsin absorption band reversibly bleaches at the expense of a new absorption band centered at 480 nm. In phosphatidylcholine vesicles, the apparent pK_a of this equilibrium is 7.7 and the titration curve shows no important hysteresis; titration curves obtained by stepwise acidification or alkalinization of the vesicles are comparable with those obtained by erratic back and forth pH jumps. This behavior is very similar to that of lipid-free bacteriorhodopsin [10] and there is little doubt that vesicular bR_{480}, like lipid-free bR_{480}, contains a protonated Schiff base chromophore and differs from bR_{570} mostly in secondary retinal–apoprotein interactions [21].

In these vesicles, the bR_{570}/bR_{480} equilibrium can be displaced towards bR_{570} if the lipid mixture from which they are prepared is modified. Thus, in Fig. 3A, it can be

seen that addition of the reconstitution mixture with tetramethyl pentadecane reduces the amount of purple bacteriorhodopsin that can be titrated to bR_{480}. Increasing amounts of tetramethyl pentadecane do not change the apparent pK_a of the phenomenon; it only induces the appearance of an increasing fraction of bacteriorhodopsin molecules where this spectral transition is absent within the pH range considered, resulting in titration curves of smaller amplitude. Such an all-or-none effect suggests a direct interaction between bacteriorhodopsin and the ramified alkane.

However, it is not the case when pentadecane is used instead of tetramethylpentadecane. In Fig. 3B, the amplitude of the titration curves corresponding to the $bR_{570} \leftrightarrow bR_{480}$ equilibrium in vesicles is plotted as a function of increasing concentration of pentadecane and tetramethyl pentadecane. The presence of pentadecane up to a mole fraction of 0.33 with respect to lipid content scarcely modify bacteriorhodopsin 'titrability' while this latter property has almost completely disappeared at a the same tetramethyl pentadecane molar fraction. The amplitude of the titration curve is reduced to 25 and 7% of its original value at tetramethyl pentadecane mol fractions of 0.17 and 0.33, corresponding to alkane/bR ratios of 42:1 and 82:1, respectively.

In the native purple membrane, the bR_{570}/bR_{480} equilibrium cannot be observed. The absorption maximum of the pigment is pH independent up to pH > 11 where chromophore deprotonation occurs [22]. The trimeric structure of bacteriorhodopsin can hardly be responsible for the absence of this equilibrium in native membranes as solvent- or anesthetic-induced formation of bR_{480} occurs in ordered hexagonal trimer lattices and disordered lattices as well [23,24]. In addition, the bR_{570}/bR_{480} equilibrium is

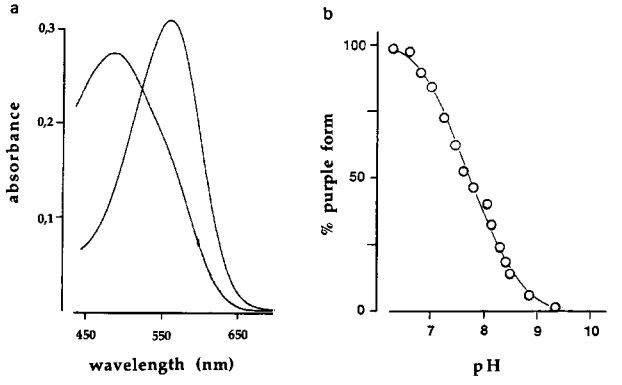

Fig. 2. The left part shows the absorption spectra of bacteriorhodopsin reconstituted into phosphatidyl choline vesicles measured at pH 6.5 (λ_{max} = 570 nm) and at pH 9.5 (λ_{max} = 480 nm) in unbuffered 150 mM KCl. In the right part, open circles correspond to the percentage of purple bacteriorhodopsin that is titrated to its 480 nm spectral form as a function of pH. Percent values were calculated from the ratio of absorbances at 570 and 480 nm after normalisation of the values of this ratio at 100 and 0 at pH 6.5 and 10, respectively. The solid line is the normalised titration curve of an hypothetic group with a pK_a of 7.7.

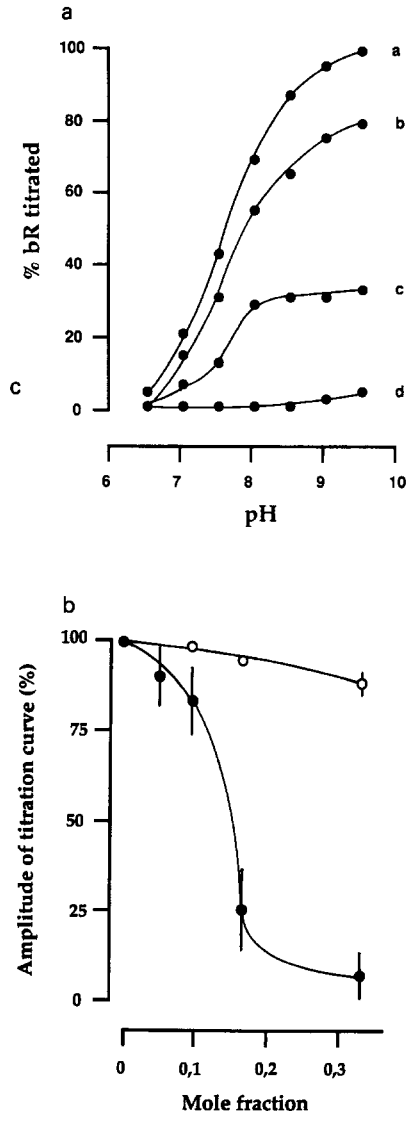

Fig. 3. (A) Inverted titration curves of bacteriorhodopsin chromophore in vesicles containing tetramethylpentadecane. Curves a through d correspond to tetramethylpentadecane mol fractions of 0, 0.05, 0.17 and 0.33, respectively. (B) Amplitude of the $bR_{570} \leftrightarrow bR_{480}$ titration curve between pH 6.5 and 9.5 in phosphatidylcholine vesicles containing increasing mol fractions of pentadecane (open circles) and tetramethylpentadecane (closed circles). Error bars represent the standard deviation measured over five sets of vesicles prepared with purple membrane from two different batches of halobacterial cultures.

also absent in many monomeric forms of bacteriorhodopsin. As a matter of fact, the chromophore equilibrium is either absent or occurs only above pH 10 in all solubilised but not delipidated bacteriorhodopsin samples. The one exception to this rule is the Triton X-100-solubilised lipid-free bacteriorhodopsin in which the purple color is stable over a large pH range [10] and, curiously, Triton is the only detergent which has multiple methyl side groups on its hydrophobic part. Together with the results presented here, it constitutes phenomenological evidence that the methyl side groups of the purple membrane alkyl chain lipids play a specific structural role in the mainte-

nance of the native lipid–protein interactions without which the pigment chromophore readily equilibrates between different spectral species.

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