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ARTICLE

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Proton binding to biological membranes

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Abstract Biological membranes contain proton-binding moieties. A laser-induced proton pulse was used to characterize the proton-binding properties of bacterioopsin-containing membranes and of sarcoplasmic reticulum. Different protonation and deprotonation processes occurred. The liberation of protons from pyranine dye and the protonation of the membranes were independent of temperature; the reprotonation of pyranine and proton release from the membranes were temperature dependent. In the cases of membrane-free and membrane-containing systems, the activation enthalpies and entropies were calculated from the decay rates. The activation enthalpy of 16 kJ/mol for reprotonation of pyranine in membrane-free solution is characteristic for a diffusion-controlled process. The value for the membrane-containing systems was nearly double, suggesting that the buffering moieties of the membrane surfaces strongly bind the protons, raising the activation enthalpies. This is possibly an effect of the Coulomb cages formed from closely located proton acceptor sites. The activation entropies were positive in all cases.

Keywords Proton jump · Pyranine dye · Arrhenius parameters · Bacterioopsin · Sarcoplasmic reticulum

Introduction

The surfaces of biological membranes contain proton-binding moieties. The review of Gutman and Nachliel (1990) discusses the dynamics of proton transfer processes in broad perspective, among others those occurring on membranes. Gutman and colleagues studied the time behavior of protonation of the free proton-accepting sites on purple membranes and on bacterioopsin-containing membranes from *Halobacterium salinarum* by the proton jump technique (Nachliel et al. 1996). The protons liberated from pyranine dye by UV laser flashes were bound to the membranes within ~1 μs and released with a lifetime of ~50 μs at room temperature. Analysis of the decay curves led to the determination of the number of proton acceptors on the cytoplasmic and extracellular sides of both membranes (Nachliel et al. 1996). It was also found that Coulomb cages, formed from closely located negative charges on the membrane, influenced the proton binding.

Contrary to the above data, the dwell time for protons on the surface of purple membranes, emitted in the photocycle of bacteriorhodopsin, was found to be about 700 μs at room temperature. From the temperature-dependent data an activation enthalpy of ~40 kJ/mol and negative activation entropy were determined (Heberle and Dencher 1992; Heberle et al. 1994). The negative activation entropy was not calculated by these authors but from the published data by us (Tóth-Boconádi et al. 2000).

The incongruity of the long proton dwell time compared to the results of the above proton-binding experiment was previously discussed (Tóth-Boconádi et al. 2000). In the present paper, we report that activation enthalpies of proton binding to bacterioopsin-containing membranes and to sarcoplasmic reticulum membrane are in the range 27–30 kJ/mol and the activation entropies are positive.

Materials and methods

Purple membrane was separated from *Halobacterium salinarum* strain R1M1 by the standard method (Oesterhelt and Stoeckenius 1974). Retinal was extracted by the method described by Dancsházy et al. (1999) to obtain membrane with bacterioopsin (white membrane). Sarcoplasmic reticulum (SR) was isolated from rabbit skeletal muscle as described by Nakamura et al. (1976). The

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SR preparations were suspended in a medium of 0.25 M sucrose, 10 mM Tris-maleate, pH 8, at a protein concentration of about 30 mg/mL, and stored at –70 °C until use. Before use, samples were thawed and diluted 20 times with 0.1 M KCl, pH 7.2 (without buffer), and centrifuged at 54,000*g* for 60 min at 4 °C. The pellets were resuspended in the same sucrose and buffer-free media. The membrane and pyranine concentrations of the solutions, as well as those of other ions, are given in the figure caption. The temperature was controlled with homemade instruments.

Proton jumps were produced by illuminating the sample containing pyranine (8-hydroxypyrene-1,3,6-trisulfonic acid, trisodium salt; Molecular Probes) with an excimer laser (Lambda Physik EMG 101 MSC, $\lambda = 308$ nm). The process is represented as $\varphi OH \rightarrow \varphi O^- + H^+$. The released protons bind to membranes ($M^- + H^+ \rightarrow MH$) and are released with a characteristic time delay and reprotonate φO^-. This process was monitored at $\lambda = 448$ nm with an argon ion laser (Stabilite 2016, Spectra-Physics). The transient proton concentration was determined using the extinction coefficient $\epsilon = 24{,}000\ M^{-1}\ cm^{-1}$ for deprotonated pyranine φO^- (Nachliel et al. 1996). Absorption changes were recorded with a photomultiplier and digitized by a computer-controlled transient recorder (Thurlby DSA-524).

Results and discussion

The temperature dependencies of the φO^- concentrations were recorded for pyranine without any membrane, for sarcoplasmic reticulum membrane, and for white membrane, at the monitoring wavelength. In all three cases, time dependencies with a fast rise (within 1 μs) and slower decay appeared. The interaction of protons with membranes is a complex process. According to Nachliel et al. (1996), different protonation and deprotonation reactions occur. We assumed that one of those is the rate-limiting step and evaluated the time dependencies by fitting two exponentials: one for rise and one for decay. Our assumption only approximates the underlying complex processes. Figure 1 shows the Arrhenius plots of the rate constants of the decays. The rates of rise, i.e. the release of protons from pyranine and the protonation of the membranes, did not exhibit any temperature dependence (not shown). The rates of decay, expressed in $M^{-1}\ s^{-1}$ units (rates of second order), i.e. the reprotonation of φO^-, allowed us to determine the activation enthalpies and entropies. The activation enthalpy ΔH and entropy ΔS were calculated by the equation:

$$k = 10^{13} \exp\left(\frac{1}{R}\left(\Delta S - \frac{\Delta H}{T}\right)\right) \quad (1)$$

where R and T are the Boltzmann constant and temperature, respectively.

The data are collected in Table 1. The rate of reprotonation of free pyranine (18 ± 1 in units of $10^{10}\ M^{-1}\ s^{-1}$ at 23 °C) coincided with the value of 18 ± 1.5 from the paper of Gutman and Nachliel (1990). The activation entropies are positive in all three cases, indicating phenomena in the direction of disorder. The activation enthalpy of 16 kJ mol^{-1} for the reprotonation of φO^- in membrane-free solution corresponds to a diffusion-limited reaction. The values of ~27–30 kJ mol^{-1} for the membranes suggest that the buffering moieties of membrane surfaces strongly bind the protons, raising the activation enthalpies above those of diffusion-controlled mechanisms. We assign this phenomenon to the Coulomb cages formed from closely located proton acceptor sites.

The two membranes studied in this work are of different composition: bacterioopsin contains only the apoprotein of bacteriorhodopsin and lipids. The SR membrane contains the main protein and Ca^+ATPase, as well as other proteins. Nevertheless, the protonation parameters of the two membranes are similar to each other. We plan to extend our studies to other membranes to determine if the protonation parameters of other protein-containing membranes are generally similar.

These results might help to separate the effect of membrane protonation from the parameters to be studied in proton jump driven reactions or reactions where protons are emitted. The timing of proton emission was studied in the experiments mentioned in the Introduction (Heberle and Dencher 1992; Heberle et al. 1994). The activation enthalpies found for the protonation of membranes (Table 1) are smaller than ~40 kJ mol^{-1} measured by these authors for assumed proton dwelling via lateral diffusion on the membrane surface after light-driven proton emission from the

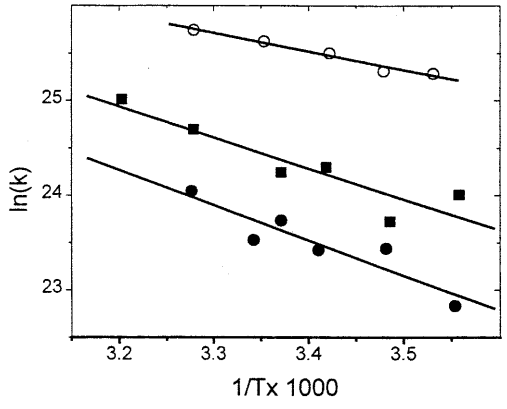

Fig. 1 Arrhenius plots of the rate constants for decay in the cases of pyranine (○), sarcoplasmic reticulum (■), and bacterioopsin-containing membrane (●). The second-order rate constant k is in units of $M^{-1}\ s^{-1}$. *Lines* are the results of linear fits. Solutions were 30 μM pyranine, 30 μM pyranine and 14 μM ATPase in the membrane of sarcoplasmic reticulum, 30 μM pyranine and 17 μM bacterioopsin, respectively. The solutions were unbuffered at pH 7.2

Table 1 Values of activation enthalpy ΔH (kJ mol^{-1}) and entropy ΔS (kJ $mol^{-1}\ K^{-1}$) for the reprotonation of φO^- in the cases of membrane-free and membrane-containing solutions

Sample	ΔH	ΔS
Pyranine	16.1 ± 1.6	$+0.018 \pm 0.002$
Pyranine + SR	27.1 ± 5.7	$+0.047 \pm 0.010$
Pyranine + white membrane	30.1 ± 6.5	$+0.048 \pm 0.010$

membrane-bound bacteriorhodopsin. Although their Arrhenius parameters were determined for membrane-containing photoexcited bacteriorhodopsin, we do not expect the proton buffering energetics to differ much between the membranes containing bacteriorhodopsin or bacterioopsin. The decisive difference between parameters for membrane protonation and proton emission is in the activation entropies. These are positive for protonation of the membranes and negative for proton emission by bacteriorhodopsin, indicating an ordering process not characteristic for diffusion. The Arrhenius parameters for the proton emission, according to our explanation based on electric measurements (Tóth-Boconádi et al. 2000, 2001), are due to the photoreaction in the bacteriorhodopsin itself. The protons are released by a proton-emitting cluster at the extracellular side and taken up by a proton-accepting cluster at the cytoplasmic side of the molecule that undergo reorganizations during the photocycle. This takes a long time, ~1 ms at room temperature, much longer than the time the protons spend on the membrane, ~50 μs, as shown in this work and also by Nachliel et al. (1996). This 50 μs is the time the emitted protons dwell on the membrane. This dwell time, convoluted with the 1 ms proton emission time, remains undetectable.

We may conclude that further, more detailed studies of this type are useful for all experiments where the proton jump technique or proton emission measurements on membrane-bound proteins are considered.

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