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The effect of lipid environment in purple membrane on bacteriorhodopsin

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Abstract

The decay rate of the Bacteriorhodopsin (BR) photocycle intermediate M_{412} and proton, the proton pump efficiency (H^+/M_{412}), the ratios of M_{412} to other intermediates and the rotational correlation time (τ_c) in purple membrane (PM) fragments treated by the zwitterionic detergent 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) with different concentrations were studied. The results show that: (1) The largest effect of CHAPS on M_{412} decay rate and proton decay rate of BR, τ_c of PM and the ratios of M_{412} to other intermediates in BR photocycle is in the range of its critical micelle concentration (CMC). This indicates that changes of the ratios of M_{412} to other intermediates, τ_c, M_{412} decay and proton decay occur and are due to the variation of the lipid environment. (2) The dependency of proton yield on CHAPS concentrations is basically consistent with that of $M_{412s\%}$. This indicates the relation between proton pumping function and M_{412}. These studies show the importance of maintaining a native environment.

Keywords: Bacteriorhodopsin; Purple membrane; CHAPS

1. Introduction

Bacteriorhodopsin (BR), the only protein in Purple Membrane (PM), has the function of a light-driven electrogenic proton pump. The protein consists of 248 mainly hydrophobic amino acids and a chromophore, all-*trans*-retinal, bound to lysine-216 via a protonated Schiff-base linkage [1,2]. Upon the absorption of yellow light, BR goes through a photochemical cycle consisting of several distinct spectroscopic intermediates, K_{610}, L_{550}, M_{412}, O_{640} [3,4]. Among them, the formation and decay of M_{412} are associated with proton pumping function [5]. In the process of M_{412} formation, M_{412} was observed to have two rise components [6]. M_{412} decay was also confirmed to have two components, a fast decaying component of M_{412} (M_{412f}) and a slow decaying component of M_{412} (M_{412s}). The event linked with the BR photocycle is the proton pumping function. The quantum yield of the photocycle has several values measured by different laboratories [7,8]. There are many factors that affect kinetics of M_{412} decay and protonation, such as lipid in PM, cations, temperature, pH, etc. One of the good methods to study the mechanism of BR photocycle is to use detergent to remove lipid in PM [9,10]. Different detergents have different effects on BR and lipid in PM. Nonionic detergent Triton X-100, β-Octylglucoside, bile salt [11–13] can remove lipid in PM to different extents and change the kinetic process of BR. About the effect of zwitterionic detergent CHAPS on the structure and function of PM and BR, few papers can be found [14].

The effect of different environment on the secondary structure shows detergent solubilization results in changing the protein conformation [15]. There are relationship between structure and function on BR, the change of structure should affect the BR photocycle process and protons pump function. This paper presents the effect of zwitterionic detergent CHAPS on the rotational correlation time (τ_c) in PM, the intermediate M_{412} decay process in the BR photocycle, the proton decay process, the ratios of M_{412} to other intermediates and the proton pumping function resulting from changing lipid environment in PM. From these results it shows there is a relation between

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proton pumping function of BR and M_{412s} decay. The largest effect of CHAPS on BR photocycle is in the range of its critical micelle concentration. Lipid environment is important for the structure and function of purple membrane.

2. Materials and methods

CHAPS was purchased from Sigma. For growth of H. halobium and isolation of purple membrane, the method of Oesterhelt [16] was used with slight improvement. The purple membrane fragments were suspended in various CHAPS concentrations (0–40 mM). The mixed membrane suspension was adjusted to pH = 6.7 and OD (568) = 0.2, then incubated for 24 h at 4°C in the dark. For the ESR measurements, purple membrane was labeled with 16-doxyl-stearic acid, free radical (from Sigma) for 3 h at room temperature, then the labeled sample was washed several times until the free spin label was removed. ESR spectra were obtained from Varian E-109, X band, center magnetic field, 3200 Gs, sweep width 200 Gs, time constant 0.1280 ms, sweep time 120 s, microwave power 20 mW, modulation amplitude 1 Gs, microwave frequency 9.13 GHz. The steady-state absorption spectra was obtained from an UVIKON-930 UV/visible spectrometer. The ratios of M_{412} to other intermediates in the photocycle were determined by laser Raman spectroscopy (JY T-800). The excitation wavelength was 488 nm. BR treated with CHAPS was illuminated in front of a 250 W tungsten lamp (10 cm) for more than 1 min before the start of the experiment for Raman measurements, flash kinetic spectroscopy measurements and the steady-state absorption measurements. All measurements were done at 25±0.1°C in a circulating water bath with temperature control (RTE-8, NESLAB). The kinetics of M_{412} decay rate was detected at the wavelength 412 nm and proton decay were assayed by probing the transient absorbance changes of illuminated BR using an average of 85 flashes at 400 nm. Transmission changes of BR and pH indicating dye were measured with a single beam flash kinetic spectrophotometer constructed in our laboratory. The measuring light source was a 250 W tungsten lamp. Illumination was induced by a photographic flash-lamp (BY-18) made by a camera manufacturer in Shanghai with a 0.4-ms flash duration. The flash beam was passed through a yellow filter (OG4). The wavelength of 412 nm will be cut off, while the wavelength of 568 can pass through. The change in proton concentration of the assay medium (H^+/mol BR) was calibrated in 0.1 mM HCl with *p*-nitrophenol as a dye indicator using continuous illumination. The differential extinction coefficient is assumed to be 23 mM^{-1} cm^{-1} at wavelength: 412 nm [17]. Proton yield is calculated from the ratio of protons released per M_{412}(H^+/M_{412}).

3. Results

3.1. The steady-state absorption of BR treated with CHAPS

The absorption maximum of BR treated with different concentrations of CHAPS is blue shifted by 2–7 nm (Fig. 1). The absorption in the UV range has little change. This shows that the retinal chromophore is affected by adding CHAPS.

3.2. Kinetics of M_{412} and proton decay

Changes were measured in the decay kinetics of intermediate M_{412} and proton in BR treated with CHAPS. For BR treated with 6 mM CHAPS concentration (this concentration is in the range of CHAPS critical micelle concentration), the decay times of M_{412} and proton increase obviously (Fig. 2). The half times of M_{412s} ($t_{1/2s}$) and proton decay ($t_{1/2H^+}$) as functions of CHAPS concentration are shown in Fig. 3. The $t_{1/2s}$ and $t_{1/2H^+}$ increase from 3.8 to 43 ms and from 6 to 97 ms, respectively, when CHAPS concentrations increase from 0 to 20 mM. The decay half times of proton and M_{412s} exhibit a sharp increase from about 2 mM CHAPS. At high CHAPS concentrations, the increase of both $t_{1/2}$ tends to saturate. This phenomenon indicates that the effects of zwitterionic detergent CHAPS on the decay of M_{412s} and proton are strongly related with its effects on the lipid environment of BR [18].

3.3. Proton pumping function

Between 2–4 mM CHAPS, the proton yield (H^+/M_{412}) of BR in 0.1 M KCl was the lowest, 0.67, which was much less than natural BR (Fig. 4). H^+/M_{412} began to increase above 4 mM CHAPS, but it was still less than that of natural BR. It is suggested that the proton pumping

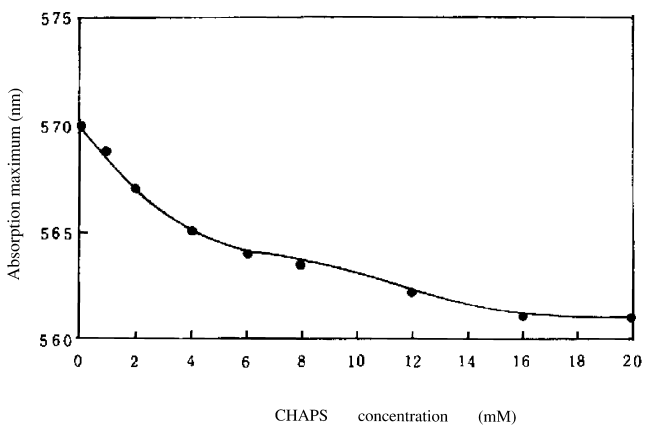

Fig. 1. CHAPS concentrations dependence of absorption maximums of BR treated with CHAPS at pH = 6.7, temperature: 25±0.1°C.

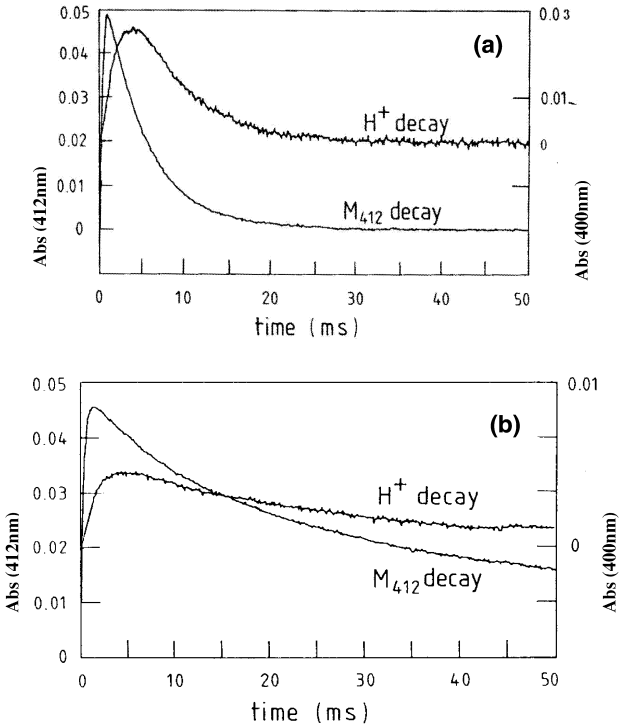

Fig. 2. Comparison of M_{412} decay of BR with those of proton decay. (a) For native BR. (b) For BR treated with 6 mM: CHAPS. Temperature: 25±0.1°C; Probe wavelength: 400 nm for proton decay and 412 nm for M_{412} decay.

function is also dependent on the lipid environment in PM. Moreover, the dependency of proton yield on CHAPS concentrations is basically consistent with that of $M_{412s\%}$ on CHAPS concentrations (Fig. 4). This indicates the relation between proton pumping function and M_{412s}.

3.4. Raman spectra

Fig. 5 shows the Raman spectra of BR treated with 0–16 mM CHAPS. The band at 1567 cm^{-1} is due to the C=C stretching vibration of M_{412} intermediate, the band at 1530 cm^{-1} is due to the C=C stretching vibration of other intermediates [19]. I_{1567}/I_{1530} cm^{-1} could show the relative quantity of M_{412} in the photocycle. The relationship of I_{1567}/I_{1530} cm^{-1} and CHAPS concentrations was shown in Table 1. When the concentration of CHAPS was near 6 mmol, the ratio of I_{1567}/I_{1530} cm^{-1} was largest. It means that near the critical micelle concentration of CHAPS, the relative quantity of M_{412} in photocycle reached the maximum.

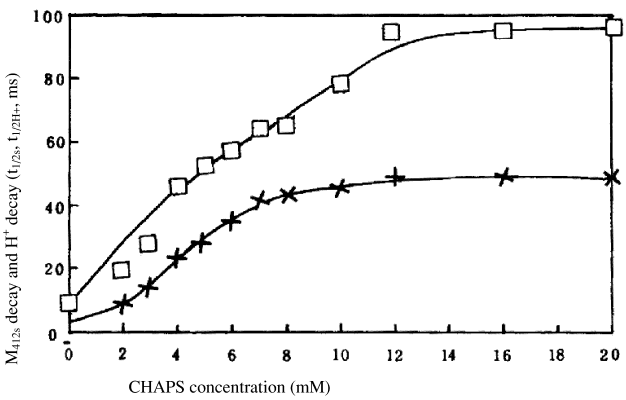

Fig. 3. CHAPS concentrations dependence of $t_{1/2s}$ (+), and $t_{1/2H^+}$ (□) in 0.1 M KCl at pH=6.7 and temperature: 25±0.1°C.

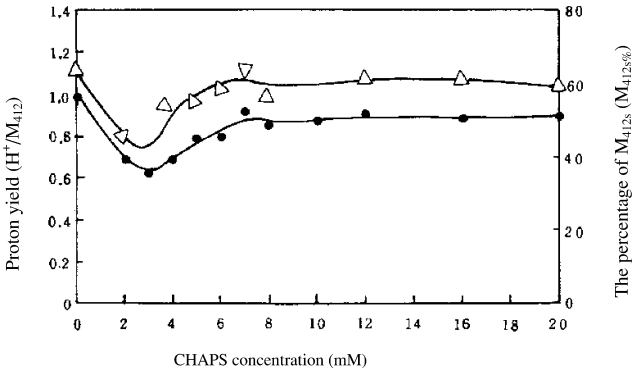

Fig. 4. CHAPS concentrations dependence of proton yield H^+/M_{412} (●) and $M_{412s\%}$ (△) in 0.1 M KCl at pH=6.7 and temperature: 25±0.1°C.

3.5. ESR spectra

Fig. 6 shows the ESR spectra of PM treated with 0–40 mM CHAPS labeled by 16-doxyl-stearic acid, free radical. It is shown that in the ESR spectra, the parameters which are used to calculate the rotational correlation time have changed when PM is treated with successive concentration of CHAPS. Fig. 7 shows the effect of CHAPS on rotational correlation time τ_c of hydrophobic inner area in PM. The τ_c increased with CHAPS concentration increasing, when CHAPS concentration was 5 mM, τ_c reached the maximum.

4. Discussion

The data above demonstrates that CHAPS has effects on the kinetics of M_{412} decay and proton pumping function. The zwitterionic detergent CHAPS is known to be effective at solubilizing lipids and membrane proteins without denaturation of proteins [20]. Since the CMC of CHAPS was reported differently from several laboratories [14,20,21], the range of CMC is from 4 to 10 mM CHAPS concentration. In this experiment, the effect of CHAPS on purple membrane is to solubilize the lipids around BR first, for lipids are solubilized more easily than proteins. CMC of the detergent provides not only a guide for the appropriate concentration of detergent, but also an approximate upper limit to the concentration of membrane to be

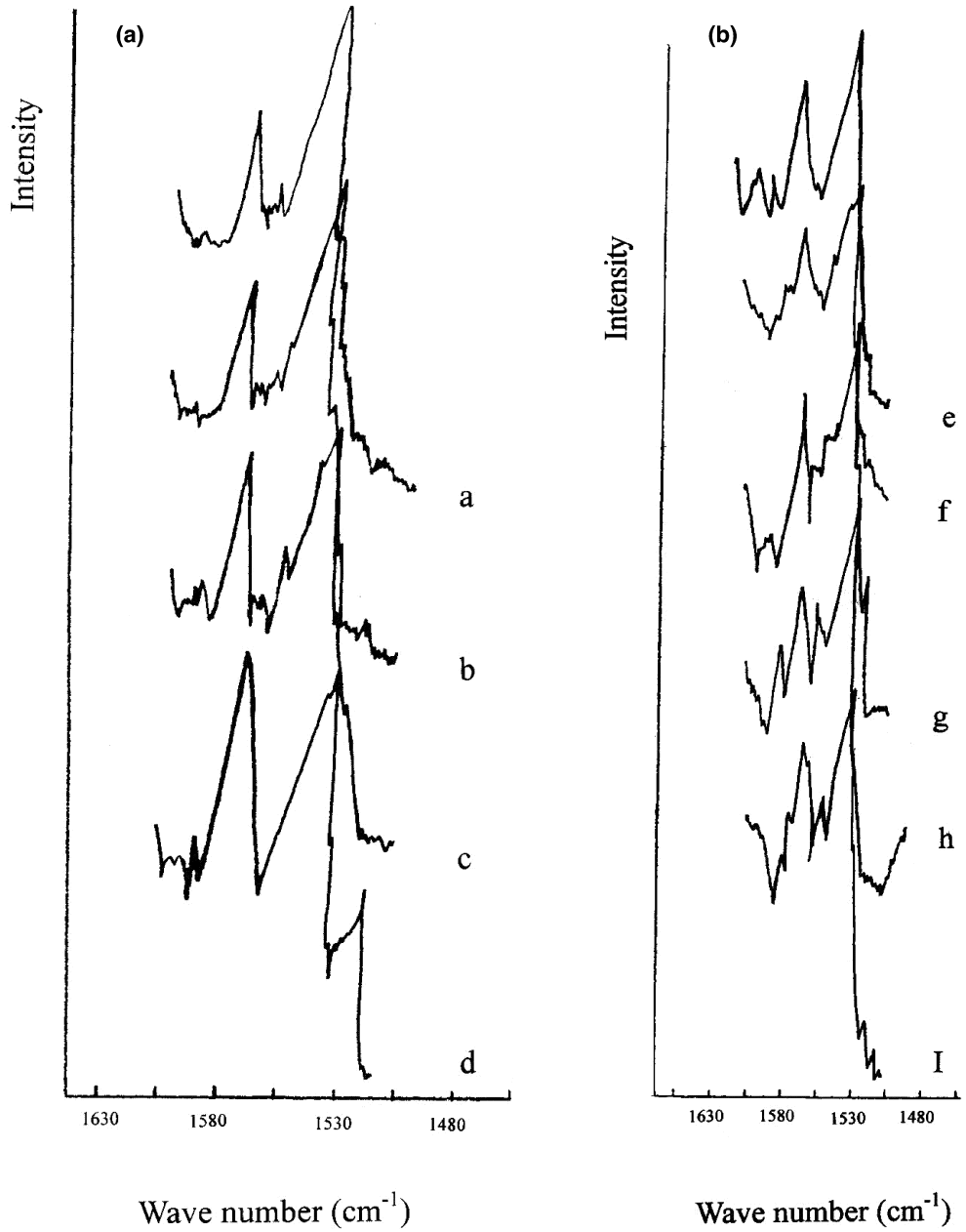

Fig. 5. Raman spectra of BR treated with 0–16 mmol/l CHAPS. (a) 0 mmol/l; (b) 2 mmol/l; (c) 4 mmol/l; (d) 6 mmol/l; (e) 8 mmol/l; (f) 10 mmol/l; (g) 12 mmol/l; (h) 14 mmol/l; (i) 16 mmol/l.

solubilized. The process of liposome solubilization and reconstitution of bacteriorhodopsin have been studied using CHAPS and CHAPSO, the process was shown to fit well to three-stage model previously proposed for other detergents [22]. According to the structural characteristics of CHAPS, we can divide its states in aqueous solutions into three parts for purple membrane solubilization [23,24].

(1) 0–4 mM: It is under the CMC of CHAPS. Because the tail of CHAPS is inserted into the inner area of the bilayer of purple membrane, a slight disturbance takes place in lipid bilayer without destroying the integrity of the bilayer. The high viscosity in these area of purple membrane bilayer is formed. The results show that rotational correlation time increases gradually. The isomerization of retinal

Table 1
Relationship between I_{1567}/I_{1530} cm^{-1} and CHAPS concentrations

CHAPS (mmol/l)	0	2	4	6	8	10	12	14	16
I_{1567}/I_{1530} cm^{-1}	0.51	0.58	069	0.94	0.58	059	0.55	0.54	0.54

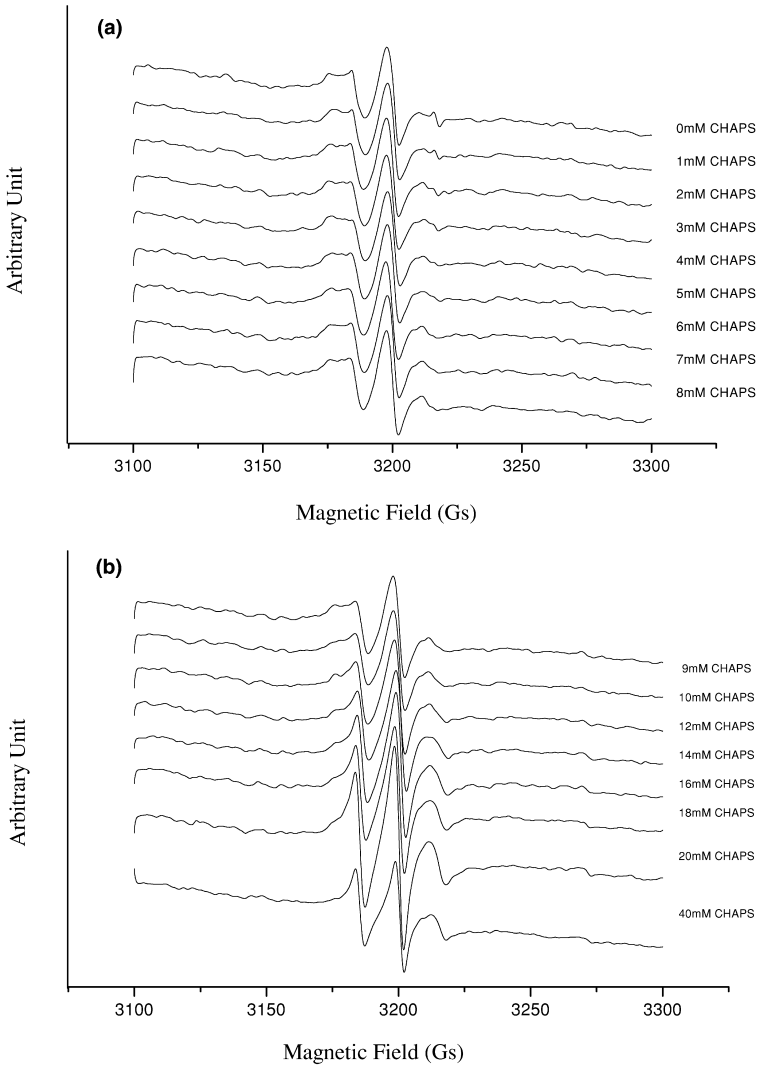

Fig. 6. ESR spectra of purple membrane labeled by 16-doxyl-stearic acid, free radical, treated with different concentrations of CHAPS.

is not easy and photochemical cycle becomes difficult. The change of lipid environment of BR in PM results in increasing of $t_{1/2s}$ and $t_{1/2H^+}$. (2) 4–10 mM: The range of formation of CHAPS micelles, both CHAPS monomers and micelles exist in equilibrium. Lipids are removed by CHAPS, and some proteins are solubilized. The integrity of the lipid bilayer is damaged, so the environment of BR greatly changes. The τ_c reached the maximum at 5 mM CHAPS concentration. It results in the sharp increase of $t_{1/2s}$ and $t_{1/2H^+}$ near 4 mM CHAPS treatment. (3) Above 10 mM: This is above the CMC of CHAPS. Lipids around the membrane protein are exchanged for detergent, resulting in formation of mixed CHAPS-lipid micelles, CHAPS-protein micelles and CHAPS-lipid-protein micelles. In the presence of excess CHAPS, micelles are in a more stable state, so both $t_{1/2s}$ and $t_{1/2H^+}$ tend to saturate.

The effects of CHAPS on the proton yield, $M_{412s\%}$ (Fig. 4) show that when CHAPS concentration reaches 3 mM, the proton yield is the lowest, the $M_{412s\%}$ reaches the minimum, above 8 mM CHAPS both proton yield and $M_{412s\%}$ tends saturate. These results also show the effect of the lipid environment of BR on the proton pump function and photocycle of BR. If the change of M_{412s} and proton decay kinetics could be traced simultaneously, the understanding of relationship between them might be obtained. Studying this problem should benefit the understanding of the mechanism of the relation between the BR photocycle and the proton pump. It was noticed, $t_{1/2s}$ and $t_{1/2H^+}$ increases when CHAPS concentration increases from 0 to 10 mM. When above 10 mM, both of them tend to saturate, but proton yield and $M_{412s\%}$ have the minimum at 3 mM, it means the proton yield and $M_{412s\%}$ are not consistent directly with the $t_{1/2s}$ and $t_{1/2H^+}$.

From ESR experiments, it is noticed that between 10 and 20 mM CHAPS concentration, τ_c decreases. It means that when CHAPS concentration reaches 20 mM, the purple membrane will be solubilized completely.

The ratios of M_{412} to other intermediates show the

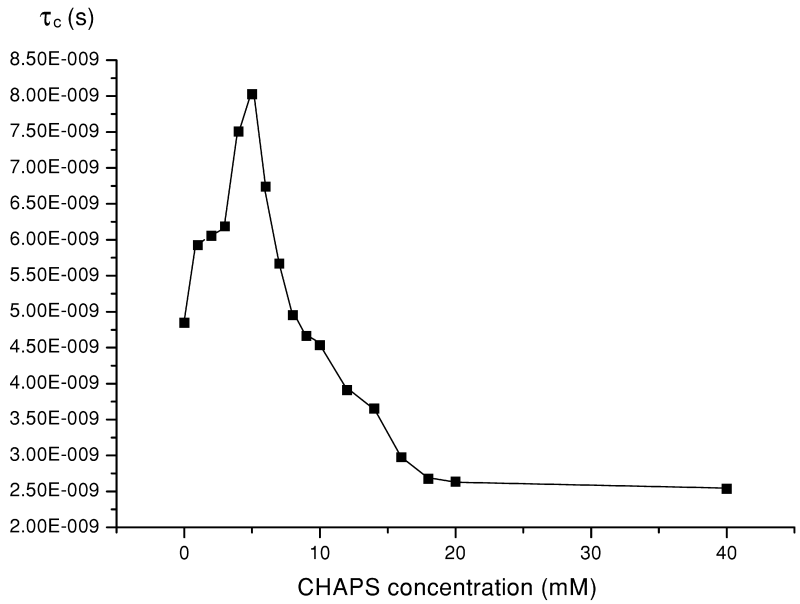

Fig. 7. Effect of CHAPS on rotational correlation time τ_c of hydrophobic inner area in purple membrane bilayer.

relative quantity of M_{412} in the photocycle of BR. It was reported that after treatment of CHAPS, the kinetics of M_{412} of BR show an increase in the rise time and much larger increase in the decay time [14]. Therefore, it is reasonable that the ratios of M_{412} to other intermediates increase when the BR is treated by CHAPS. From our experiments, the M_{412} decay increases obviously from 2 mM CHAPS concentration, the ratios of M_{412} to other intermediates also increase from 2 mM CHAPS concentration. The M_{412} decay sharply increases from 2 to 6 mM CHAPS concentration and the relative quantity of M_{412} in the photocycle reaches the maximum near 6 mM CHAPS. After 10 mM CHAPS concentration, both the M_{412} decay and ratios of M_{412} to other intermediates tend saturate.

The study of the effect of environment on the structure shows detergent solubilization results in changing the protein conformation [15]. Upon partial delipidation or detergent solubilization, a significant conformational change occurs [25]. The change of absorption maximum of BR (Fig. 1) also means the change of protein structure. In this work we have investigated more thoroughly the profound influence on BR photocycle, proton pump function and membrane viscosity by treatment of purple membrane with different CHAPS concentrations. We suggest CHAPS affects the lipid environment of BR, the effect of environment results in the conformation change of BR. The change of conformation leads the change of photocycle and proton pump function of BR.

Some other detergents, such as Triton X-100, and melittin that affect the lipid environment of BR also lead the change of photocycle and proton pump function of BR [26–29]. These studies show the importance of maintaining a native lipid environment of BR.

5. Conclusions

1. Further confirm the association between M_{412s} and proton pumping function.
2. In the range of its critical micelle concentration (CMC), CHAPS makes largest effect on M_{412} decay and proton decay of BR.
3. The proton yield and $M_{412s\%}$ are lowest when CHAPS concentration reaches 3 mM. Largest effect of CHAPS on proton yield and $M_{412s\%}$ occurs before its critical micelle concentration. They are not consistent with the M_{412} decay and proton decay.
4. CHAPS makes the changes of rotational correlation time (τ_c) of hydrophobic inner area in PM and ratios of M_{412} to other intermediates. The τ_c reaches the maximum at 5 mM CHAPS concentration, and the ratios reache the largest at 6 mM CHAPS concentration. Both of the largest effects occur in the range of CMC.
5. The studies of the effect of lipid environment in purple membrane show the importance of maintaining a native lipid environment of BR.

6. Abbreviations

PM	Means purple membrane
BR	Means bacteriorhodopsin
τ_c	Means rotational correlation time
CHAPS	Means (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate)
$t_{1/2s}$	Means the half time of M_{412} slow-decaying component
$t_{1/2H^+}$	Means the half time of proton decay

$M_{412s\%}$	Means the percentage of M_{412s} in total M_{412}
H^+/M_{412}	Means the proton pump efficiency
CMC	Means critical micelle concentration

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