Study on the Long Lifetime of the M State in Chemically Modified Bacteriorhodopsin Film

Bing Liang, Baofang Li,* and Long Jiang

Laboratory of Colloid and Interface Science, Center for Molecular Science, Institute of Chemistry, The Chinese Academy of Sciences, Beijing 100101, China

Received January 31, 2001
Revised Manuscript Received June 25, 2001

In recent years, bacteriorhodopsin (BR) has been extensively investigated on account of its attractive properties, such as large quantum efficiency, long-term stability to thermal and photochemical degradation, fast photoisomerization response, high two-photon efficiency, and reasonable diffraction efficiency, all of which can be fully utilized in making optical and optoelectronic devices (for a review, see refs 1−3).

BR is the key protein in the purple membrane (PM) isolated from *Halobacterium salinarum*.[4] Upon absorption of light, the BR molecules in the PM act as light-driven proton pumps, with a net transport of protons from the inner (cytoplasm) to the outer (extracellular) side of the cell.[5−8] BR has a photocycle that contains a series of states with different absorption spectra and various lifetimes.[9] The B state is the ground state and has a wide absorption band in the neighborhood of 570 nm. When a molecule in the ground state absorbs a photon, it moves through a series of intermediate states into the M state, which has a lifetime of 10 ms under natural conditions.[10] The M state is the only photointermediate that has a deprotonated Schiff's base, and as a result of the deprotonation, its absorption spectrum is significantly blue-shifted to 412 nm from the 570 nm of the initial ground state (B state). Thus, between the M and B states, the system can be approximated macroscopically by a photochromic two-level model (M_{412} ↔ B_{570}). This photochromic model (M_{412} ↔ B_{570} cycle of 1 million times has been reported[11]) is quite unique and provides a mechanism for optical applications.

However, some applications require significantly longer M-state lifetimes to obtain greater information storage time, higher light sensitivity, higher contrast ratios, and higher contrast decay times.[12] Hence, the lifetime of 10 ms of the M state is too short for actual use, and methods for extending the M-state lifetime under ambient conditions continue to be a focus of investigations of BR materials. In the past two decades, a number of methods have been developed to extend the lifetime of the M state by changing the environment of BR (humidity,[13] pH,[14] temperature,[15] chemical additive,[16−22] etc.), modifying the structure of the molecule itself by genetic variants,[23,24] or replacing the retinal chromophore by a synthetic analogue.[25] Of all of these methods, using a chemical additive to prolong the M-state lifetime is the most convenient, and it has an attractive potential because of the variety of agents available for selection.

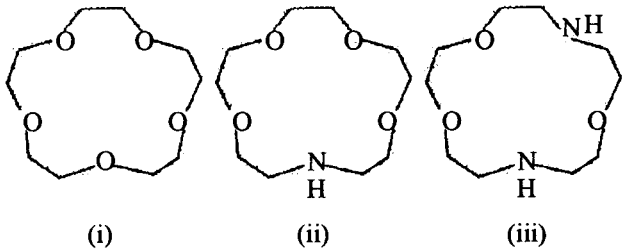

Figure 1. Structures of (i) 15-crown-5 (1,4,7,10,13-pentaoxacyclopentadecane), (ii) aza-15-crown-5 (1,4,7,10-tetraoxa-13-azacyclopentadecane), and (iii) diaza-15-crown-5(1,4,10-trioxa-7,13-diazacyclopentadecane).

Because crown ether complexes strongly with cations such as H^+, Li^+, Na^+, and K^+,[26] it is possible that, by using crown ether as a chemical additive, the photocycle of BR could be modified partially and subsequently the M-state lifetime could be extended. In the present study, we attempted to prolong the lifetime of the M state by using 15-crown-5, 1,4,7,10-tetraoxa-13-azacyclopentadecane (aza-15-crown-5), and 1,4,10-trioxa-7,13-diazacyclopentadecane (diaza-15-crown-5) as chemical additives (structures shown in Figure 1), and a long lifetime for the M state was conveniently obtained. Thus, this effort expands the use of BR materials in optical applications.

* Corresponding author. Address: Institute of Photographic Chemistry, the Chinese Academy of Sciences, Beishatan, DeWai, Beijing, 100101, P. R. China. Telephone: +86-10-64888175. Fax: +86-10-64879375. E-mail: bfli@ipc.ac.cn.

(1) Brauchle, C.; Hampp, N.; Oesterhelt, D. *Adv. Mater.* **1991**, *9*, 420.
(2) Lindvold, L. R.; Ramanujam, P. S. *J. Sci. Ind. Res.* **1995**, *54*, 55.
(3) Hampp, N. *Chem. Rev.* **2000**, *100*, 1755.
(4) Birge, R. R. *Biochim. Biophys. Acta* **1990**, *1016*, 293.
(5) Kuhlbrant, W. *Nature* **2000**, *406*, 569.
(6) Subramaniam, S.; Henderson, R. *Nature* **2000**, *406*, 653.
(7) Lanyi, J. K. *Biochim. Biophys. Acta* **2000**, *1459*, 339.
(8) Balashov, S. P. *Biochim. Biophys. Acta* **2000**, *1460*, 75.
(9) Gergely, C.; Zimanyi, L.; Varo, G. *J. Phys. Chem. B* **1997**, *101*, 9390.
(10) Birge, R. R. *Annu. Rev. Phys. Chem.* **1990**, *41*, 683.
(11) Chen, Z.; Lewis, A.; Takei, H.; Nebenzahl, I. *Appl. Opt.* **1991**, *30*, 5188.
(12) Seitz, A.; Hampp, N. *J. Phys. Chem. B* **2000**, *104*, 7183.
(13) Korenstein, R.; Hess, B. *Nature* **1977**, *270*, 184.
(14) Bacon, M.; Wang, C. H.; Kar, A. K.; Baxter, R. L.; Wherrett, B. S. *Opt. Commun.* **1996**, *124*, 175.
(15) Hampp, N.; Popp, A.; Brauchle, C.; Oesterhelt, D. *J. Phys. Chem. B* **1992**, *96*, 4679.
(16) Brith-Lindner, M.; Avi-Dor, Y. *FEBS Lett* **1979**, *101*, 1132.
(17) Avi-Dor, Y.; Rott, R.; Schnaiderman, R. *Biochim. Biophys. Acta* **1979**, *545*, 15.
(18) Nakasako, M.; Kataoka, M.; Tokunaga, F. *FEBS Lett.* **1989**, *254*, 211.
(19) Uruga, T.; Hamanaka, T.; Kito, Y.; Uchida, I.; Nishimura, S.; Mashimo, T. *Biophys. Chem.* **1991**, *41*, 157.
(20) Lee, K.-H.; Mcintosh, A. R.; Boucher, F. *Biochem. Cell Biol.* **1991**, *69*, 178.
(21) Bandrovskaya, I. K.; Shershun, Y. D.; Batori-Tartsi, Z. I.; Frolova, N. P.; Korposh, O. I.; Sharkany, J. P. *Proc. SPIE* **1997**, *3055*, 91.
(22) Batori-Tartsi, Z. Ludmann, K.; Varo, G. *J. Photochem. Photobiol. B* **1999**, *49*, 192.
(23) Oesterhelt, D.; Brauchle, C.; Hampp, N. *Q. Rev. Biophys* **1991**, *24*, 425.
(24) Marinetti, T.; Subramanian, S.; Mogi, T.; Marti, T.; Khorana, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 529.
(25) Jaaskelainen, T.; Leppanen, V. P.; Parkkinen, S.; Parkkinen, J. P. S.; Khodonov, A. *Opt. Mater.* **1996**, *6*, 339.
(26) Izatt, R. M.; Bradshaw, J. S.; Nielsen, S. A.; Lamb, J. D.; Christensen, J. J. *Chem. Rev.* **1985**, *85*, 271.

10.1021/cm010109l CCC: $20.00
Published on Web 08/08/2001

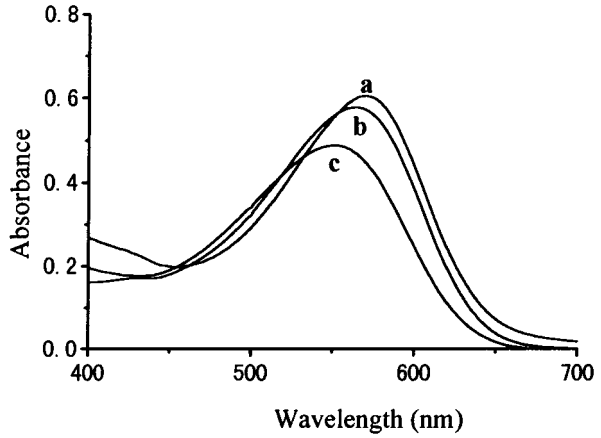

Figure 2. Absorption spectra of (a) BR suspension, (b) BR−PVA film, and (c) BR−PVA film with a BR/diaza-15-crown-5 molecular ratio of 1:250.

We prepared different BR−PVA [poly(vinyl alcohol)] films using 15-crown-5, aza-15-crown-5, and diaza-15-crown-5 as chemical additives. Tris [tris(hydroxymethyl)-amino methane] was used as the buffer in these films. The molecular ratio of BR/additive was in a range of 1:100−1:300. Spectral and kinetic transformation studies of BR−PVA films were carried out using absorbance spectroscopy. All of the samples were light adapted prior to the measurement.

The absorption spectra of a BR suspension and of a BR−PVA film both with and without chemical additive are presented in Figure 2. It is well-known that the absorption maximum of a BR−PVA film without any chemical additive (Figure 2, curve b) has a slight blue shift compared to the absorption peak wavelength of BR in suspension (Figure 2, curve a) because of the influence of the dehydration on the Schiff base of the retinal chromophore of BR.[27] As the chemical additive, for example diaza-15-crown-5, was added to the BR−PVA films, another blue shift of about 10 nm (Figure 2, curve c) compared to BR−PVA film occurred. It could be argued that diaza-15-crown-5 might interact with the BR molecules in a dried film. When the molecular ratio of BR/diaza-15-crown-5 reached 1:300, BR was partially denatured (not shown here). It can therefore be concluded that BR/diaza-15-crown-5 above a ratio of 1:300 had a strong effect in denaturing the purple membrane. The same effect of a blue shift from 570 nm was observed upon addition of aza-15-crown-5 or 15-crown-5 to the BR−PVA film, but it was not as obvious as that for diaza-15-crown-5 in the molecular ratio range of 1:100−1:300 for BR to crown ether.

The decay kinetics of the M state in the BR−PVA films with these three different chemical additives in the molecular ratio range of 1:100−1:250 was investigated. Figure 3 shows the typical decay kinetics curves of the M state in BR−PVA films at different BR/diaza-15-crown-5 molecular ratios. With increasing additive content, the decay of the M state was gradually slowed with all three different additives, and different lifetimes of the M state ($\tau_{1/e}$) were found to exist separately. Figure 4 shows a comparison of these three additives' effects on the prolongation of the M-state lifetime. The results indicate that the structure of crown ether is

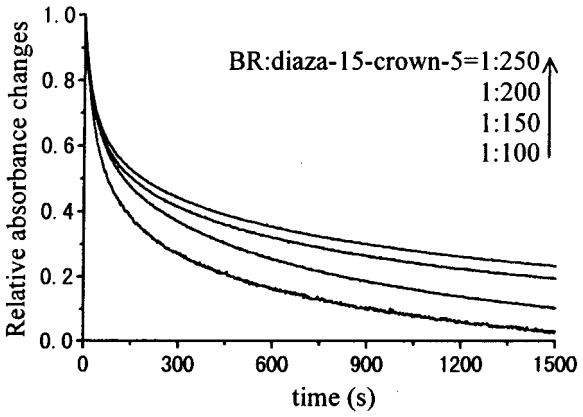

Figure 3. Decay kinetics curves of the M state in a BR−PVA film at different BR/diaza-15-crown-5 molecular ratios.

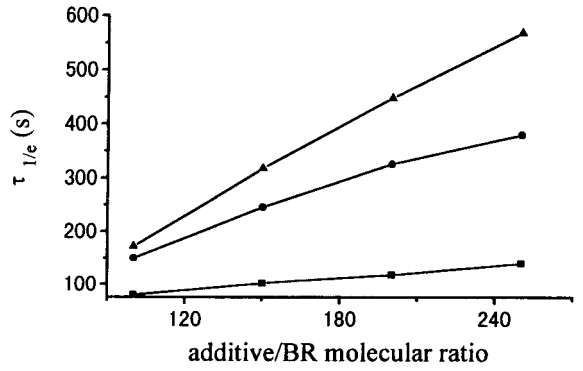

Figure 4. Comparison of three additives' effects on the prolongation of the M-state lifetime (−■−, 15-crown-5; −●−, aza-15-crown-5; −▲−, diaza-15-crown-5).

important in the prolongation. When 15-crown-5 was added into BR−PVA films, the lifetime of the M state was extended to about 100 s at a BR/15-crown-5 molecular ratio of 1:250. When the oxygen heteroatom on the ring of 15-crown-5 was replaced by a −NH− group, the effect of the new agents on the prolongation was enhanced, and this effect also increased with increasing number of −NH− groups. Aza-15-crown-5 could extend the M-state lifetime to 384 s when its content was at a BR/aza-15-crown-5 molecular ratio of 1:250. Furthermore, the effect of diaza-15-crown-5 on the photocycle of BR was the most marked among these three, and the lifetime of the M state could be prolonged to 568 s at the highest diaza-15-crown-5 content level of BR/diaza-15-crown-5 molecular ratio of 1:250, which represents over a 4-order-of-magnitude improvement on the 10-ms lifetime of the native BR protein. This long lifetime of the M state indicates that diaza-15-crown-5 is a competent chemical additive, and even compared with the results for other kinds of chemical additives, such as triethanolamine and arginine,[18,22] this lifetime is much longer and more attractive for optical uses.

This remarkable prolongation might originate from two aspects of the additives. First, these three crown ethers all have strong complexing properties toward H^+; thus, the number of protons available for the M-state BR molecules to recapture to relax back to the B state is reduced, and the rate of reprotonation of the Schiff base is significantly decreased. Because the pK_a of diaza-15-crown-5 is the largest among these three,[26] its complexing property toward H^+ is also the strongest. Thus, the M-state lifetime with this chemical additive

(27) Hildebrant, P.; Stockburger, M. *Biochemistry* **1984**, *23*, 5539.

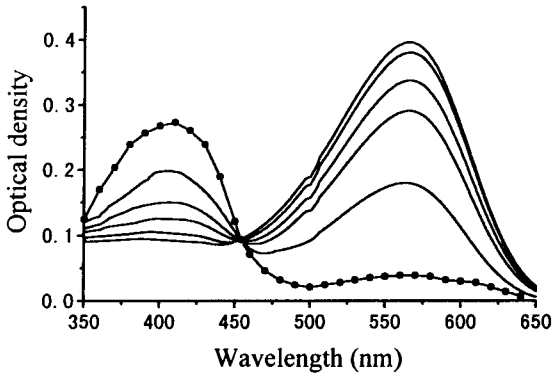

Figure 5. Photochromic property of a BR−PVA film with a BR/diaza-15-crown-5 molecular ratio of 1:250 (from top to bottom at 410 nm, the curves correspond to times of 0 min, 4 min, 10 min, 40 min, 90 min, and 3 h, respectively).

is surely the longest. Second, the −NH− group that plays a part of the ring has strong alkaline properties. It is well-known that increasing the pH of the environment of BR can increase the M-state lifetime.[14] This not only decreases the number of H^+ around the M-state BR molecules to extend the lifetime of the M state, but also partly accounts for the fact that the effect on prolongation of 15-crown-5 is the weakest whereas that of diaza-15-crown-5 is the strongest.

Figure 5 shows the absorption spectra of the BR−PVA film with a BR/diaza-15-crown-5 molecular ratio of 1:250 at various times during conversion of the M state to B state. The spectra were obtained by first exciting the BR film with 560-nm light for 30 s to reach a high M-state accumulation and then recording the subsequent absorption spectra as the M state relaxed back to the B state. The spectrum of zero time was obtained by the measurement of the absorbance at each wavelength just after the light source was turned off. From Figure 5, we can see that the M state was not yet depleted completely even after 1.5 h. In addition, the photochromism between yellow (M state) and violet (B state) can also be observed visually. Thus, the present BR−PVA film with the BR/diaza-15-crown-5 molecular ratio of 1:250 provides significant potential for BR optical applications even under ambient conditions.

In summary, the use of crown ether as an additive in a BR−PVA film has the effect of prolonging the lifetime of the M state significantly. When the BR/diaza-15-crown-5 molecular ratio reaches 1:250, the M state in the BR−PVA film has the longest lifetime ($\tau_{1/e}$) of 568 s. The results herein provide a potential for crown ether to affect the photocycle period of BR, and this saliently long lifetime of the M state establishes BR as an ideal material for optical applications. Many new and interesting applications in optics are now awaiting the exploitation of this material.

Experimental Method

Purple membranes were isolated from *Halobacterium salinarum* with the procedures described by Oestehelt and Stoeckenius[2828] and were suspended in distilled water at a concentration of 10 mg/mL. 15-Crown-5 and diaza-15-crown-5 were purchased from Aldrich. Aza-15-crown-5 was purchased from Acros. These chemicals were of analytical grade and were used without further purification. The average molecular weight of PVA was 30 000−50 000.

We prepared different BR−PVA films with and without the chemical additives of 15-crown-5, aza-15-crown-5, or diaza-15-crown-5. PVA was dissolved by boiling in a solution of 50 mM Tris at a concentration of 15% (w/v). The BR suspension was combined in a 1:1 volume ratio with PVA solution. Then, the BR−PVA mixture was spun at 5000 rpm for 15 min to remove residual bubbles. Films were prepared separately by dropping BR + PVA and BR + PVA + one of the chemical additives on clean quartz substrates, which were placed on a leveled plate at room temperature. The molecular ratio of BR/additive was in the range of 1:100−1:300. The pH of the solution was about 8.0. Then, the films were dried in air for more than 24 h under ambient conditions. Because of the low water content of the dried BR films, it is not feasible to give a pH value for them. Typically, dried films had an optical density (OD) between 0.4 and 0.5.

Spectral and kinetic measurements were carried out on a Javco V-530 UV/vis spectrophotometer at ambient conditions. The excited light source was a 300-W xenon lamp equipped with a glass optical filter (λ = 560 nm). The maximum power density of the excitation light was approximately 5 mW/cm^2. All films were light adapted for 10 min, and then they were exposed to 560-nm light for 30 s to reach a high M-state accumulation.

Abbreviations

15-crown-5 = 1,4,7,10,13-pentaoxacyclopentadecane
aza-15-crown-5 = 1,4,7,10-tetraoxa-13-azacyclopentadecane
diaza-15-crown-5 = 1,4,10-trioxa-7,13-diazacyclopentadecane
PVA = poly(vinyl alcohol)
Tris = tris(hydroxymethyl)-aminomethane

Acknowledgment. This work was supported by the National Natural Science Foundation of China (Contract 69731010) and the Chinese Academy of Sciences (Contract KJ-951-A$_1$-501-05).

CM010109L

(28) Oesterhelt, D.; Stoeckenius, W. *Meth. Enzymol.* **1974**, *31*, 667.