Effect of Cyclen on the Prolongation of M Intermediate Lifetime in the D96N Mutant of Bacteriorhodopsin

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A new kind of chemical additive—cyclen (1,4,7,10-tetraazacyclododecane) was used in BR_{D96N}-PVA films in an attempt to obtain longer M state lifetime. At the highest cyclen concentration of 1 : 250, the lifetime of M state could be prolonged to 936 s. Comparing the lifetimes of the M state in BR_{D96N} suspension and BR_{D96N}-PVA film with the same cyclen content, it is the strong complexing properties of cyclen towards H^+ that plays the most important role in this prolongation.

Bacteriorhodopsin (BR) is the key protein in the purple membrane (PM) isolated from *Halobacterium salinarium* and acts as a light-driven proton pump.[1–3] BR has been extensively studied over the last three decades, and a vast amount of data has been obtained on its structure, the main steps in proton transport, the role of light-induced configurationally changes of the chromophore and conformational changes of the protein, the function of certain residues, etc.[4–6]

Photoexcitation of the chromophore initiates a photocycle which involves several intermediate states labeled by J, K, L, M, N, and O, and returns finally to the initial BR state (B state).[7,8]

The M state is the only photointermediate that has a deprotonated Schiff base and its absorption spectrum is significantly blue-shifted to 412 nm from 570 nm of the B state. This photochromism of $B_{570} \leftrightarrow M_{412}$ is quite unique and provides a mechanism for optical applications.[9,10]

However, in wild-type BR protein, the M state lifetime is only 10 ms in ordinary conditions and is considered to be too short for optical applications. It has been known that BR_{D96N} has longer M state lifetime due to the replacement of aspartic acid (Asp-96) by asparagines (Asn-96),[11,12] and using chemical additive, such as guanidine hydrochloride,[13] arginine,[14] triethanolamine,[15] and diaza-15-crown-5[16] (1,4,10-trioxa-7,13-diaza-cyclopentadecane), is another useful method to prolong the lifetime of M state. In this work, we used a new kind of chemical additive—cyclen (1,4,7,10-tetraazacyclododecane, the structure was shown in Figure 1) and reported its significant effect on the prolongation of the M state lifetime in the D96N mutant of bacteriorhodopsin.

BR_{D96N} was a kind gift from Prof. D. Oesterhelt (Max-Planck-Institute of Biochemistry, Matrinsried, Germany) and Prof. N. Hampp (University of Marburg, Marburg, Germany). The powder of BR_{D96N} was suspended in distilled water for film preparation. The BR_{D96N}-PVA films with different cyclen concentration in a range of BR_{D96N}/cyclen molecule ratio of 1 : 100–1 : 300 were prepared as our previously reported method.[16]

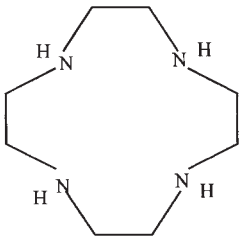

Figure 1. Structure of cyclen (1,4,7,10-tetraazacyclododecane).

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

The absorption spectra of BR_{D96N} suspension and BR_{D96N}-PVA films with and without cyclen as chemical additive were presented in Figure 2(a). Without the additive, the absorption maximum of BR_{D96N}-PVA film was located at 565 nm (shown in Figure 2(a), curve (ii)). Compared with the absorption peak wavelength of BR_{D96N} in suspension (Figure 2(a), curve (i)), a slight blue shift about 5 nm occurred, which was due to the same influence of the dehydration on the Schiff base of the retinal chromophore of BR_{D96N} as BR_{wt} molecules in a dry film.[17] When cyclen was added into the film with BR_{D96N}/cyclen molecular ratio of 1 : 250, another blue shift about 4 nm appeared. This 4 nm blue shift could be argued that cyclen might have weak interaction with BR_{D96N} in such dry films.[18] When the molecular ratio of BR_{D96N}/cyclen reached 1 : 300, BR_{D96N} was denatured partially (not shown here). Thus it may therefore be concluded that the highest molecular ratio of BR_{D96N}/cyclen was 1 : 250. Figure 2(b) shows the absorption spectra of M state in BR_{D96N}-PVA films with and without cyclen. The absorption maximums of the M state were also blue shifted as the B state had appeared. The absorption spectrum of M state in BR_{D96N} suspension was not shown here, because the M state lifetime of BR_{D96N} suspension was very short and its absorption spectrum was not suitably got by UV/Vis Spectrophotometer.

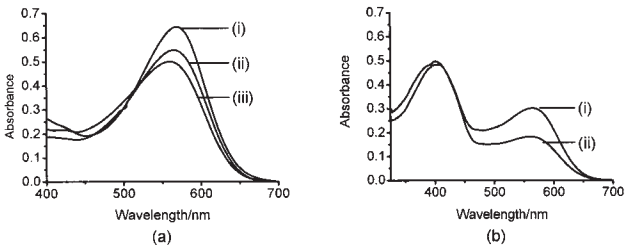

Figure 2. Absorption spectra of (a) B state; (b) M state. (i) BR_{D96N} suspension; (ii) BR_{D96N}-PVA film; (iii) BR_{D96N}-PVA film with BR_{D96N}/cyclen molecular ratio of 1 : 250.

The decay kinetic curves of the M state in the BR_{D96N} films with different cyclen concentrations were investigated, as shown in Figure 3. The curves were obtained by first exciting the BR_{D96N}-PVA film for 30 s to reach a high M state accumulation and then recording the relative absorbance changes at 408 nm as the M state relaxed back to the B state. With increasing cyclen content, the decay of the M state was slowed down gradually, and different M state lifetime ($\tau_{1/e}$) of 56 s, 216 s, 510 s, 690 s, and 936 s were got separately, corresponding to no additive and BR_{D96N}/cyclen molecular ratio of 1 : 100, 1 : 150, 1 : 200, and 1 : 250 respectively. Compared with the lifetime of 56 s of the BR_{D96N}-PVA film without the additive, the long-lived M state lifetime of 936 s represented over one order of magnitude improvement.

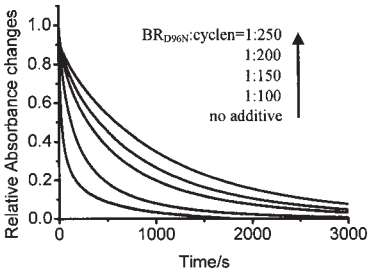

Figure 3. Decay kinetics of the M state in BR_{D96N}-PVA film at different BR_{D96N}/cyclen molecular ratio.

The reason for this remarkable prolongation might originate from these two facts. First, in D96N mutant of BR, substitution of Asn-96 for Asp-96 makes the process of the proton translocation step from Asp-96 to the Schiff base lack, and the deprotonated Schiff base is directly reprotonated from the medium during the M state relax back to the B state. Since cyclen has strong complexing property towards H^+,[19] the number of protons in the medium which were available for the M state BR molecule to recapture to relax back to the B state was apparently reduced and the rate of reprotonation of the Schiff base was significantly decreased. Second, as R. Korenstein and B. Hess had reported,[20] dehydration of BR_{wt} in films can increase, by 2–3 orders of magnitude, the lifetime of the M state. In BR_{D96N}-PVA film the lifetime of M state of BR_{D96N} can also be prolonged by the same dehydration reason.

To determine what the main reason on the prolongation in BR_{D96N}-PVA film with cyclen as chemical additive is, the lifetime of M state in BR_{D96N} suspension with BR_{D96N}/cyclen molecular ratio of 1 : 250 was investigated.

Figure 4 shows the decay kinetic curve of BR_{D96N} suspension with BR_{D96N}/cyclen molecular ratio of the above highest addition of 1 : 250. It could be concluded that the lifetime ($\tau_{1/e}$) of M state was about 200 s. Comparing the lifetime of 936 s in BR_{D96N}-PVA film with the same addition, only about five-fold of the M state lifetime was extended due to the dehydration of BR_{D96N}. Thus it may be concluded that not the effect of dehydration but the strong complexing property towards H^+ of cyclen play the main reason in the prolongation of M state lifetime in BR_{D96N}-PVA film with cyclen as chemical additive.

In summary, the lifetime of M state in BR_{D96N}-PVA film with BR_{D96N}/cyclen molecular ratio of 1 : 250 could be extend to

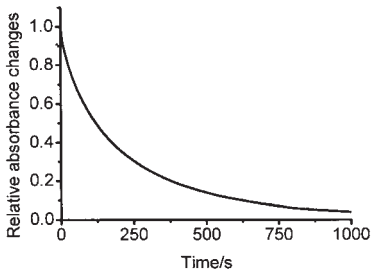

Figure 4. Decay kinetics of the M state in BR_{D96N} supension with BR_{D96N}/cyclen molecular ratio of 1 : 250.

936 s, which is over one order of magnitude longer than BR_{D96N}-PVA film without cyclen. At this highest addition, comparing the lifetimes of M state in BR_{D96N} suspension and BR_{D96N}-PVA film with cyclen it may be concluded that the strong complexing property towards H^+ of cyclen plays the main reason in the M state lifetime prolongation. This long-lived M state expands the use of BR material on optical applications.

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