Photoinduced transformation of 14-F-bacteriorhodopsin gelatin films based on both wild type and D96N mutant

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Abstract

Spectral and kinetic transformations were studied in gelatin films made with 14-F wild type (WT) bacteriorhodopsin (BR) and 14-F D96N mutant BR. Unlike the recent study of water suspensions of the same pigments, where a red shifted species at 660 nm was shown to form under the light in 14-F WT only, there are no drastic differences in photoinduced behavior between gelatin films based on 14-F WT and 14-F D96N. It is not observed any photoinduced formation of red shifted species at 660 nm for both types of films as it is observed for corresponding pigments in water suspension. The observed results are explained in a terms of relationship between the rates of two photoinduced processes that occur in suspensions and films of corresponding pigments. Kinetic characteristics of the photoinduced processes for the films with chemical additives suggest that there are no advantages in using 14-F D96N films when compared to films based on 14-F WT. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Over the last few decades the example of bacteriorhodopsin (BR) has demonstrated that biological systems potentially can be used to solve material requirements in technical devices (Oesterhelt et al., 1991; Birge, 1995). Indeed, photochromic, electrochromic and non-linear-optical properties of BR allow the potential utilization of this naturally found molecular device (Druzhko et al., 1995; Hampp and Silber, 1996; Kolodner et al., 1997; Hampp, 2000).

BR is a unique light-energy-transducing molecule, that is the photocycling protein in the purple membrane of the bacterium Halobacterium salinarium (Ebrey, 1992). It has a remarkable feature of forming a hexagonal crystalline array of BR molecule trimers. Seven transmembrane α-helices are arranged in a circular manner with
chromophore inside the pore (Grigorieff et al., 1996). In native non-modified BR chromophore is retinal bound to the protein via the protonated Schiff base at the z-helixG (Henderson et al., 1990). Under the light BR molecules undergo a photocycle with a number of intermediates (J, K, L, M, N and O). The intermediate M is a key intermediate, where the release and uptake of the proton occur. The proton acceptor D85 is protonated in the L-to-M reaction, in which the Schiff base becomes deprotonated, whereas the donor D96 is deprotonated in the M-to-N transition, in which the Schiff base is reprotonated. For the wild-type BR protein in aqueous solutions at room temperature, it takes around 50 μs for the M-state to accumulate following the initial photon absorption event (the B-to-M photoreaction, where B is initial state of BR) (Birge, 1990). After the formation of the M-state further thermal relaxation occurs and the original B-state is regenerated on a timescale of 10 ms (Birge, 1990), with associated spectral recovery. This time (10 ms) is considered to be too short for cache-memory applications. Dehydration of BR in air to yield either polymer- or non-polymer-based films increases, by several orders of magnitude, the lifetime of the M-state (Korenstein and Hess, 1977; Vsevolodov et al., 1989). Moreover, speaking about applications, it is preferential to have BR in the form of thin films, as it is easier to manipulate with such type of sample. Chemical modification of polymer-based BR films increases the lifetime of the M-state (information storage time) to minutes (Dyukova and Vsevolodov, 1996).

Some applications require significantly greater information storage time than minutes. We have attempted to solve this problem by substituting artificially synthesized analogs for the natural chromophore in native BR (Druzhko and Zhamakhmedov, 1985). This provides a range of novel retinal complexes, that possess photochromic characteristics different from those in native BR. Polymeric films based on 4-keto BR (BR with 4-keto retinal as the chromophore) have a decay time of M-state intermediate not less than 30 min (Druzhko et al., 1995).

Combination of the two approaches — specific replacement of the chromophore in some mutants and then comparison of photoinduced characteristics of 4-keto WT BR and 4-keto D96N BR demonstrates an increase in the contribution of the most long-lived component of the M-state decay for the films based on 4-keto D96N as compared to those for 4-keto WT (Druzhko and Weetall, 1997). It is this increase that extends the operating range of this film and therefore, it may offer an advantage as a recording media, when compared to films based on the 4-keto WT.

There are advantages and disadvantages in using of 4-keto BR. The absorption maximum of 4-keto BR at 508 nm (Druzhko and Chamorovsky, 1995) is blue shifted as compared to that in native BR. This is a major disadvantage of this analog as a photochromic material because of the smaller photochromic shift — only about 90 nm between ground state and M-state (for comparison about 150 nm for native BR). A greater photochromic shift would result in a better image contrast. In addition the greater photochromic shift would enable one to use inexpensive semi-conductive lasers for recording of information, which begin to irradiate more than 600 nm range. This indicates that an absorption maximum at a wavelength longer than 600 nm would be wanted and thus red shifted pigments would be more desirable.

There have been several efforts to prepare unusually red-shifted pigments (Singh et al., 1996; Gat et al., 1997; Hoischen et al., 1997). We have recently demonstrated that 14-F retinal when incorporated into apomembranes WT and D96N, produces pigments with drastically different photoinduced behavior (Druzhko et al., 1998). Red-shifted pigment (λ_max ≤ 680 nm) has been previously observed as a minor component of the major 587-nm pigment in 14-F BR made with white membrane JW2N (Tierno et al., 1990). A similar red shifted pigment is formed under yellow light (λ > 500 nm) only in the 14-F analogs derived from WT BR (Druzhko et al., 1998). Measurements of the photoinduced transformation in 14-F WT analogs indicate, that the photocycle of the major pigment occurs simultaneously with the process in the red region and is partially masked by the formation of the red shifted species. The 14-F D96N samples have a significantly
slower and more complicated photoinduced behavior. Results of differential absorbance spectra, kinetic measurements together with pH-dependencies of light-on and light-off kinetics suggest existence of two processes initiated in both 14-F WT and D96N by yellow light. For 14-F WT they occur simultaneously with the pH-dependent equilibrium between them. For 14-F D96N the photocycle with ground state at 588 nm is followed by the formation of the red species at 660 nm, which begins only after the light is turned off (Druzhko et al., 1998). Because of these unique properties of these analogs in suspension, it is of interest to determine how the peculiarities of photoinduced behavior of these two 14-F pigments — WT BR and D96N BR — manifest themselves for gelatin films, based on these pigments.

In the present work we have studied photoinduced transformation of 14-F BR gelatin films based on both wild type and D96N mutant to learn whether either of these materials are preferable as a media for recording and processing of optical information.

2. Experimental details

Apomembranes (AM) from WT and D96N mutant were prepared in the course of reaction of photoinduced hydroxylaminolysis: aqueous suspensions of WT and D96N BR were bleached by illumination with wavelength \( \lambda > 500 \) nm in the presence of 0.5 M \( \text{NH}_2\text{OH} \), pH 8.2–8.4, at 7°C. The further purification of AM was performed as previously described (Druzhko and Weetall, 1997). The activity of AM was tested by reconstitution with \textit{all-trans} retinal (Sigma, St. Louis, MO). The 13-cis-14-F retinal and \textit{all-trans}-14-F retinal were prepared as previously reported (Francesch et al., 1997). Retinal analogues dissolved in \textit{iso}-propanol (TLC Grade) were used for reconstitution. The reconstitution procedure was carried out under dim red light at 25°C and described in detail in (Druzhko et al., 1998). Photochromic polymer (gelatin) films were prepared using a casting procedure, where the photosensitive mixture of BR derivatives, gelatin binder and chemical additives was introduced between two glass supports separated by 1080 \( \mu \)m spacers. The process of film preparation has been previously described in detail (Dyukova and Vsevolodov, 1996). The thickness of the dried samples were approximately 70 \( \mu \)m. Chemical additives dianinopropan (DAP) and guanidine hydrochloride (GuHCl) were of reagent grade (Sigma, St. Louis, MO). All UV/VIS absorption spectral data and photoinduced absorption changes were measured with diode-array UV/VIS spectrophotometer (HP 8452A). The maximum power density of the excitation light (Kodak 300 W, 120 V bulb Ektagraphic projector, equipped with a yellow long-pass filter (\( \lambda > 500 \) nm) was approximately 30 mW cm\(^{-2}\).

3. Results and discussion

The absorption spectra of gelatin films made with 14-F WT and 14-F D96N BR are similar to the data observed for water suspensions of the same pigments (Druzhko et al., 1998). As indicated earlier, the absorption maxima of spectra for the water suspensions were red shifted relative to these pigments with natural chromophores (Druzhko et al., 1998). The same red shift has been observed for 14-F WT and D96N embedded into gelatin films. Both spectra have quite wide absorption maxima at 584–586 nm. Similar to the spectra for suspensions there are no sufficient differences between the initial spectra of different isomers 14-F pigments, WT as well as D96N. Most likely, there is an equilibrium between the 13-cis 14-F and \textit{all-trans} 14-F chromophore isomers after 1 h of reconstitution similar to Tierno et al.’s observation about distribution of isomers of 14-F retinal chromophore during reconstitution with white membranes under conditions similar to ours (Tierno et al., 1990). Actually it is more appropriate that our resulted pigments would not be further called as ‘\textit{all-trans}’ and ‘13-cis’.

If gelatin films based on 14-F derivatives of WT and D96N have been illuminated by yellow light the photoinduced changes of absorbance are absolutely different when compared with those for the water suspensions of the same pigments. The differential absorbance spectra (light minus dark)
of 14-F WT and 14-F D96N measured in wide pH range (5–9), show a rise in absorbance at 660 nm only for 14-F WT BR water suspension and no rise in this spectral range for 14-F D96N water suspension (Druzhko et al., 1998). The differential absorbance spectra ‘light minus dark’ measured for gelatin films (Fig. 1) demonstrate no rise in 600-nm range for both WT and D96N of 14-F derivatives of BR. Thus, unlike the suspensions, the corresponding gelatin films demonstrate no drastic differences in photoinduced transformations between 14-F WT and 14-F D96N.

Comparison of kinetic curves confirms this observation. We have recently presented the kinetic curves of the photoinduced absorbance changes in 14-F WT and 14-F D96N water suspensions monitored at 412, 588 and 660 nm (Druzhko et al., 1998). In the case of the 14-F WT water suspension, when the light is turned on, the 412 nm absorbance rises, the 588 nm absorbance disappears and 660 nm absorbance again rises (Druzhko et al., 1998). In contrast, for 14-F D96N water suspension, when the light is turned on, the 660 nm absorbance disappears, and when the light is turned off, the 660 nm absorbance reforms and then decays very slowly (Druzhko et al., 1998). The kinetic curves of the photoinduced absorbance changes in 14-F WT and 14-F D96N gelatin films monitored at 412, 588 and 660 nm are presented in Fig. 2. As indicated in the Fig. 2, there are no drastic differences in photoinduced absorbance changes between 14-F WT and 14-F D96N gelatin films, nothing of the kind to those for suspensions. Unlike the rise of absorbance at 660 nm for suspensions — light-on for WT and light-off for D96N (Druzhko et al., 1998) — there is no rise at all in case of both types of films. Furthermore we have observed disappearance of absorbance at 660 nm. Chemical additives for film preparations (GuHCl and DAP) were used as in ref. Dyukova and Vsevolodov (1996). Kinetics for the films with chemical additives are presented in Fig. 3. This figure shows no principal differences in kinetics for gelatin films with chemical additives compared with those films with no chemical additives. So, the main result of this study of films is that there is no formation of a red shifted species at 660 nm for both types of gelatin films as opposed to the observations of a red shift in the same pigments in water suspension. In addition, there are no fundamental differences in the photoinduced behavior between WT and D96N as observed with the water suspensions.

An explanation for these differences between the gelatin films and the water suspensions may be as follows. There was evidence of the existence of two processes initiated in both 14-F WT and D96N by yellow light (Druzhko et al., 1998). The one process is a photoinduced transformation of
the initial ground state at 588 nm, and the another one is a formation and decay of red-shifted species at 660 nm. The relationship between the rates of these two processes might be the possible reason of the observed differences in photoinduced behavior between 14-F WT and 14-F D96N in water suspension. In the case of fluorinated analog based on D96N the photocycle with the ground state at 588 nm could not be as fast as that for WT. In the case of WT we couldn’t not observe the M-intermediate accumulation because of high rate of photocycle and it made possible to observe the red species formation and decay. Actually the photocycle at 588 nm was partially masked by the formation of the red shifted species (Druzhko et al., 1998). For 14-F D96N photocycle was much more slower and therefore accumulation of M-state made impossible the observation of the red shifted species formation as long as photocycle was incomplete. All these considerations are confirmed by the results observed with corresponding gelatin films. Embedding fluorinated derivatives of BR into gelatin slows down the photocycle at 588 nm much more actively than it makes in D96N water suspension. This effect, therefore, levels all these differences between WT and D96N and we don’t observe red species at all.
The time constants and corresponding relative amplitudes of exponential fittings of the light-on and light-off processes in 14-F WT and 14-F D96N gelatin films are presented in Table 1. The data characterize the numerical differences that the chemical additives contribute to the photoinduced behavior of these two types of gelatin films. The effect of chemical additives is especially in evidence for light-off processes in 14-F WT films and is almost absent for 14-F D96N films. Earlier chemical additives as classified as di- and tri- amines were used for increasing the life time of the M-state of BR (Dyukova and Vsevolodov, 1996). Chemically modified films based on WT and D96N with native chromophore had a 9- to 12-fold increase in the lifetime of the M-state as compared with non-modified films. As for pigments with modified chromophores, chemically enhanced BR films based on 4-keto BR WT have been shown to exhibit the same tendency — 2- to 3-fold increase in the lifetime of the M-state (Druzhko and Weetall, 1997). Moreover, such a combination of chemical additives as GuHCl and DAP caused an increase in the contribution of the most long-lived component of the M-state decay for the films based on 4-keto D96N as compared

Fig. 3. Photoinduced (λ > 500 nm) absorbance changes vs. time of 14-F WT and 14-F D96N gelatin films with chemical additives (guanidine hydrochloride + diaminopropan) monitored at 412, 588 and 660 nm. † denotes light is turned on, ‡ denotes light is turned off.
Table 1
Time constants of the one-, two- or three-exponential fits of the kinetic curves monitored at 412, 588 and 660 nm under the yellow light ($\lambda > 500$ nm) and after light exposure.*

<table>
<thead>
<tr>
<th></th>
<th>14-F WT, no adds, $(t_1, t_2, t_3 \pm S.D.)$ (s)</th>
<th>14-F WT, with adds, $(t_1, t_2, t_3 \pm S.D.)$ (s)</th>
<th>14-F D96N, no adds, $(t_1, t_2, t_3 \pm S.D.)$ (s)</th>
<th>14-F D96N, with adds, $(t_1, t_2, t_3 \pm S.D.)$ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>412 nm light-on</td>
<td>2.31 ± 0.28</td>
<td>1.65 ± 0.18</td>
<td>0.15 ± 0.22</td>
<td>0.75 ± 0.017</td>
</tr>
<tr>
<td>588 nm light-on</td>
<td>1.47 ± 0.17</td>
<td>1.37 ± 0.25</td>
<td>0.87 ± 0.017</td>
<td>0.98 ± 0.017</td>
</tr>
<tr>
<td>660 nm light-on</td>
<td>23.49 ± 1.4</td>
<td>5.44 ± 0.45</td>
<td>2.24 ± 1.8</td>
<td>6.23 ± 0.55</td>
</tr>
<tr>
<td>412 nm light-off</td>
<td>3.57 ± 0.4</td>
<td>5.17 ± 0.4</td>
<td>9.8 ± 1.9</td>
<td>8.1 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>23.4 ± 1.9</td>
<td>41.4 ± 3.9</td>
<td>18.08 ± 1.9</td>
<td>16.4 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>351.9 ± 24</td>
<td>411.9 ± 24</td>
<td>285.8 ± 27.5</td>
<td>255.0 ± 23.8</td>
</tr>
<tr>
<td>588 nm light-off</td>
<td>3.1 ± 0.31</td>
<td>5.1 ± 0.47</td>
<td>6.8 ± 0.57</td>
<td>6.5 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>26.35 ± 2.4</td>
<td>25.36 ± 2.8</td>
<td>14.6 ± 1.6</td>
<td>12.48 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>474.4 ± 29.8</td>
<td>574.4 ± 29.8</td>
<td>207.8 ± 21.8</td>
<td>207.4 ± 21.8</td>
</tr>
<tr>
<td>660 nm light-off</td>
<td>29.6 ± 2.8</td>
<td>31.7 ± 2.8</td>
<td>6.51 ± 0.57</td>
<td>8.17 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>267.7 ± 29.8</td>
<td>287.7 ± 22.8</td>
<td>145.1 ± 14.8</td>
<td>141.4 ± 16.8</td>
</tr>
<tr>
<td></td>
<td>0.3 ± 0.25</td>
<td>0.69 ± 0.03</td>
<td>0.49 ± 0.05</td>
<td>0.49 ± 0.05</td>
</tr>
</tbody>
</table>

* The light-on kinetic changes of absorbance were analyzed using one-exponential model. This function fits the experimental curves with sufficiently small residuals 1–2%. For the second portion of the photocycle, when the protein donates a proton to the periplasm and the Schiff base takes the proton to the cytoplasm, more than one exponential fit is required (Varo and Lanyi, 1990). The sum of two or three exponentials fits the light-off kinetics with residuals about 1–3% of the measured value. The S.D. is from three measurements. The relative amplitudes $A_i$ are given in brackets under the corresponding time constants $t_i$. The experiments were performed at room temperature ($22 \pm 1°C$) and room relative humidity of 45 ± 4%.

with the observations for 4-keto WT, and it may offer an advantage as a recording media when compared to films based on the 4-keto WT (Druzhko and Weetall, 1997). In case of 14-F D96N this combination of chemical additives does not affect the lifetime of the M-state in a similar fashion (Table 1), thus it means the lack of possible advantages of 14-F D96N films when compared to films based on 14-F WT.

References


