Blue light regeneration of bacteriorhodopsin bleached by continuous light

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Abstract Photobleaching of bacteriorhodopsin (BR) by continuous light has recently been demonstrated. This bleaching consists of at least two subsequent product states. One of them is absorbing maximally in the blue spectral region. Our present study shows that upon illumination of the bleached sample with blue light a back photoprocess appears, resulting in regeneration of the original BR state. From a technical point of view, the observed phenomenon is similar to the reverting effect of blue light on the photocycle. An important difference is that the photobleached state of BR is much more stable than any of the photocycle intermediates, and may provide an advantage for several technical applications.

Key words: Blue light effect; Stability in vivo; Technical application of bacteriorhodopsin; Photoreaction; Thermal denaturation

1. Introduction

Bacteriorhodopsin (BR) is a light-driven proton pump. Its photo and thermal reactions are summarized in numerous recent reviews [1–3]. BR absorbs light by its chromophore, retinal [4]. Retinal undergoes conformational changes, and due to coupling with the protein, a part of its energy stored from the absorbed light results in translocation of protons across the membrane (see e.g. [5]). Only approximately 20% the energy of the absorbed photon energy is stored, the rest is converted into heat [6].

For a long time it was thought that BR has a practically unlimited active lifetime, but we recently showed that under continuous illumination (for e.g. with yellow light), ranging in the tens or hundreds of mW/cm^2, it undergoes a considerable bleaching process [7]. pH and, especially, the temperature of the sample were important factors.

Our data suggested a mechanism of photobleaching, in which local warming around the light absorbing retinal allows the appearance of a new, denaturation pathway. The first reaction is an equilibrium, driven forward exclusively by light. The second reaction seems to be a unidirectional reaction, resulting in a form that absorbs in the blue range. This state is stable in the dark [7]. The present paper deals with the blue light sensitivity of this form.

The photocycle of BR contains a long living intermediate M [8], that absorbs in the blue range with a maximum at 412 nm. It has been shown that the M state can be reconverted to the ground state by blue light through a fast pathway [9]. The photocycle, with this fast recovery pathway, has been suggested for numerous different technical applications (e.g. [10]), especially with some mutants (e.g. D96N [11]) where the lifetime of the M intermediate is increased [12].

The idea that a form absorbing in the blue range can be converted by blue light was tested, and our measurements indicate that a relatively weak blue light can be used for the, at least partial, regeneration of photobleached BR. Our observations suggest that the energy difference between the forms, which results in the irreversibility of the second step of the photobleaching pathway, can be covered by the energy of a blue photon, making this step of the bleaching photoreversible.

2. Materials and methods

The purple membranes were prepared according to standard procedures, and prepared for measurements in the same way (incorporation into 2 mm thick, 10% polyacrylamide gel, 30 mM universal buffer, etc.) as for the study of photobleaching [7].

The measuring system was also the same. For bleaching, the light of the high-pressure mercury lamp (200 W, HBO 200, NARVA, Berlin, Germany) was filtered through heat filters and glass optical filters transparent in the yellow spectral range ($\lambda > 500$ nm). For the regeneration experiments, the yellow filter was exchanged for a filter transparent in the blue spectral region ($\lambda < 450$ nm). The light intensity was reduced by neutral optical filters. The maximum intensity of the yellow and blue light was approximately 400 and 100 mW/cm^2, respectively, measured with an Alphametrics DC 1010 photometer (Karl Lamber, Chicago, USA).

The absorption spectra were recorded by a Shimadzu UV-160 spectrophotometer (Shimadzu, Kyoto, Japan).

3. Results

The fact that blue light can partially regenerate the ground state of bleached BR can be seen in Fig. 1. The original absorption of BR (trace 1) decreases considerably after 30 min of illumination by yellow light (trace 2). After switching off the bleaching light, the sample consists of three different forms and some recovery of the ground-state appears over time, as shown by traces 3 and 4, due to the decay of the form with an absorption maximum at about 490 nm (see [7]). Illumination of this sample by blue light for 10 min causes a considerable increase in the absorption in the 430–650 nm spectral range (trace 5). However, we may not state that this would completely explain the regeneration of the ground state, due to the distorted spectral shape. However, this alteration of the shape disappears in the 450–750 nm spectral range, and the resulting spectrum (trace 6) has the same shape in that range as the BR ground state. This indicates that a transient form, accumulated during illumination with blue light, decays into the BR ground-state completely, within 30 min.

The regeneration of the bleached samples were studied at

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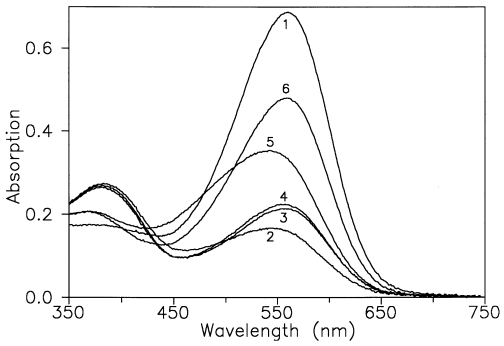

Fig. 1. Bleaching (by yellow light, 400 mW/cm^2, 30 min) and regeneration (by blue light, 1 mW/cm^2, 10 min) of BR at 60°C, pH 9.5. The procedure was: the measurement of the spectrum before bleaching (trace 1); after bleaching (trace 2); 20 and 60 min later (traces 3 and 4); after regeneration with blue light (trace 5); and 30 min later (trace 6).

different intensities of blue light. In the intensity range studied, the less intense blue light caused a higher degree of regeneration. Quantitatively, at maximal light intensity (approximately 100 mW/cm^2), at 1/10 and at 1/100 of that, the bleached samples with 33% (24% immediately after bleaching) of their original absorption were regenerated to 43, 64 and 68%, respectively. The regeneration by blue light is also as effective at 20°C as at 60°C (data not shown).

Except the smallest blue light intensity, a decay of the chromophore was observed, as indicated by a decrease in the absorption below 450 nm, parallel to decreased recovery of the main absorption band. Thus, the data measured at the weakest blue light (Fig. 1) were used for further evaluations.

The spectra of the different forms were derived by appropriate subtractions of the data. These spectra are shown in Fig. 2. The spectrum of the form with an absorption maximum of 495 nm was derived from the spectrum of the BR ground-state, and the spectrum obtained immediately after switching off the regenerating blue light. The spectrum of the 395 nm form was calculated from the spectrum of the sample measured after bleaching by using the spectra of BR and the 495 nm form.

An additional spectrum was also calculated (from the spectra of the sample regenerated by blue light, and of BR before bleaching (traces 6 and 1 in Fig. 1, respectively)) that corresponds to an unregenerable form, having maximal absorption at 370 nm. This spectrum seems to be a mixture (see the band in the 450–600-nm range), probably due to the presence of a small amount of the 495 nm form in the sample measured after regeneration.

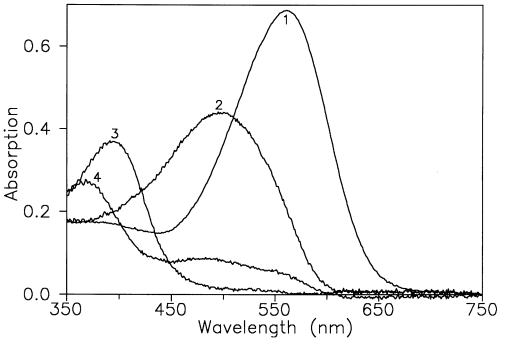

Fig. 2. The spectra of the different forms (1, ground-state; 2, 495-nm form; 3, 395-nm form; 4, 370-nm form) appearing during bleaching and regeneration of BR calculated from the data shown in Fig. 1.

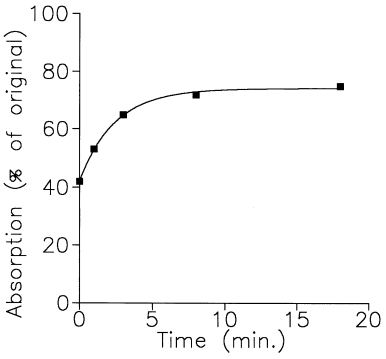

Fig. 3. The absorption of the sample after different durations of illumination of the bleached sample with blue light. The light intensity was about 10 mW/cm^2.

The dependence of the regeneration versus the duration of the illumination with blue light is shown in Fig. 3. It is a monoexponential function characterized by a baseline of $74.6 \pm 0.6\%$ (maximal absorption achievable after regeneration), time constant of 2.5 ± 0.2 min, and amplitude of $32 \pm 0.9\%$. The blue light intensity was about 10 mW/cm^2.

4. Discussion

Our present measurements indicate that blue light can partially regenerate BR after the bleaching, caused by continuous illumination of the sample with yellow light. According to our control measurements, and as expected from the model for the bleaching published in [7], blue light itself is able to generate bleaching of the sample (data not shown).

As the result of these two processes generated by a blue light, a complete sample regeneration cannot be expected. Moreover, as our data indicate, the regeneration process has a much higher efficiency with weaker blue light.

These findings can be described by the three-state model ($A \leftrightarrow B \rightarrow C$) suggested in our paper describing the photobleaching [7], and with the following: (1) the illumination of the 395 nm form C with blue light converts it into the 495 nm form B allowing a regeneration of the BR ground-state A by the $B \rightarrow A$ thermal process; and (2) the excitation of the 395-nm form may also result in either the destruction of the molecule into a spectrally invisible form or, less likely, into the 370-nm state. These are the pathways that result in the non-regenerable part of the sample.

According to our data (as the 370-nm band disappeared at the 1.0 blue light intensity, but this did not result in the increase in the regeneration of BR) the illumination of the 370-nm form probably does not result in the regeneration of either the 395- or 495-nm form, rather it is converted into spectrally invisible forms.

Our observation on the bleaching and regeneration of BR by continuous light may have potential practical and technical importance. Data can be stored in BR by a yellow light and it can be deleted by a blue one. This can be done several times (data not shown). Thus, through these processes, BR can be

used as rewriteable storage material. An additional advantage is the relatively long data storage time, which is much less achievable by other processes observed in BR.

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