Preparation of (1,1,5-tri-demethyl)bacteriorhodopsin pigment and its photocycle study

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Halobacterium halobium *Absorption maximum* *Steric hindrance* *Opsin shift* *Photolysis study* *Photoproduct*

1. INTRODUCTION

Bacteriorhodopsin, the pigment protein in the purple membrane of *Halobacterium halobium*, contains an all-*trans*-retinyl chromophore bound to the protein via a protonated Schiff base linkage with a lysine residue [1]. The absorption maximum of this pigment is substantially shifted to the red (570 nm) compared to a protonated Schiff base of all-*trans*-retinal (SBH^+) in solution (440 nm). The effect is quantitatively measured via the 'opsin shift' [2] defined as the energy difference between the absorption maximum of SBH^+ (in cm^{-1}) and the corresponding pigment. Authors in [2] proposed a molecular model accounting for the absorption maximum of bacteriorhodopsin in terms of two protein charges (or dipoles) in the neighbourhood of the polyene moiety: one, the counterion to the iminium nitrogen and the second in the vicinity of the ring. The changes undergone by the pigment during the photocycle are due to two kinds of transformations: changes localized in the retinyl molecule and others involving the protein. Both kinds of transformations are closely interrelated.

We have recently shown that the introduction of steric hindrance in the vicinity of the β-ionone moiety of bacteriorhodopsin (by substitution on the ring with various bulky groups) drastically changed both the absorption maxima of the pigment and its photocycle [3]. The purpose of this work was to investigate the opposite effect; i.e., that of releasing steric hindrance at the ring by removing from the natural pigment all of its ring methyl substituents. We have thus prepared (1,1,5-tri-demethyl)-all-*trans*-retinal and studied its binding to bacterioopsin and its photocycle process. Recently, a (5-demethyl)retinal analogue was reported by other laboratories [4,5].

2. MATERIALS AND METHODS

2.1. *Preparation of (1,1,5-tri-demethyl)-all-*trans*-retinal* 1

The compound was synthesized as described in scheme 1. 1-Cyclohexane-1-carboxaldehyde [6] was condensed with the sodium salt of triethyl-4-phosphono-3-crotononitrile followed by reduction with diisobutylaluminium hydride to give after separation of isomers, aldehyde 2. Similar steps (condensation and reduction) with 2 gave (1,1,5-tri-demethyl)-all-*trans*-retinal 1 (scheme 1).

2.2. *Preparation of Schiff base*

The Schiff base of aldehyde 1 was prepared by mixing the aldehyde with 1.5 equivalents of *n*-$BuNH_2$ in EtOH at 0°C for 1 h. The solvent and

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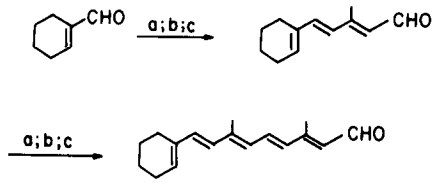

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Scheme 1. (a) $(EtO)_2POCH_2C(CH_3)$ = CHCN/NaH/THF, room temperature, 1 h. (b) Dibal/hexane, −78°C, 30 min/oxalic acid, ether, room temperature, 3 h. (c) Separation of isomers/flash chromatography.

excess of amine were evaporated, and the crude oil was dissolved in dry EtOH. The Schiff base was protonated by passing HCl gas through its EtOH solution at 0°C.

2.3. *Binding of 1 to bacterioopsin*

The artificial pigment was prepared by reconstitution of the apomembrane with the synthetic retinal, as in [7] at pH 7, using Hepes buffer.

2.4. *Pulsed laser photolysis studies*

Pulsed laser photolysis of membrane suspension was carried out, using a UV-14 DL-200 Molectron dye laser system (8 ns, 0.5 mJ) described in [8]. Data digitized using a Biomation 8100 transient recorder were averaged in a Nicolet 1170 computing system.

3. RESULTS

Binding of 1 to apomembrane resulted in the formation of a pigment (denoted as I), exhibiting a visible absorption band, peaking at 535 nm (dark-adapted). The protonated Schiff base of 1 (derived from *n*-$BuNH_2$) in MeOH, absorbed at 457 nm. Thus, in the case of I, the opsin shift is 3200 cm^{-1} as compared with 4900 cm^{-1} in the natural system.

Exposure of the dark-adapted form of I (λ_{max} = 535 nm) to visible light red shifts the absorption to 545 nm. Dark adaptation shifted the maximum back to its original value. These effects are analogous to those obtained in natural bacteriorhodopsin.

3.1. *Pulsed laser photolysis studies*

Pulsed laser photolysis experiments carried out

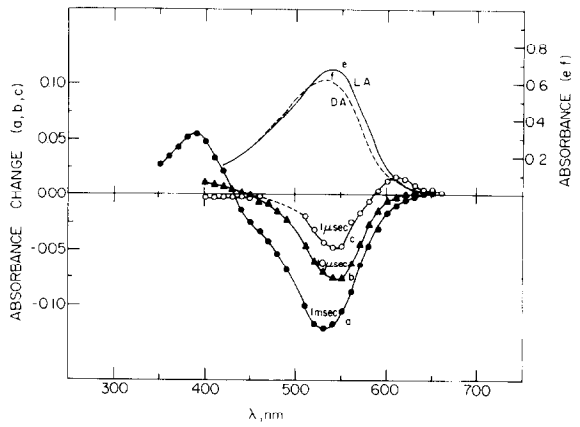

Fig.1. Absorption spectra and difference spectra in laser photolysis of pigment I. (– – –) Dark-adapted; (——) light-adapted. Time after laser pulse: (○) 1 μs; (▲) 10 μs; (●) 1 ms.

with the light-adapted pigment I indicated general patterns similar to those characteristic of bR_{570}. The main phenomena are shown by the light-induced difference spectra presented in fig.1. The first detectable intermediate is a red-shifted species (K_{590}) analogous to the K_{610} photoproduct of bR_{570}. The energy differences $\Delta\nu$(bR/K), between the maximum depletion of the pigment band and the maximum absorption growing in the red is 2125 cm^{-1}. The value is essentially identical to that found in natural bR. The long-lived blue-shifted intermediate (M) absorbs at 410 nm, similarly to the M_{412} intermediate in bR photocycle.

4. DISCUSSION

The absorption maximum of SBH^+ of 1 in MeOH is 457 nm. Its 20 nm red shift, relative to SBH^+ of all-*trans*-retinal, is accounted for by the absence of 1,1, and 5 methyl groups. The chromophore in 1 is allowed to adopt a more planar conformation around the 6–7 single bond than that of all-*trans*-retinal due to the release of steric hindrance between 5-CH_3 and 8-H and 1,1 methyls and 7-H. The planar conformation is associated with an absorption shift to longer wavelength [9], although reduced by an opposing shift originating from the omission of the 5-Me (which red shifts the absorption maximum of all-*trans*-retinal [10]. The net result is a red shift relative to SBH^+ of all-*trans*-retinal.

In variance with the red shift observed in SBH^+ of the synthetic chromophore 1, the corresponding pigment (I) is blue-shifted by about 30 nm relative to the natural bacteriorhodopsin chromophore. The overall effect is a smaller opsin shift (3200 cm^{-1} *vs* 4900 cm^{-1}).

On the basis of the models in [2], we attribute the reduced opsin shift to an increased separation between the polyene and the ring charge, made possible by the planarity of the chromophore, or by additional freedom in the binding site originating from the absence of the methyl groups. The increased distance from the ring charge will result in a blue spectral shift.

The parameter $\Delta\nu$(bR/K) in the case of I is similar to that of bacteriorhodopsin. It is thus evident that the spectral shift associated with the formation of K cannot be due to geometrical changes involving the ring charge. This conclusion is in keeping with the model attributing the initial photoinduced red shift to a separation of the Schiff base from its counterion [11]. Similar considerations also apply to the M intermediate.

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