13-Acetoxy-13-desmethylretinal: Synthesis, Incorporation into Bacteriorhodopsin, and Its Apparent Inactivating Effect

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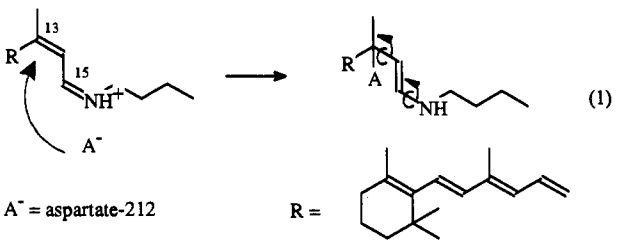
Received July 12, 1994

Bacteriorhodopsin (bR), the protein pigment of the purple membrane (PM) light-driven proton pump, is a single polypeptide chain of 248 amino acids.[1] It traverses the membrane to form seven rods[2] of high α-helical character.[3] PM's color results from the presence of an equivalent of retinal, bound as a protonated Schiff base (PRSB) at lysine 216, and its interaction with the protein. Light initiates a photocycle where the first step is a photoisomerization of *all-trans*-retinal to the 13-*cis* isomer. All subsequent steps in the cycle are thermal dark reactions. The *all-trans* → 13-*cis* photoisomerization has been shown to be obligatory for proton pumping,[4] and consequently, the thermal reisomerization of 13-*cis* → *all-trans* in the latter part of the cycle is required for continual turnover. We report herein the synthesis and incorporation into bacterioopsin of a novel analogue, 13-acetoxy-13-desmethylretinal, **1**, designed to probe the mechanism of dark *cis–trans* isomerization.

Thermal *cis–trans* isomerization also occurs upon dark adaptation[5] (*all-trans*,15-*anti* → 13-*cis*,15-*syn*) and is dynamic (13-*cis*,15-*syn* ⇌ *all-trans*,15-*anti*) while in the dark-adapted state (bR^{DA}).[6] These double *cis–trans* isomerization reactions apparently proceed by a concerted one-step bicycle-pedal mechanism.[7]

The chromophore, except for the Schiff base proton, appears to be well shielded from solvent by the protein and lipid bilayer and suggests that the protein itself catalyzes dark *cis–trans* isomerization. We have noted previously that catalysis could be achieved by two mechanisms: (1) removal of the counteranion (aspartate 212) from the vicinity of the protonated Schiff base nitrogen and (2) the introduction of a negative charge or the addition of a nucleophile at C13 of the PRSB.[8] Similar schemes with variation have been advanced by others.[9] The catalytic effect of removing the counteranion has been demonstrated in a model system[10] and more recently in a bR mutant where the counterion could be partially neutralized.[11] We have suggested,[6,8] however, that the two types of catalytic enhancements could be accomplished in one act by the addition of aspartate 212[12] to the PRSB at C13 to provide an intermediate which could undergo internal rotation about the C15–N and/or the C13–C14 bonds (eq 1). Asp-212, shown by FTIR studies to be deprotonated,[13]



(1)

and Lys-216 are one above the other on the same side of the helix in close proximity. Recent structural data show Asp-212 as part of a complex counteranion of the positively charged Schiff base nitrogen where its nearest oxygen is 3.6 Å from nitrogen.[14] That same oxygen is also within 4 Å of retinal's C13 and could, by a microconformational change, move closer to add to C13. Such a mechanism involving reversible addition of a nucleophile to C13 provides a rationale for the regiospecificity of isomerization (only 13-*cis* and *all-trans* are observed in this system) and is similar to the mechanism encountered in enzyme-catalyzed *cis–trans* isomerization where bicycle-pedal double isomerization has also been observed.[15] Previous studies reported from this laboratory support a nucleophilic mechanism for thermal PRSB *cis–trans* isomerization.[8,10,16]

We reasoned that if Asp-212 participated as proposed, a retinal analogue, **1**, possessing a good leaving group at C13, might trap the nucleophile and lead to a cross-linked chromophore to render the membrane inactive (Scheme 1). Nucleophilic addition of Asp-212's carboxyl to **1** at C13 would provide an almost symmetrical intermediate **2** where loss of acetate could compete with loss of aspartate. 13-Acetoxy-13-desmethylretinal (**1**) was synthesized from β-ionone according to Scheme 2.[17] The C15 aldehyde (**3**), synthesized by methods reported in the literature,[18] was treated with acetylacetaldehyde dimethyl acetal in THF and 2 equiv of NaH to obtain the 13-keto 15-dimethyl acetal (**4**). Treatment of **4** with lithium diisopropylamide generated its enolate, which was then acetylated with Ac_2O/DMAP to furnish 13-acetoxy-13-desmethylretinal 15,15-dimethyl acetal (**5**). Gentle hydrolysis of **5** in acetone, catalyzed by Bio-Rad AG 50W-X1 (H^+ form) and monitored by HPLC, furnished a mixture of several products where two aldehyde components (δ 10.16, d, and 9.77, d) and the 13-keto 15-enol acetate (**6**, δ 8.22, d) were detected by NMR (acetone-d_6). Acids and acidic media, e.g., silica gel, readily catalyze the conversion of **1** to **6**. 13-*cis*-**1**, however, was purified by HPLC on a cyano column (Et_2O/hexane) end-capped with TMS groups.[19] Hydrolysis at the keto dimethyl acetal (**4**)

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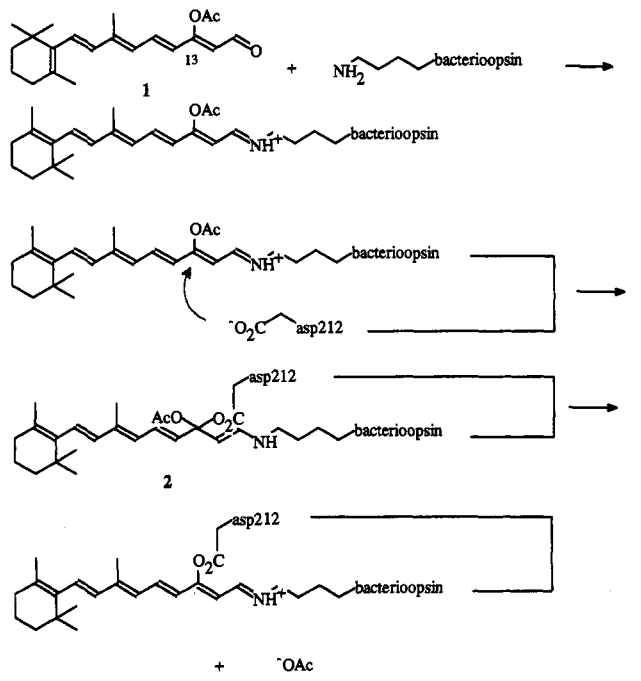
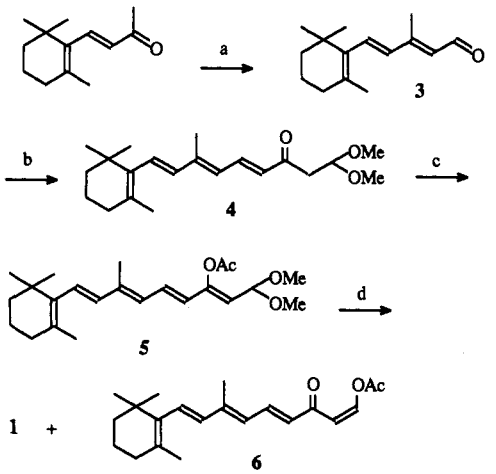
Scheme 1



Scheme 2[a]



[a] (a) Reference 17; (b) $CH_3COCH_2CH(OMe)_2$/NaH (2 equiv)/THF, −78 °C, 10 min at 0 °C; (c) (1) LDA/THF, −78 °C, (2) Ac_2O/DMAP; (d) Bio-Rad AG 50W-X1/acetone.

stage and attempted acetylation of the anticipated 13-keto,15-enol/15-aldo,13-enol mixture under varied conditions, however, resulted instead in only the formation of the 13-keto 15-enol acetate (**6**). MM2 molecular mechanics coupled with AM1 semiempirical quantum calculations indicate that **6** is 4.8 kcal/mol *more stable* than **1**, providing a rationale for the ready conversion of **1** to **6**.

13-*cis*-**1** forms a pigment (13-Ac-bR) with bacterioopsin in the dark which initially absorbs at 559 nm, but within 1 h in the dark its maximum moves to 573 nm, 15 nm red-shifted with respect to that for native bR^{DA}. The λ_{max} (EtOH) of 13-*cis*-**1** is 383 nm. The substantial red shift indicates protonated Schiff base formation between 13-*cis*-**1** and the protein and that the chromophore fits well into the retinal binding pocket.[21] The 559 → 573 shift is reminiscent of the shift observed upon dark adaptation of native bR initially formed from 13-*cis*-retinal. Synthetic bR chromophores often undergo replacement by *all-trans*-retinal. Addition of excess *all-trans*-retinal to 13-Ac-bR results in the slow replacement of **1** by the native chromophore as evidenced by a shift of the maximum to 558 nm and an increase in absorbance. The non-pseudo first order kinetics exhibit a half-time of about 12 h at ambient temperature.

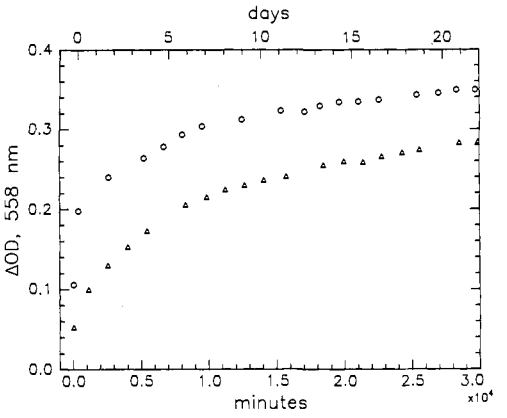


Figure 1. The course of replacement of **1** in 13-Ac-bR by *all-trans*-retinal. All operations are carried out in the dark under dim red light. 13-Ac-bR is fully formed, and the sample is divided exactly in half. To one (circles) is added excess *all-trans*-retinal immediately. The other (triangles) is allowed to sit for a week before the same excess amount of *all-trans*-retinal is added.

Upon long standing in the dark the 573 nm absorption of 13-Ac-bR slowly decreases with a concomitant increase in absorption at 406 nm indicative of a transformation. At ambient temperature about half of the 573 nm absorption is lost in 8 days. Addition of equal amounts of excess *all-trans*-retinal to equal aliquots of (a) freshly formed 13-Ac-bR and (b) an identical sample after 1 week in the dark indicates that the 13-Ac-bR aliquot suffering a substantial loss of its 573 nm absorption also loses a substantial amount of its ability to bind *all-trans*-retinal (Figure 1) by forming an intermediate which is not readily hydrolyzed.[22] The nature of this inactivation reaction is under investigation and will be reported in the future.

Acknowledgment. This reseach was carried out under Contract DE-AC02-76CH00016 with the U.S. Department of Energy and supported by its Division of Chemical Sciences, Office of Basic Energy Sciences. The author is pleased to acknowledge discussion with Prof. F. W. Fowler. The author thanks Elinor Norton for mass spectra and Dr. Robert Rieger for HRMS.

(19) The elution order is **6**, *all-trans*-**1**, 13-*cis*-**1**. H-12 for 13-*cis*-**1** is at δ 7.22, indicative of a 13-*cis* geometry.[20] *all-trans*-**1** has not yet been successfully separated from a coeluting impurity.

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