Chemoenzymatic Synthesis of 11-cis-Retinal Photoaffinity Analog by Use of Squid Retinochrome

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Chemoenzymatic Synthesis of 11-*cis*-Retinal Photoaffinity Analog by Use of Squid Retinochrome

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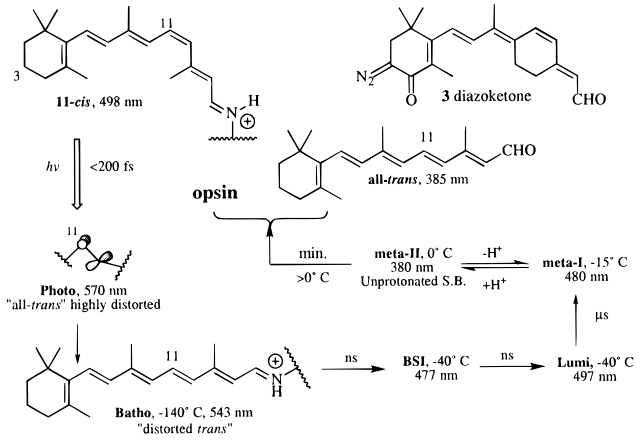
We expect the photoactive 11-*cis*-3-diazo-4-oxoretinal (**1**) to be crucial in clarifying the visual transduction process, but its synthesis has not been successful. However, as described below, the preparation has been achieved by incubation of *trans* isomer **2** with squid retinochrome which smoothly performed the critical *trans* → 11-*cis* isomerization to yield **1**.

The visual transduction process is initiated by isomerization of the 11-*cis*-retinal chromophore in rhodopsin to *all-trans*, cleavage of the chromophore/opsin bond and terminates in *all-trans*-retinal and opsin.[1] The *cis* → *trans* isomerization triggers a chain of conformational changes in the opsin which induces an enzymatic cascade leading to vision.[1a,2] Scheme 1 depicts the intermediates which have been identified based on low-temperature spectroscopy.[3] However, the movement of the chromophore relative to the receptor opsin along the isomerization pathway remains unknown.

Our objective is to clarify this crucial aspect on a molecular structural basis using photoaffinity labeling as the main tool. Photolysis of a pigment incorporating the nonisomerizable 11-*cis*-locked retinal analog **3** (Scheme 1) resulted in clear-cut cross-linking to Leu-266, thus revealing that the ionone C-3 is in close contact with helix F of rhodopsin in the dark.[4] On the other hand, studies using a photoreactive 11-*cis*-retinal analog in which the 11-ene is not locked showed that the C-3 region cross-linked to both helices C and F;[5] recent studies with spin labels[6] also showed that movements of these two helices were involved in the light activation process. It is thus possible that the *cis* → *trans* isomerization results in a "flip-over motion of the ring" from the proximity of helix F to helix C as well as movements of helices F and C and that this induces the conformational changes responsible for the enzymatic cascade. This scheme has been further corroborated by Sakmar and co-workers who have demonstrated that the relative movements of helices C and F is required for activation of the G-protein-coupled receptor rhodopsin.[7]

Scheme 1



We deemed it necessary to follow the isomerization pathway in a temperature-resolved manner by using, in contrast to locked analog **3**, the rhodopsin analog incorporating unlocked retinal **1**. After irradiation at 500 nm (−140 °C, "batho"), the chromophore is cross-linked (254 nm) and the amino acid(s) are sequenced. Similiar experiments will be performed at −40 °C ("lumi"), −15 °C ("meta-I"), etc. Since the photoaffinity label should traverse the same path of 11-*cis*-retinal isomerization, such sequential cross-linking experiments should allow one to trace the relative movements of the chromophore and the receptor opsin.[8]

Synthesis of 11-*cis*-retinal analogs, however, face problems due to the chromophore instability. The problem is magnified for analogs with functional groups which have to be introduced after formation of the 11-*cis*-ene. Most schemes geared toward introducing the 11-*cis* geometry close to the end of the synthesis have either failed or resulted in low yields and formation of complex isomeric mixtures.[9] In contrast to 11-*cis* analogs, it is known that synthesis of the more stable *all-trans* analogs are more straightforward. It would therefore be advantageous to synthesize the corresponding *all-trans* isomers and then introduce the 11-*cis* geometry at the end of the synthesis. Herein, we report a method for enzymatically introducing the 11-*cis* moiety at the last stage of the synthesis from an *all-trans* retinal, a reaction that could be performed with the necessary tritiated analog and could be quite general.

The visual cells of cephalopods contain in addition to rhodopsin, a photosensitive isomerase, retinochrome, which performs the *all-trans*-retinal → 11-*cis*-retinal regeneration.[10] In view of the efficiency of retinochrome in isomerization of

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(8) Preliminary experiments have shown that photoactivation of the diazoketo group in **1** does take place at the low temperature of −140 °C.

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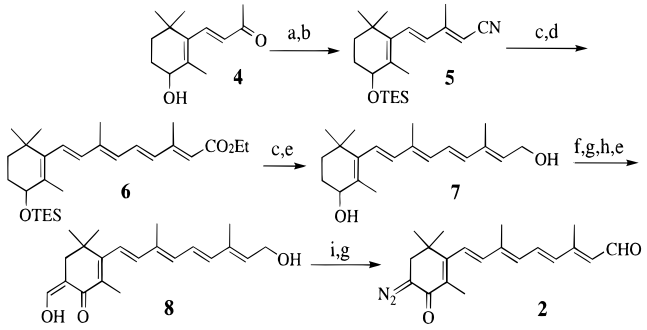
Scheme 2[a]



[a] Conditions: (a) TESCl, Im, DMF; (b) cyanomethyldiphenylphosphate oxide, NaH, THF; (c) Dibal-H, ether; (d) triethyl 3-methyl-4-phosphonocrotonate, NaH, THF; (e) nBu_4NF, THF; (f) TBSCl, Im, DMF; (g) MnO_2, CH_2Cl_2; (h) ethyl formate, NaH, THF; (i) 4-carboxybenzenesulfonazide, NaH, THF.

all-trans-retinal, the possibility of using it for introducing the 11-*cis* geometry at the last synthetic step was investigated. *All-trans*-3-diazo-4-oxoretinal (**2**), made in a straightforward manner, was thus subjected to isomerization by retinochrome isolated from the retina of the squid *Todarodes pacificus*.[11]

Synthesis of **2** (Scheme 2) was achieved by slight modification of a previous route.[12] The hydroxyl group of 4-hydroxy-β-ionone (**4**)[13] was protected with triethylsilyl chloride (TESCl). HWE olefination with the sterically hindered cyanomethyldiphenylphosphate oxide[14] led to the isolation of **5** in 72% yield (91% *E*-isomer). Subsequent Dibal-H (diisobutylaluminum hydride) reduction of the cyano and further HWE olefination with triethyl 3-methyl-4-phosphonocrotonate yielded **6** with all carbons in place in 50% yield for both steps. The functional group interconversions were easily performed by Dibal-H reduction of **6** followed by removal of the TES protecting group and selective protection of the primary alcohol. The 4-hydroxyl was oxidized by MnO_2, and the resultant 4-oxo species was formylated at C-3. Next, the silyl protecting group was removed and diazotization of **8** was accomplished by 4-carboxybenzenesulfonazide[15] and **2** was obtained by MnO_2 oxidation of the primary alcohol.

All-trans-3-diazo-4-oxo-retinal (**2**) was added to a suspension of apo-retinochrome at pH 6.5 to yield a stable pigment (Figure 1) with kinetics similar to *all-trans* retinal.[16] Formation of the protonated Schiff base (PSB) chromophore within the protein red-shifted the absorption to 474 nm. The photoisomerization of **2** was accomplished by irradiation of a suspension of **2** in phosphate buffer pH 6.5 at 4 °C in the presence of catalytic amounts of apo-retinochrome (2 mol%) with a xenon lamp behind a 540 nm wavelength "cutoff" filter for 40 min.[16] Successive hexane extractions yielded a mixture containing 75% 11-*cis*-3-diazo-4-oxo-retinal (**1**) (Table 1). HPLC separation of this mixture afforded pure 11-*cis* product in 45% yield.[17] As seen from Table 1, the retinochrome photoisomerization of **2** led to a 75:5:20 ratio of 11-*cis*/13-*cis*/*all-trans* isomers (ratio determined by HPLC analysis). The 11-*cis* isomer (**1**) was

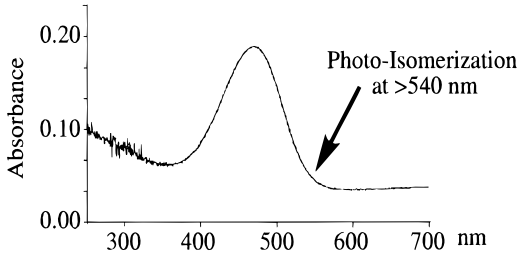
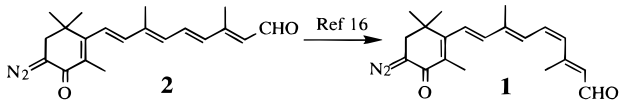


Figure 1. UV spectrum of retinochrome pigment of **2** formed when apo-retinochrome was incubated with substiochiometric amount of retinal analog **2** (1:0.8) in phosphate buffer prior to irradiation in the dark. The pigment absorbs at 474 nm. Photoisomerization of **2** to the 11-*cis* isomer was effected by irradiation with a Xenon lamp behind a 540 nm "cutoff" filter.

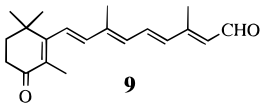
Table 1. Percentage of Isomers Isolated from Photoisomerization of **2** and **9** with apo-Retinochrome



all-trans retinal	11-*cis*	13-*cis*	*all-trans*
2	75	5	20
9	83	6	11

easily identified by its expected H-12 *cis J* coupling (11.2 Hz), as compared to the H-12 *trans J* coupling of **2** (15.4 Hz).

To demonstrate the utility of this protocol, *all-trans*-4-oxo-retinal (**9**), prepared by MnO_2 oxidation of **7**, was isomerized under similar conditions. Retinochrome photoisomerization of **9** led to a 83:6:11 ratio of 11-*cis*/13-*cis*/*all-trans* isomers (Table 1). The *all-trans* isomer can be readily recycled (especially since it is the last step of synthesis) in order to increase the efficiency of this scheme.



It is also well-known that apo-retinochrome can isomerize various geometrical isomers of retinal to 11-*cis*-retinal. Although not pertinent to our synthesis, possibly other retinal analogs could be isomerized to 11-*cis*. It is also noteworthy that the photoisomerization with retinochrome does not effect the photocleavable diazoketone functional group in **2**, as evidenced by the strong 2072 cm^{-1} IR band of the 11-*cis* product **1** obtained following the isomerization.

In conclusion, photoisomerization of *all-trans*-retinal analogs to the 11-*cis* isomer with retinochrome can be utilized as an efficient synthetic method. This is because the 11-*cis* geometry is established at the last stage of synthesis with high stereoselective output; moreover, it is applicable to radioactive analogs containing, e.g., tritium, which would be essential for sequencing the cross-linked sites. The low-temperature photolytic cross-linking of **1** to rhodopsin and sequencing of the cross-linked amino acid(s) will be reported in due course.

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(16) *All-trans*-4-oxo-3-diazo-retinal (0.107 mg, 0.33 μmol) as a solution in ethanol (0.2 mL) was added to the suspension of apo-retinochrome (2.2 mol %, 0.0071 μmol) in phosphate buffer (24 mL, pH 6.5) containing 0.1% BSA at 4 °C. The suspension was stirred at this temperature and irradiated with a xenon lamp (Ushio, 300 W) equipped with a 540 nm wavelength cutoff filter for 40 min. The mixture was then extracted with cold hexane (5 × 10 mL). The organic layer was separated by centrifugation at 10 000 rpm for 10 min. The pellets of apo-retinochrome separated under these conditions and could be reused, and the aqueous phase was discarded. Combined organics were dried with anhydrous sodium sulfate, and the solvent was evaporated to yield the crude mixture of isomers.
(17) Normal phase HPLC (Cosmosil Si-60, 4.6 × 250 mm) purification of the crude isomeric mixture (80% hexane, 20% ethyl acetate, 3 mL/min, detection at 370 nm) led to the isolation of **1**: ^{1}H NMR ($CDCl_3$, 400 MHz) 1.22 (6H, s), 1.93 (3H, s), 2.01 (3H, s), 2.35 (3H, d, J = 1.2 Hz), 2.67 (2H, s), 6.03 (1H, d, J = 11.2 Hz), 6.07 (1H, d, J = 8.3 Hz), 6.25 (1H, d, J = 16.1 Hz), 6.30 (1H, d, J = 16.1 Hz), 6.60 (1H, d, J = 12.7 Hz), 6.67 (1H, dd, J_1 = 11.2 Hz, J_2 = 12.7 Hz), 10.09 (1H, d, J = 8.3 Hz); IR (cm^{-1}) 1354.3, 1614.7, 1656.2, 2072.3, 2855.9, 2926.2; UV (hexane) 361 nm, (methanol) 377 nm; HRMS calcd for $C_{20}H_{25}O_2N_2$ $[M + 1]^+$ 325.1916, obsd 325.1918.