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Enantioselective Binding of an 11-*cis*-Locked Cyclopropyl Retinal. The Conformation of Retinal in Bovine Rhodopsin

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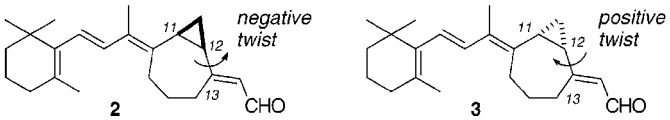
ABSTRACT



Binds to Opsin Does Not Bind to Opsin

The conformation of the retinal chromophore in rhodopsin is central for understanding the visual transduction process. The absolute twist around the 12-*s* bond of the chromophore in rhodopsin has been determined by studies with 11-*cis*-locked 11,12-cyclopropylretinal analogues (11*S*,12*R*)-2 and (11*R*,12*S*)-3, enantioselectively synthesized with the aid of an enzyme. The finding that enantiomer 2 binds to opsin while the other 3 does not defines the absolute sense of twist around the 12-*s* bond.

Rhodopsin, the photoreceptor protein responsible for dim light vision belongs to a family of G-protein coupled receptors (GPCR) including β-adrenergic receptors, olfactory receptors, and chemokine receptors.[1] GPCRs, consisting of seven transmembrane α-helices, are targets of many important drugs and are of great importance. Rhodopsins are the most thoroughly studied GPCR due to their essential biological functions and availability.[2] In the case of bovine rhodopsin, the ligand 11-*cis*-retinylidene chromophore **1** is covalently attached to the ϵ-amino group of Lys-296 in the seventh helix through a protonated Schiff base (PSB) linkage (Figure 1).[3]

The retinal chromophore adopts a 6-*s*-*cis* and 12-*s*-*trans* conformation in rhodopsin. Furthermore, due to steric interactions between 5-Me/8-H and 13-Me/10-H, the chromophore is twisted around the C6/C7 and C12/C13 single bonds, i.e., planes A/B and B/C are not coplanar (Figure 1).[4] Light induces isomerization of 11-*cis*-retinylidene chromophore to its *all-trans* form, thus triggering changes in the conformation of rhodopsin which initiate a cascade of enzymatic reactions leading to the generation of a neural signal.[5] This 180° isomerization of 11-*cis* to *all-trans* is accompanied by a flip-over of the ionone ring, rather than the PSB moiety, and involves a movement of the 13-Me away from 10-H.[6] Determination of the absolute sense of twist around the 6-*s* and 12-*s* bonds of the retinal chro-

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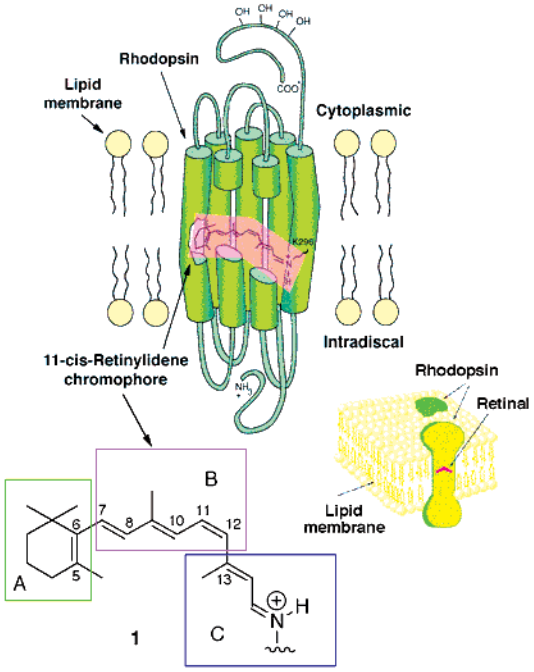

Figure 1. The 11-*cis*-retinylidene chromophore **1** of rhodopsin is not planar, that is, planes A, B, and C are not coplanar.

mophore in rhodopsin is central for understanding the changes in chromophore/receptor interactions along the visual transduction pathway. In addition, the nonplanar arrangement of the retinal conformation together with other factors, such as the distance between the PSB and its counterion and the electrostatic charge distribution within the retinal binding site, plays a critical role in regulating the absorption maxima of visual pigments covering the wide range of wavelengths from the UV region to around 640 nm.[7]

The nonplanar conformation of the retinal chromophore also accounts for the unique circular dichroic (CD) spectrum of rhodopsin. Native rhodopsin exhibits two positive Cotton effects (CE) in its CD spectrum at 480 nm ($\Delta\epsilon = +2.8$, α-band) and 337 nm ($\Delta\epsilon = +9.8$, β-band) (Figure 2). On the basis of the chiroptical data of rhodopsin pigments incorporating retinal analogues with either coplanar A/B or

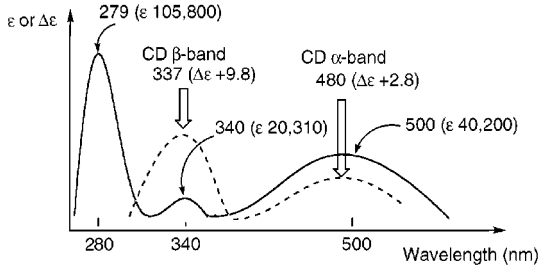

Figure 2. UV/vis (—, ϵ) and CD (- - -, $\Delta\epsilon$) spectra of bovine rhodopsin in 23 mM octyl glucoside buffer solution (pH 7.0).

B/C structures, the origin of α- and β-bands has been assigned to twists around C12/C13 and C6/C7 bonds, respectively.[8] Although it is generally accepted that the two positive CEs reflect the interaction between the retinal chromophore and its protein environment as well as chromophoric twists of polyene chain, the absolute sense of twist around the 6-*s* and 12-*s* bonds, or the absolute conformation of the 11-*cis*-retinylidene chromophore in rhodopsin around these bonds, remained to be established.

We recently investigated the absolute sense of twist around the 12-*s* bond based on exciton-coupled CD of 11,12-dihydrorhodopsin pigments.[9] Namely, since this CD method depends on the through-space coupling of two or more isolated chromophores,[10] the single twisted polyene chain in native 11-*cis*-retinal was separated into two independent conjugated systems by saturation of the central 11-ene. The triene and diene thus generated interacted through space within the retinal binding site to give a negative exciton CD couplet reflecting the negative C12/C13 absolute twist in the dihydroretinal analogues (Figure 3).

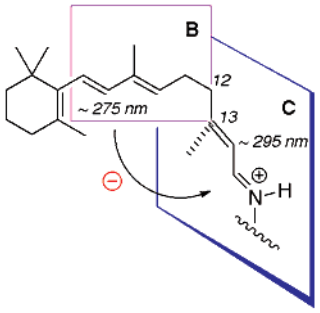

Figure 3. Absolute sense of twist around C12/C13 bond of the retinal chromophore in rhodopsin deduced by negative CD couplets of 11,12-dihydrorhodopsin pigments.

This negative helicity of the retinal chromophore agrees with theoretical calculations by Kakitani et al. and solid-state NMR studies by Han and Smith.[11] However, in a recent ab initio study,[12] the chiroptical data of PSB formed from methylamine and 11-*cis*-retinal with four arbitrarily assigned

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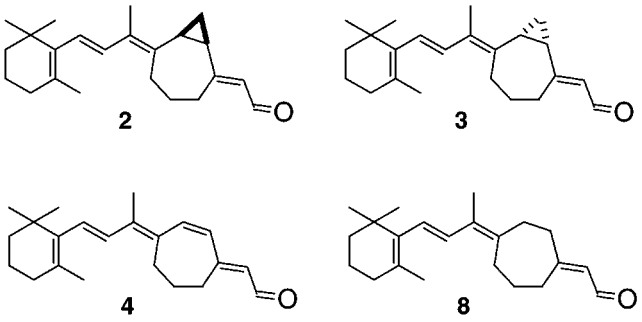
geometries were calculated, whereupon a positively twisted C12/C13 geometry gave rise to a positive CE which implied a positive helicity around the C12/C13 bond. However, the effect of the protein, such as the PSB counterion and electrostatic field within the retinal binding site, was not considered in this calculation. The following bioorganic experiments were performed in order to obtain additional evidence regarding the absolute conformation of the retinal 12-*s* bond in rhodopsin.

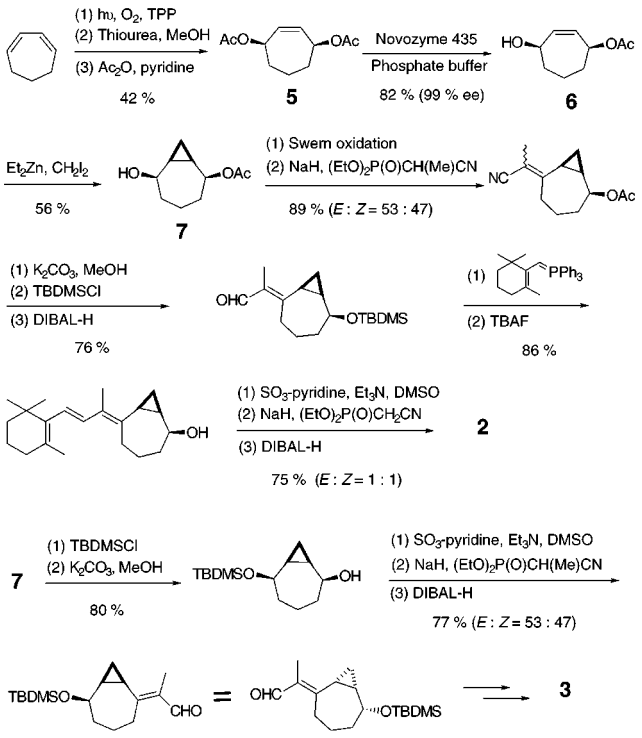


11-*cis*-Seven-membered-ring-locked retinal analogues with a cyclopropyl ring incorporated into the C11/C12 bond (**2** and **3**) were designed for the opsin binding studies. The rationale for choosing a retinal with locked 11-*cis* configuration via a seven-membered ring lies in earlier observations that both the UV/vis and CD data of rhodopsin pigment incorporating 11-*cis*-cycloheptatrienylideneretinal (**4**) closely resembled those of native rhodopsin, indicating similar ligand conformations in protein.[13] Incorporation of a cyclopropyl ring at the C11/C12 bond yields a retinal analogue with a rigid structure where the twist around the 12-*s* bond is predetermined by the absolute configuration of the analogue. Namely, enantiomers **2** and **3** adopt opposite twists around the C12/C13 bond; it was therefore expected that binding of the enantiomer to bovine opsin with the right absolute geometry would occur preferentially over the other enantiomer.

The cyclopropyl retinal analogues were synthesized as enantiomerically pure isomers (Scheme 1). The key step involves enantioselective hydrolysis of diacetate **5** by lipase Novozyme 435 to give monoacetate **6** in 82% yield (99% ee).[14] Simmons-Smith reaction of **6** affords a single diastereomer of cyclopropyl alcohol **7** (de > 20:1) which can be converted to both **2** and **3** through separate reaction sequences (Scheme 1).[15] The final products were further purified by HPLC (normal phase, 5−10% EtOAc/hexanes, 2 mL/min) before being subjected to opsin binding studies.

Analogues **2** and **3** possess absorption maxima at 266 nm in MeOH, while their *n*-butylamine Schiff base absorbs at

Scheme 1



260 nm and the corresponding PSB absorbs at 284 nm in MeOH, which is red-shifted compared to the PSB of 11,12-dihydroretinal analogue **8** (λ_{max} 272 nm)[9] due to the partial conjugation effect of cyclopropyl ring. CD of analogues **2** and **3** measured in methanol showed opposite CEs with similar intensities (Figure 4). An excess of retinal **2** or **3**

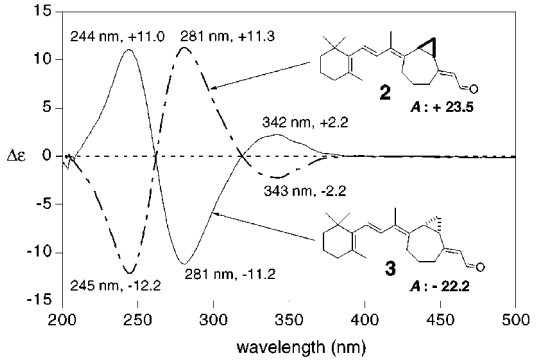


Figure 4. CD spectrum of cyclopropyl retinal analogues **2** and **3**, measured in MeOH.

was separately incubated with bovine opsin, and the binding was monitored by UV.[16] The initial concentrations of retinal analogues in the two binding experiments were about the same. Generally, the binding of retinal analogues to opsin gives rise to a bathochromic shift in the UV/vis absorption originating from the formation of PSB within the retinal

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(15) (a) **6** was coupled to both (*S*)- and (*R*)-α-methoxyphenylacetic acid, and the enantiomeric purity of **6** was determined by ^{1}H NMR of its MPA ester derivatives. The optical rotation value of **6** agrees with literature value (see ref 14). (b) The *cis* geometry between hydroxyl group and cyclopropyl ring in **7** was confirmed by NOESY, HMQC, and COSY NMR experiments.

binding site of rhodopsin. This was also the case in the binding of **2** to opsin; a new band at 312 nm was formed over a 1 h incubation period at room temperature (Figure 5,

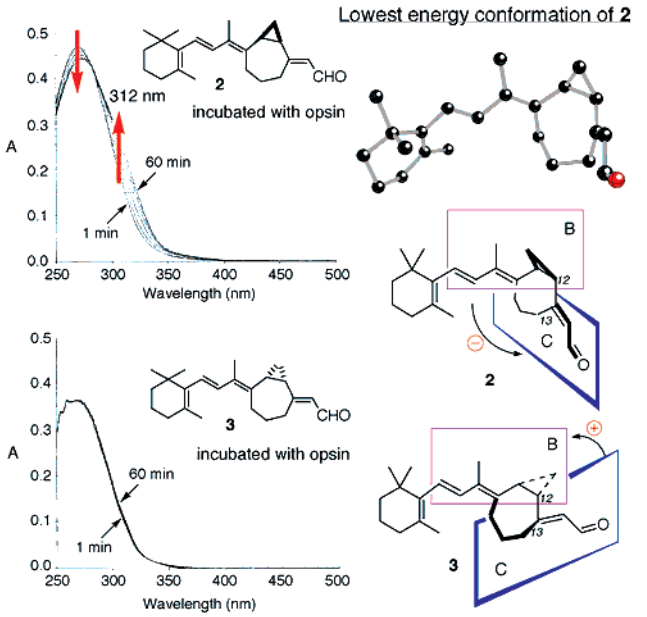

Figure 5. Retinal analogues **2** and **3** were separately incubated with opsin, and the progress of binding was monitored by UV. The lowest energy conformation of the cyclopropylretinal analogues were calculated by MacroModel.

colored arrow), indicating formation of the cyclopropyl rhodopsin pigment. In contrast, with analogue **3** no change in the UV was observed over 2 h, thus indicating that it does not bind to opsin (Figure 5). Again, due to the partial conjugation effect of the cyclopropyl ring, the 312 nm absorption maximum of the pigment derived from **2** is red-shifted by more than 25 nm in comparison to the pigment incorporating retinal analogue **8** (λ_{max} 285 nm).[9]

The cyclopropyl rhodopsin pigment derived from **2** was further purified by concanavalin A affinity chromatography to remove excess retinal chromophore.[17] The purified pigment absorbs at 280 nm with a shoulder around 312 nm, arising, respectively, from the rhodopsin aromatic amino acids and the bound pigment. It exhibits a positive CE in CD at 284 nm accompanied by a weak shoulder around 300 nm in nonyl glucoside solution. This further confirmed the pigment formation since the apoprotein opsin has negligible CD above 270 nm (spectra not shown).

The binding experiments of **2** and **3** with opsin demonstrate the steric preference of the retinal binding site. This also represents the first case of enantioselective binding of a chiral retinal analogue to bovine opsin. Previous bioorganic studies with allenic and adamantyl allenic retinal analogues had shown that the cyclohexene ring binding site in rhodopsin is lenient and exhibited no chiral preference between the enantiomers of allenic retinal analogues.[18] The enantioselective binding of retinal analogue **2** suggests a close steric fit of the chromophore in rhodopsin around the middle moiety of the molecule. This enantioselective fit is critical for subsequent rhodopsin activation triggered by 11-ene isomerization.[6]

While analogue **2** binds to opsin, **3** does not bind at all. Therefore, the negative twist between planes B/C of **2** (Figure 5) should represent the geometry of the retinal binding pocket in rhodopsin. Molecular modeling by MacroModel (MM3 force field) yielded the lowest energy conformation of **2** as depicted in Figure 5. The twist around the C12/C13 bond in **2** is −110°, i.e., C13 to PSB moiety is twisted toward the front of plane B (Figure 5). This negative helicity is in agreement with our earlier conclusion based on negative exciton CD couplets of 11,12-dihydrorhodopsin pigments (Figure 3).[9] Determination of the absolute twist around the 6-*s*-*cis* bond and rationalization of the 6-*s* and 12-*s* bond absolute twists with respect to subsequent ligand/receptor movements are currently ongoing. Theoretical treatment of the chiroptical data of cyclopropylretinal analogues are also under study (V. Buss and N. Harada, private communications).

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Supporting Information Available: Experimental procedures for syntheses of **2** and **3** and spectroscopic data.

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(16) A stock solution of opsin in 10 mM CHAPSO/HEPES buffer (pH 7.0) was added to a pair of UV cuvettes (1 mL solution each), and the background of the UV spectrometer was scanned. A solution of retinal analogue **2** or **3** in EtOH (5 μL) was added to the sample cuvette, and 5 μL of EtOH was added to the control cuvette; UV were measured every 10 min over a 1 h period. The initial band (taken within 1 min of mixing) in UV corresponds to unbound retinal analogues in buffer. The binding of chromophore to opsin is accompanied by a decrease in the intensity of the unbound chromophore band and a growth of a new band at longer wavelength corresponding to PSB formation. Unchanged UV indicates that the chromophore remains unbound throughout the whole period. The binding of **2** was further confirmed by incubating the pigment with 11-*cis*-retinal. No new band at 500 nm in UV/vis was detected, suggesting that the retinal binding site was occupied by **2**.

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