Irregularity in Opsin Shifts of Hydroretinochromes

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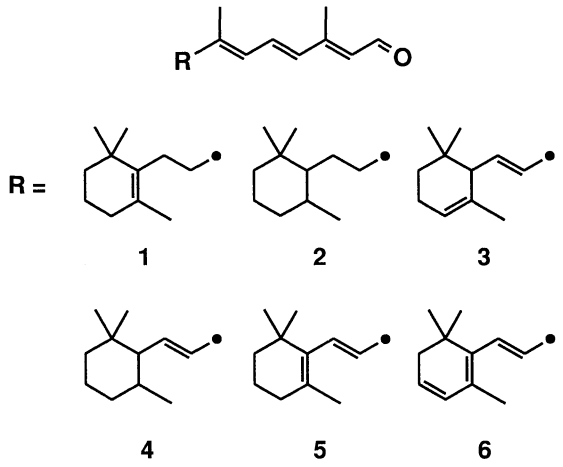
(Received February 25, 1997; CL-970137)

Hydroretinochromes were reconstituted with aporetinochrome and hydroretinal analogues; 7,8-dihydroretinal, 5,6-dihydroretinal, dehydroretinal, and their analogues. The 'opsin shifts' were 2200 ± 100 cm^{-1} for trienals and pentaenals, whereas 1600 ± 100 cm^{-1} was the value for tetraenals.

Deep color of retinal protein comes out when the retinal protein is reconstituted with apoprotein and retinal. The chemical explanation of the bathochromic coloration had involved one of the important problems unsolved. Therein, the concept, 'opsin shifts', has been proposed for accounting for the phenomena.[1] If the retinal analogue instead of native retinal can form artificial pigment with apoprotein, the pigment acquires the similar properties as native pigments except those in the chromphore. The discussion on the opsin shifts of the artificial pigments have been developed in protonation and chrompohoric difference.[1]

For examination of π-conjugation in the chromophore of retinochrome,[2] hydroretinals were selected as one of the suitable candidates.[3] No papers have been appeared in the study of bathochromic shifts in hydroretinochromes.[4,5]

The hydroretinal analogues were synthesized according to the ordinary retinoid syntheses.[3] Retinochrome was isolated from squid's eye (squid's name is *Todarodes pacificus*) in the dark under dim light by Hara's method.[6] The protonated Schiff base (SBH^+) was formed as equimolar mixture of the analogues, butylamine and hydrogen chloride in methanol.[7] The peak maxima of all the spectra were confirmed with their second derivatives.



For the determination of pigment formation, a digitonin solution of aporetinochrome was titrated with a methanolic solution of the analogue. The obtained titration curve consisted of two straight lines, a steep line ranging from 0 to 1.0 in ratio and a flat line above the ratio of 1.0, as in the case of 7,8-dihydroretinal (**1**) in Figure 1. This kink point means that dihydroretinochromes can be formed in an equimolar ratio with

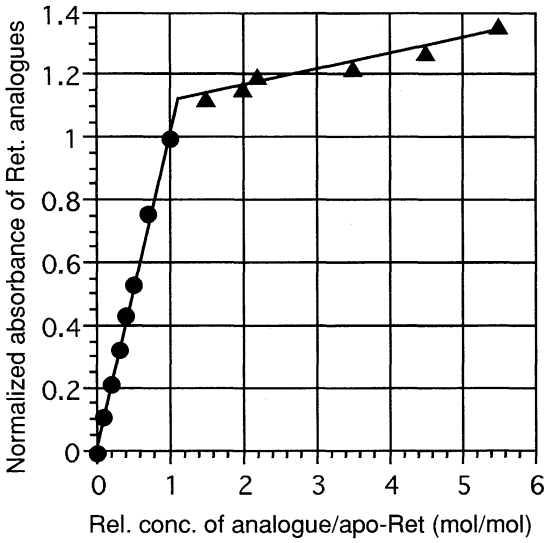


Figure 1. Titration curve in formation of 7,8-dihydroretinochrome.

Table 1. Opsin shifts in hydroretinochromes

Analogue	No. of Conjug-ation	λ_{ret}[a] /nm	λ_{SBH^+}[a] /nm	λ_{Ret}[a] /nm	Δν /cm^{-1}[b]
1	4	340	389	425	2300
2	4	338	387	421	2100
3	5	366	426	460	1700
4	5	364	425	456	1600
5	6	381	444	491	2200
6	7	395	461	504	1900

[a]The symbols, λ_{ret}, λ_{SBH^+} and λ_{Ret} mean the wavelengths of the retinal analogues, the protonated Schiff bases and the retinochromes at the absorption maxima, respectively. [b]The values of Δν were calculated as follows; $\Delta\nu = 1/\lambda_{SBH^+} - 1/\lambda_{Ret}$.

retinal analogue and aporetinochrome.

The absorption maxima for the retinal analogues, their butylamine Schiff bases, protonated Schiff bases, retinochrome analogues and the values of opsin shifts are shown in Table 1. No linear relationship between the number of conjugated double bonds and the shifts was found in the Table. Thus the opsin shift (2300 ±100 cm^{-1}) in 7,8-dihydroretinochrome is close to that in native retinochrome (2200 ±100 cm^{-1}), whereas the significantly low value (1600 ±100 cm^{-1}) was obtained for 5,6-dihydroretinochrome. The similar tendency was observed for 5,6,7,8-tetrahydroretinochrome (2100 ±100 cm^{-1}) and 4,5-didehydro-5,6-dihydroretinochrome (1700 ±100 cm^{-1}). These results can be classified in two groups, e.g. a trienal or pentaenal group with 2200 cm^{-1}, and a tetraenal group with 1600 cm^{-1}. The difference of 600 (=2200-1600) cm^{-1} means that the artificial retinochrome with a tetraenal is slightly destabilized in the excited state in comparison with the case of native retinochrome.

The irregularity in the opsin shift is independent of photoisomerization reaction or binding. In the analogue **1**, pigment was quantitatively formed as a 1:1 adduct at the similar rate to that in retinal. However the photoisomerization of the retinochrome analogue was less regioselective for its 11-cis isomer (regioselectivity in 60%). The same rate was observed in 4,5-didehydro-5,6-dihydroretinal, whereas the regioselectivity of photoisomerization was in 90%.[8]

The present work is partly defrayed by the Grant-in-Aid for Scientific Research on Priority-Area-Research "Photoreaction Dynamics" from the Ministry of Education, Science, Sports, and Culture of Japan (06239106). We gratefully acknowledge to Professor J. Lugtenburg as a visiting professor sponsored by JSPS (S96100) for helpful discussion.

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