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FORMATION AND 'OPSIN SHIFTS' OF ARTIFICIAL RETINOCHROMES

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Retinochromes in which the retinal is an artificial analog were synthesized, and their 'Opsin Shifts' were calculated. The values were compared with those of the natural retinochrome.

Retinochrome is a photosensitive chromoprotein found in the visual cells of cephalopods.[1] The most noticeable difference from rhodopsin lies in the geometrical configuration of the retinal chromophore, which is 11-cis in rhodopsin, but all-trans in retinochrome.[2,7] On irradiation of retinochrome, its chromophore changes from the all-trans to the 11-cis form, which assists to regenerate rhodopsin.[7] On the other hand, bacteriorhodopsin, a third retinoidal protein, contains all-trans retinal (or an equimolar mixture of all-trans and 13-cis retinals) and bacterioopsin.[3]

These retinoidal proteins show individual colors. Although all the retinal isomers are yellow, their couplings with also colorless apoproteins often result in the formation of deep-colored pigments (red to purple). The reason why the absorption spectra of the pigments show such bathochromic shifts has so far remained as an unsolved problem.

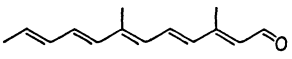
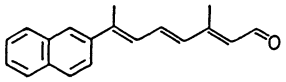
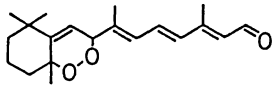
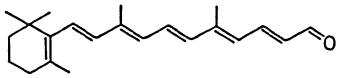
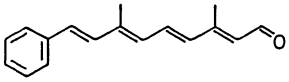
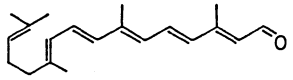
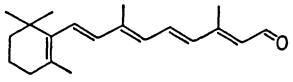
Recently Nakanishi et al. has advanced an invaluable suggestion that the bathochromic shifts in rhodopsin and bacteriorhodopsin can be accounted for in terms of a point charge model.[4] Here we report the evaluation of the protein shifts[5] in retinochromes by use of retinal analogs, and the shifts are compared with those on rhodopsin and bacteriorhodopsin.

All retinal analogs were prepared by the Wadsworth-Emmons reaction from the

corresponding ketones or aldehydes.[6] All-trans retinal analogs were separated by means of HPLC in the final step of the synthesis. Aporetinochrome was isolated from the squid (*Todarodes pacificus*) eyes, according to the Hara's method.[7] Artificial retinochromes with a retinal analog and the aporetinochrome were reconstituted by adding the all-trans analog in ethanol to the aporetinochrome in a 2% digitonin solution. The protonated Schiff-base of a retinal analog was formed by adding butylamine and dry HCl in succession, both in methanol, to a methanol solution of the retinal analog.

The absorption maxima (λ_{max}) and the protein shifts of the pigments are shown in the Table 1.

Table 1. Reconstituted Retinochromes

Retinal analog	λ_{max}/nm (MeOH)	λ_{max}/nm SBH	λ_{max}/nm Retinochrome	$\Delta\tilde{\nu}$/cm^{-1} Protein shift
	403	465	490	1 100
	383	425	458	1 700
	336	377	≈390[a]	880
	405	448	≈480[a]	1 490
	394	455	482	1 230
	400	475	520	1 800
	390	440	496	2 600

a) Slow formation of pigment.

The chemical structure of each retinal analog has a great influence on the reconstitution of the artificial retinochrome. For instance, the rate of pigment formation is extremely slow, when C_{22}-aldehyde is mixed with aporetinochrome. Aporetinochrome seems to be capable of recognizing the length of the conjugated double bonds system.

As shown in the Table 1, all the protein shifts in the artificial retinochromes have smaller values than those in the natural retinochrome. This fact strongly suggests that the excited state of artificial pigments is less stabilized than that of the native pigment and the hydrophobic binding of the cyclohexene ring with the protein plays an important role for the stabilization.[8] The molecular weight of retinochrome has been estimated to be 24 000, which is fairly close to that of bacteriorhodopsin (26 000) but is lower than that of rhodopsin (38 000).[9] However, some properties of retinochrome are apparently similar to bacteriorhodopsin and others to rhodopsin. For instance, in the bound form of retinal to the protein, retinochrome with only all-trans retinal behaves like bacteriorhodopsin but not like rhodopsin with 11-cis retinal. Furthermore, it is wellknown that bacterioopsin, an apoprotein, is able to reconstitute the pigment with 13-desmethyl retinal in all-trans form,[10] whereas the same apoprotein cannot form an artificial bacteriorhodopsin with all-trans 13-desmethyl-14-methyl retinal. In the case of an artificial retinochrome, an addition of 13-desmethyl retinal to aporetinochrome immediately gave the corresponding pigment(488 nm). However, this was not the case with 13-desmethyl-14-methylretinal. Based on these experiments on pigment formation, we conclude that the properties of retinochrome in the ground state is similar to those of bacteriorhodopsin.

On the other hand, the above-mentioned protein shift of retinochrome was 2 600 cm^{-1}, and it is close to that of rhodopsin (2 700 cm^{-1}) but far from that of bacteriorhodopsin (4 900 cm^{-1}). From the extent of protein shift of each pigment, it is suggested that the stabilizing effect of protein on the excited state of retinochrome is similar to that of rhodopsin but not to that of bacteriorhodopsin.

In conclusion, (1) several artificial retinochromes with retinal analogs were newly synthesized. (2) The synthesized retinochromes always showed smaller protein shifts than the natural retinochrome. The difference was accounted for

in terms of hydrophobic binding. (3) Reconstituted retinochromes represent the chemical structures of the retinal moiety and their properties are similar to those of bacteriorhodopsin in the ground state and to rhodopsin in the excited state, respectively.

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References

1) T. Hara and R. Hara, Nature, 206, 1331(1965).

2) G. Wald, Comp. Biochem., 1, 311(1960).

3) D. Oesterhelt and W. Stoeckenius, Nature, 233, 149(1971).
The dark-adapted bacteriorhodopsins contains a chromophore which is a mixture of all-trans and 13-cis retinals (1:1), while the light-adapted bacteriorhodopsin contains all-trans retinal only.

4) M. G. Motto, M. Sheves, K. Tsujimoto, V. Balogh-Nair, and K. Nakanishi J. Am. Chem. Soc., 102, 7947(1980).

5) The opsin shift has been defined as the wavelength difference in the absorption spectra between the protonated Schiff-base and the rhodopsin (or bacteriorhodopsin). The concept of 'Opsin Shift' can be extended to similar shifts in other retinoidal proteins. Then, they are called 'protein shift'.

$$1/\lambda(\mathrm{SBH})-1/\lambda(\mathrm{protein})=\text{protein shift}$$

6) W. S. Wadsworth and W. D. Emmons, J. Am. Chem. Soc., 83, 1733(1961).

7) T. Hara and R. Hara, Methods Enzymol., 81, 190(1982).

8) The stabilization depends on the delocalization of the positive charge in the protonated Schiff-base of the protein in the excited state. Hydrophobic binding can be envisioned as the most important factor, taking account of the structural comparison of the native chromophore with that of the analog lacking an intact ring.

9) K. Nashima, S. Mitsudo, and Y. Kito, Biochim. Biophys. Acta, 536, 78(1978).

10) W. Gaertner, P. Towner, H. Hopf, and D. Oesterhelt, Biochemistry, 22, 2637(1983).

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