Contribution of Methyls in Retinal Side Chain to Regioselective Photoisomerization of Retinochromes

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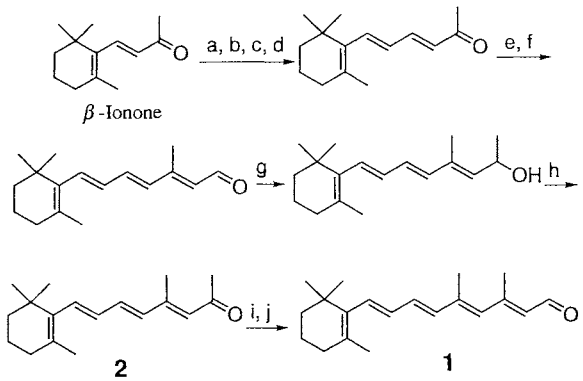
All-*trans*-9-demethyl-11-methylretinal was synthesized to elucidate the chromophoric interaction between hydrophobic part in retinochrome and 9-methyl substituent of retinal. Photoirradiation of its pigment efficiently gave the 11-*cis*-isomer in 90% regioselectivity, whereas that of 9-demethylretinochrome provided the 11-*cis* isomer in 49% selectivity. Structural contribution to regiospecific photoisomerization was proved, in comparison with the other retinochrome analogues, that 9- or 11-methyl substituent is a requisite for highly regioselective photoisomerization, but 13-methyl group is not.

Visual process is started with photoactivation of rhodopsin. Light-irradiation induces configurational change of a chromophore in rhodopsin from 11-*cis* to all-*trans* configurations in high regioselectivity and quantum yield.[1] On the contrary, the *in-vivo* system involves rhodopsin-generating reaction from all-*trans* to 11-*cis* isomer. In 1965, Hara and Hara found the photochemically reverse reaction from all-*trans* to 11-*cis* in a retina of a squid (*Todarodes pacificus*) as well as new photopigment retinochrome was isolated.[2] The reaction is characteristic of catalytic property as an enzyme in the regiospecific isomerization with a photopigmental protein. The enzymatic property of the protein enabled us to elucidate relationship of chromophoric structure to function of the protein by the use of retinal analogues.[3] The photochemical reaction takes place in the rotation about the 11-12 double bond, whose feasibility should be influenced by the methyl groups in the side chain of retinal chromophore. In particular, the photoisomerization is capable of being affected by protein pocket or hydrophobic interaction between the methyls and the protein. For estimation of the efficiency in methyl substituents, artificial retinochromes were regenerated with mixing aporetinochrome and the all-*trans* -retinal analogues.[4]

Effect of 9-methyl group on selectivity in photoisomerization was examined in 9-demethylretinochrome. The formation of 9-demethylretinochrome with addition of 9-demethylretinal to aporetinochrome was evidenced in production of a pigment whose electronic spectrum is closely similar to that of native retinochrome in shape and extinction coefficient. The rate of the pigment formation also agreed with that of the native pigment (ratio of the rates close to unity).[5] These facts suggest that the absence of 9-methyl group is independent of the pigment formation. However, photoisomerization of 9-demethylretinochrome provided 11-*cis*-9-demethylretinal in low regioselectivity (11-*cis*:all-*trans*:13-*cis*:others=49:35:13:3), whereas the high selectivity (>99%) is accomplished in the case of native retinochrome. From this fact, the following hypothesis is proposed that regioselective isomerization would require 9-methyl group.

Hence the synthesis of 9-demethyl-11-methylretinal (**1**) contributes to estimation for justification of the hypothesis. The all-*trans*-analogue was synthesized and isolated in total yield of 5%, as shown in following Scheme 1; the modified procedure was



a) NaClO, MeOH; b) $LiAlH_4$, Et_2O, 0°C; c) active MnO_2, Et_2O;
d) acetone, 1M NaOH solution (in the dark);
e) $(EtO)_2P(=O)CH_2CN$, NaH, DME, 0°C (f) DIBAL, Et_2O, -70°C;
g) MeLi, Et_2O, 0°C (h) active MnO_2, Et_2O;
i) $(EtO)_2P(=O)CH_2CN$, NaH, DME, 0°C (j) DIBAL, Et_2O, -70°C

Scheme 1. Synthesis of 9-demethyl-11-methylretinal (**1**).

optimized for the synthesis of C18-ketone (**2**). Formation of all-*trans*-9-demethyl-11-methylretinochrome was confirmed as in the case of 9-demethylretinochrome (shown in Table 1). Contrary to prediction, photoirradiation of the pigment resulted in highly regioselective formation of 11-*cis*-9-demethyl-11-methylretinal (90%).

The fact suggests that substitution of methyl at 9-position is not requirement for regioselective reaction to 11-*cis*-isomers. Here the relationships between methyl substitutions at 9-, 11- and/or 13-position and their selectivities were examined and summarized in Table 2.

On the basis of the regioselectivities in Table 2, the 9-methyl group is a keystone substituent to induce high regioselectivity in photoisomerization. The methyl group at 11-position is also important in the regioselective photoisomerization. As a summary, the high selectivity in the photoisomerization requires a methyl group at 9- or 11-position. Therein, one question arises for the selectivity of 9-demethyl-11-methylretinal, that is, presence of 13-methyl group could assist the high selectivity. For the question, synthesis of 9,13-didemethyl-11-methylretinal affords a positive answer; highly regioselective photoisomerization was observed for 9,13-didemethyl-11-methylretinal. Conclusive aspect describes that regioselective isomerization is independent of 13-methyl group, but dependent on 11-methyl group.

For estimation of hydrophobic interaction from absorption spectra, 'analogue shifts' were calculated in analogy with opsin shifts. The analogue shift depicts energetic difference between retinal analogue and retinal in the same circumstances.[6] The shift values are determined in ethanol or pigment. If two values are similar each other, the structure of analogue is close to that of the native state before and after formation of pigment. Thus, the shift

Table 1. Absorption spectra of all-*trans*-retinal and 9-demthyl-11-methylretinal

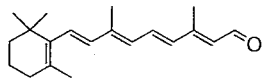
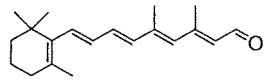
	absorption maximum / nm	
	in ethanol	in retinochrome
	381	492
	372	466

Table 2. Methyl(s) substitution and regioselectivities in photoisomerization

	9-Me	11-Me	13-Me	Selectivities
9-deMe	X	X	O	49
13-deMe	O	X	X	93
9,13-deMe-11-Me	X	O	X	91
9-deMe-11-Me	X	O	O	90
retinal	O	X	O	99
13-deMe-11-Me	O	O	X	90
11-Me	O	O	O	90

The symbol X means lack of a methyl group, while O shows presence of the group.

value of 9-demethyl-11-methylretinal in ethanol showed 640 cm^{-1}, whereas that of 9-demethyl-11-methylretinochrome furnished 1100 cm^{-1}. The difference of these values, 500 cm^{-1} means hydrophobic interaction of protein cavity and methyl substitution.[7]

In conclusion, the regioselective photoisomerization of retinochrome is controlled by the methyl group at the 9- or 11-position. These facts suggest that retinochrome has the hydrophobic binding site around the 9- and 11-position in the chromophore. There appears a hook in the protein for regioselective photoisomerization. On the other hand, the role of 13-methyl group is not dependent on regioselective photoisomerization. Therefore, the formation and regioselective photoisomerization of 9-demethyl-11-methylretinochrome are similar to that of native retinochrome.

Moreover, the relationship between the binding ability with apoprotein and the size of 9-substituent was discussed. 9-Ethyl- and 9-isopropylretinals are able to bind to aporetinochrome, but 9-*sec*-butyl-, 9-butyl-, or 9-benzylretinal formed no pigment.[8] These facts suggest that aporetinochrome recognizes moderately sized 9-substituent for pigment formation. The formed pigments were photobleached upon light to give the corresponding *11-cis* isomers in high regioselectivity. Conceptually, it is proposed that 9- or 11-methyl group plays an important role of 'Latchet mechanism' for highly selective photoisomerization, as shown in Figure 1.

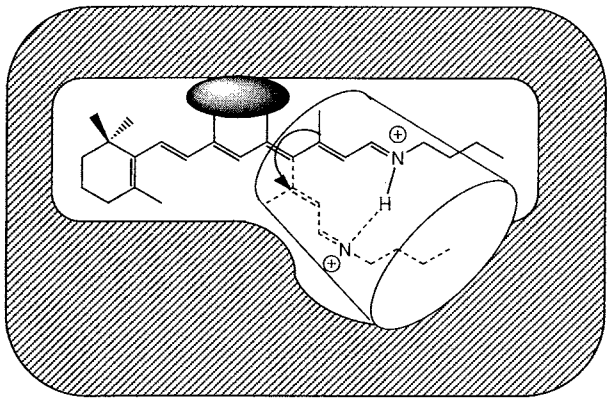


Figure 1. Latchet model for regioselective photoisomerization.

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References and Notes

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5 The ratio of the rate constants in the regeneration of the retinochromes was 1.1 based on the constant of native retinochrome. The similar value was obtained for 13-demethylretinochrome.
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8 The following retinal analogues were synthesized by Professors A. Wada and M. Ito at Kobe College of Pharmacy; 9-ethyl-, 9-isopropyl- 9-*sec*-butyl-, and 9-benzylretinal. The absorption maxima of 9-ethyl- and 9-isopropylretinochromes are 497 and 489 nm, respectively.