  Bull. Chem. Soc. Jpn., 59, 2157—2164 (1986)

Spectroscopic and MO Study of Peptide Models for the M_{412} Intermediate of Bacteriorhodopsin. N^{ε}-Retinylidenelysyl Peptides Containing Aromatic Amino Acids

Minoru SAKURAI,* Satoshi TOMOMASA, Yûji SEIYA, Yoshio INOUE, and Riichirô CHÛJÔ

Department of Polymer Chemistry, Tokyo Institute of Technology, 12-1 Ookayama 2-Chome, Megro-ku, Tokyo 152

(Received April 30, 1985)

N^{ε}-Retinylidenelysyl peptides containing Tyr and Phe were prepared in order to investigate the effect of the surrounding microenvironment on the absorption maximum of the chromophore of bacteriorhodopsin. The absorption maximum of the retinylidene Schiff base of the lysyl peptide, Boc-Lys-Phe(or -Gly)-Tyr-OMe, was shifted to the red relative to the peptides lacking Tyr, Boc-Lys-Phe-Phe-OMe and Boc-Lys-Phe-OMe. UV and ^{13}C NMR studies on the Tyr-containing lysyl-peptide have confirmed an intramolecular interaction between Tyr and the retinylidene portion in low dielectric media. A hydrogen-bond formation of the Schiff base with a Tyr side chain is supported by a theoretical estimation of the transition energy and the shielding constants for some models of Tyr-Schiff base complexes. The side-chain conformation of the Tyr-contaning lysyl-peptide is discussed in terms of the ^1H NMR data. The hydrogen-bonding shift induced by Tyr was estimated as being no less than 10 nm.

Bacteriorhodopsin (bR) acts as a light-driven proton pump in the plasma membrane of halobacteria.[1] The chromophore of bR is a retinal Schiff base formed with Lys 216.[2,3] It is protonated in the initial state[4] and becomes deprotonated upon being illuminated (intermediate M_{412}).[5-7] The absorption maximum of the M_{412} is shifted considerably towards the red compared with an unprotonated retinal Schiff base, e.g., *N*-retinylidenebutylamine. This shift should be induced by neighboring amino acids of the chromophore.

Recent reports on the chemical modifications of bR[8-10] and the pH dependence of the M_{412} formation[11-14] have shown that tyrosine is one possible candidate near the Schiff base in bR. Following Engelman's model,[15] which was built on the basis of X-ray diffraction data of bR, helices A, B, and F were formed to lie close to helix G containing Lys 216 and involve several tyrosine residues. Therefore, it is interesting to study to what extent the absorption maximum is shifted by one tyrosine spatially arranged near a Schiff base using artificial pigments.

It has been demonstrated[16-18] that N^{ε}-retinylidenelysyl peptides are useful models to study specific interactions between a Schiff base and an amino acid. However, the mechanism of the spectral shift in such an artificial pigment has not been fully clarified. Preliminary consideration using the CPK model showed that several types of interactions involving a hydrogen bond are possible between Tyr and the Schiff base in tripeptide Lys(Ret)-X-Tyr, where Ret and X stand for the N^{ε}-retinylidene group and one of the common amino acids, respectively. We prepared Boc-Lys(Ret)-Phe-Tyr-OMe **1**, Boc-Lys(Ret)-Gly-Tyr-OMe **2**, Boc-Lys(Ret)-Phe-Phe-OMe **3**, Boc-Lys(Ret)-Phe-OMe **4**, where Boc is an abbreviation for a *t*-butoxycarbonyl group.

Here, we report that the absorption maximum of an unprotonated retinal Schiff base is shifted to the red by the perturbation of one tyrosine. Furthermore, we discuss the origin of the red shift on the basis of UV, ^1H and ^{13}C NMR spectra, and a MO calculation.

Theoretical

A ^{13}C NMR chemical shift was estimated according to a theory of Karplus and Pople[19] incorporated into the CNDO/S method.[20] The average excitation energy, ΔE, was taken as 10.0 eV. The details of the procedure were given in a previous paper.[21] The calculation of the π-π^* transition energy was carried out using the CNDO/S method. Some modifications have been proposed for the CNDO/S parameters from the originating laboratory.[22,23] According to these reports, coulomb integrals were estimated using the Nishimoto-Mataga formula and reevaluated values were used for core and bonding parameters. The lowest energy (60 configurations) of all single excitations were included in the configuration-interaction calculations.[23]

These calculations would have been computationally intractable if the entire framework of the retinylidene peptide was included. It has been shown that neglecting the C_9 and C_{13} methyl groups and the saturated carbon region of the β-ionylidene ring does not introduce any serious error to the results concerning the electronic structure.[24,25] We, therefore, approximate the retinal portion as *all-trans N*-undecapentylidenemethylamine (hereafter abbreviated as NUPM). The molecular geometry was derived from crystallographic data.[26] The other portion of the retinylidene peptide was represented by the phenol ring of Tyr. The orientation of the phenol ring to the polyene chain was determined based on a

CPK model of the peptide containing all atoms.

Experimental

All peptides were synthesized by conventional solution phase procedures, using dicyclohexylcarbodiimide as a coupling regent. The Boc group was used for α-amino group protection and methyl ester for the carboxyl group. The ε-amino group of the lysine residue was protected with a benzyloxycarbonyl group during the coupling reaction. The protecting group on the lysine side chain was removed in the final step by catalytic hydrogenation using palladium-charcoal in a methanol-acetic acid 1:1 solvent. The final products were recrystallized from ethyl acetate-hexane. All the intermediate and final products were identified by their ^{13}C NMR spectra and their purities were checked by TLC. Retinylidene peptides were prepared by keeping a mixture of *all-trans* retinal and the peptide (1:1 mole ratio) in ethanol with anhydrous potassium carbonate. The products were recrystallized from ethanol-hexane. *All-trans* retinal and *all-trans* *N*-retinylidenebutylamine (NRB) were synthesized according to the procedure given in a previous paper.[27] All the Schiff bases were handled under a dim red light.

Absorption spectra were recorded on a Beckman model 25 spectrometer. ^{13}C NMR spectra were recorded on a JNM JEOL PS-100 spectrometer equipped with a PFT-Fourier transform system at 25.15 MHz. ^{1}H NMR spectra were recorded on a JEOL FX-400 spectrometer at 399.65 MHz. Unless otherwise noted, all spectroscopic measurments were made at 31±1 °C. Solvents (CCl_4, $CHCl_3$, and CH_3OH) used in absorption studies were spectral grade and were purchased from Tokyo Kasei Co., Tokyo. Perdeuterated dimethyl sulfoxide (DMSO-d_6), CD_3OD, and $CDCl_3$ were purchased from Merck Sharp & Dohme Canada, Ltd.

^{13}C NMR signals of the peptide portion of compounds **1**—**4** were assigned by comparisons with the spectra of their fragment peptides and the monomers.[28] The signals of the retinylidene portion were easily assigned by comparisons with the spectrum of NRB.[27,29] ^{1}H NMR resonances were assigned on the basis of their multiplet structure, selective homo-spin decoupling and a comparison with the literatures.[30,31]

The calculation was carried out with a HITAC M-200H computer system.

Results and Discussion

Identification of Retinylidenelysyl Peptides 1—4 by ^{13}C NMR and UV Spectroscopy. Figure 1 shows the ^{13}C NMR spectra of compund **1** together with that of NRB in CD_3OD. The signals corresponding to the peptide carbons (except the C_ε of Lys) were assigned by comparisons with the spectrum of compound **1'**, Boc-Lys-Phe-Tyr-OMe, under the assumption that the chemical shifts of these carbons are little changed by a Schiff base formation. The other signals were assigned by comparisons with the spectrum of NRB. As shown in Fig. 2, the signals corresponding to C_{10} and C_5 overlap with those of the $C_{2,6}$ of Tyr and Phe, respectively. There are no signals suggesting to the free retinal nor the C_ε of Lys having a free ε-amino group. This fact indicates the complete conversion of retinal to the Schiff base form. The chemical shifts of the retinylidene portion are in good agreement with those of the corresponding carbons for *all-trans* NRB. Therefore, the polyene chain was identified as an all-trans configuration. Similar results were also obtained for compounds **2**—**4**. The

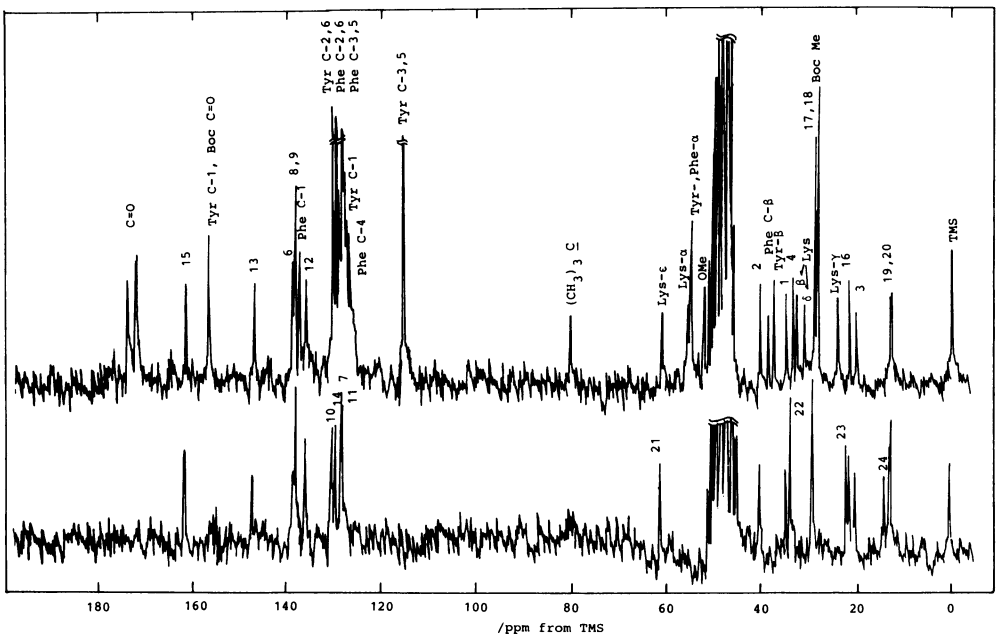


Fig. 1. ^{13}C NMR spectra of compound **1** and NRB at concd 0.1 mol dm^{-3} in CD_3OD.

peak assignments of compounds **1**—**4** are summarized in Table 1 together with those of compound **1′**, retinal and NRB.

Figure 3-a shows the absorption spectrum of compound **1** in methanol. The main peak is shifted to the blue by 15 nm compared with the *all-trans* retinal. This is consistent with the blue shift observed in the formation of NRB. The peak at 275 nm was assigned to the π-π^* transition of the aromatic rings by a comparison with the absorption spectrum of compound **1′**.

UV and ^{13}C NMR Spectra of the Retinylidene-lysyl Peptides in Low Dielectric Media. Table 2 summarizes the absorption maxima of compounds **1**—**4** and NRB in different solvents. In the halogenated solvents and in hexane, the absorption maxima of compounds **1** and **2** (which contain Tyr) were shifted to the red compared with other compounds. The concentration dependence of the absorption maximum of compound **1** was examined in carbon tetrachloride. There was no change in the band position in the observed concentration range(10^{-4}—10^{-5} mol dm^{-3}). Figure 4 shows the temperature dependence of the absorption maxima of compounds

Table 1. The Peak Assignments of Retinylidenelysyl Peptides **1**—**4**, *all-trans* NRB and *all-trans* Retinal in CD_3OD at 0.1 M[a)]

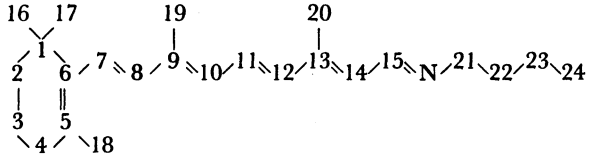
a. Retinylidene portion

Carbon No.[b)]	1	2	3	4	NRB[c)]	retinal[c)]
1	35.2	35.2	35.1	35.2	35.2	35.2
2	40.6	40.7	40.6	40.8	40.7	40.7
3	20.3	20.3	20.2	20.3	20.3	20.3
4	34.0	33.9	33.9	34.0	33.9	34.0
5	—[d)]	130.5			130.5	130.8
6	139.4	139.4	139.4	139.5	138.5	138.6
7	128.9	129.0	—[d)]	128.9	128.9	130.5
8	138.9	138.9	138.9	139.0	139.0	138.6
9	138.9	138.9	139.0	139.0	138.0	142.4
10	—[d)]	131.2	131.1	131.2	131.2	130.8
11	128.7	128.7	128.6	128.7	128.7	134.3
12	136.8	136.7	136.7	136.8	136.8	135.7
13	147.6	147.7	147.7	147.7	147.6	157.9
14	130.1	130.0	—[d)]	—[d)]	130.0	129.7
15	162.4	162.4	162.3	162.4	162.2	193.2
16	29.4	29.4	29.4	29.4	29.4	29.4
17	29.4	29.4	29.4	29.4	29.4	29.4
18	22.0	22.0	21.9	22.0	22.0	22.0
19[e)]	12.9	12.9	12.9	12.9	12.9	13.0
20[e)]	13.2	13.2	13.1	13.1	13.1	13.2
21					61.5	
22					33.9	
23					21.3	
24					14.1	

b. Peptide portion

Residue	atom	1	2	3	4	1′[f)]
Lys	α	56.0	56.3	56.2	55.9	55.5
	β	31.5	31.4	31.4	31.5	30.4
	γ	24.5	24.5	24.3	24.4	24.0
	δ	33.1	32.9	33.0	33.2	32.9
	ε	61.5	61.5	61.4	61.5	41.4
Phe	α	55.4		55.3[g)]	54.9	55.5
	β	39.1		38.9	38.4	39.0
				38.4		
	C-1	138.0		137.9	137.9	138.0
				137.7		
	C-4	127.6		127.8	127.8	127.7
				127.6		
	C-2, 6	130.4		130.1[g)]	130.3	130.4
	C-3, 5	129.3		129.3[g)]	129.4	129.4
Tyr	α	55.4	55.8			55.5
	β	37.7	37.9			37.7
	C-1	128.2	128.5			128.4
	C-4	157.5	157.8			157.3
	C-2, 6	131.2	130.8			131.2
	C-3, 5	116.3	116.6			116.2
Gly	α		43.2			
	C=O	174.6	175.6	174.6	174.8	174.7
		173.0	173.6	172.8[g)]	173.1	173.0
		172.8	171.1			172.8
	Boc C=O	157.5	157.9	157.6	157.4	157.3
	$(CH_3)_3\underline{C}$	80.6	80.6	80.6	80.6	80.7
	Ester Me	52.5	52.5	52.6	52.6	52.6
	Boc Me	28.7	28.7	28.7	28.7	28.8

a) Chemical shifts were measured as downfield shifts from internal tetramethylsilane (TMS) and are expressed in terms of ppm. The estimated error in the chemical shift is less than 0.1 ppm. b) The numbering of the carbons is given as shown in the following.



c) The assignments of these compounds were taken from Ref. 27 and 29. d) Overlapping with the peak of the aromatic carbons. e) The assignments to these carbons are tentative each other. f) Overlapping peak corresponding to two carbons in different residues.

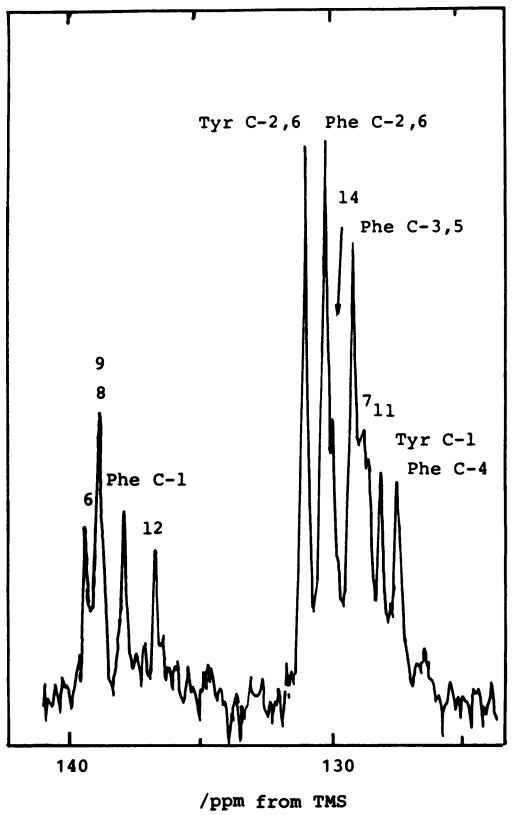


Fig. 2. Expanded spectrum of the 125—140 ppm region of compound **1**.

Table 2. Absorption Maxima (nm) of Retinylidenelysyl Peptides **1**—**4** and NRB in Different Solvents

Solvent	1	2	3	4	NRB
Carbon tetrachloride	375	370	368	363	365
Chloroform	375	372	367	366	367
Dichloromethane	375	370	366	365	363
Hexane	368	365	357	358	355
Methanol	365	364	365	364	365

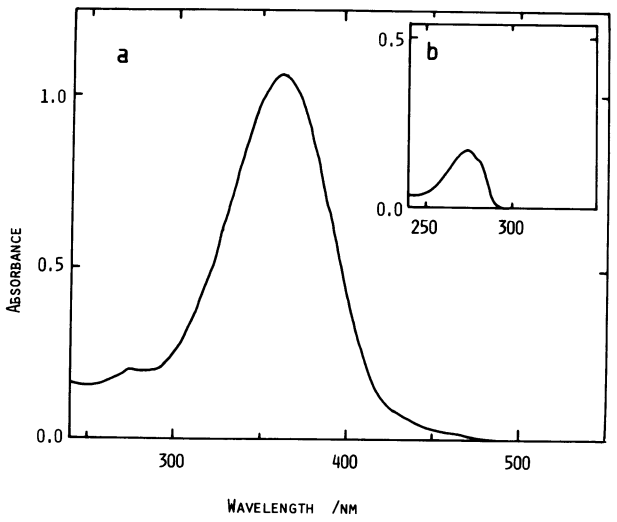


Fig. 3. (a) Absorption spectrum of compound **1** in methanol. (b) Absorption spectrum of the peptide, Boc-Lys-Phe-Tyr-OMe.

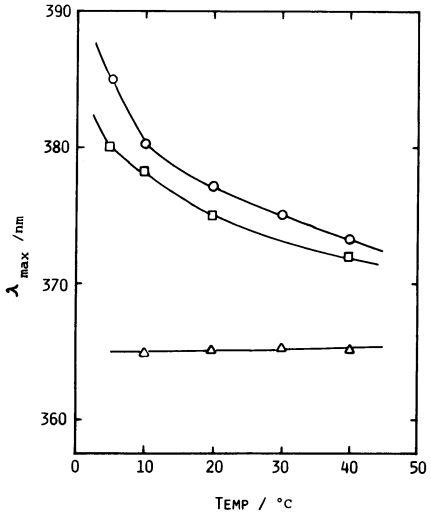


Fig. 4. Temperature dependence of the absorption maxima of compounds **1**, **2**, and **4** in carbon tetrachloride. ○: Compound **1**, □: compound **2**, △: compound **4**.

1, **2**, and **4** in carbon tetrachloride. Upon lowering the temperature from 40 to 5°, the absorption maxima of compounds **1** and **2** shifted to the red by 10 nm, but no apparent shift was detected for compound **4**. These results clearly indicate an intramolecular interaction between the retinylidene chromophore and Tyr in compounds **1** and **2**.

This interaction is also supported by the difference in the chemical shifts between compound **1** and NRB. Figure 5-a shows the chemical-shift difference($\Delta\delta$) along the polyene chain going from NRB to compound **1** in carbon tetrachloride. The magnitude of the chemical-shift difference alternates between odd- and even-numbered carbons, and the amplitude of the alternation decreases in going from the terminal nitrogen to the β-ionylidene ring. This behavior of the chemical shifts suggests an increase in the electronic polarization of the C=N bond going from NRB to compound **1**. There may be an electron-withdrawing group or a positive charge near the Schiff base nitrogen.

The absorption spectrum of a mixture of NRB and the protected tyrosine (Boc-Tyr-OMe) in carbon tetrachloride was observed. No apparent shift was detected within an observed molar ratio (Boc-Tyr-OMe/NRB) of 1—1000, with the concentration of NRB being kept constant at 3×10^{-5} mol dm^{-3}. This means that the intermolecular interaction is less

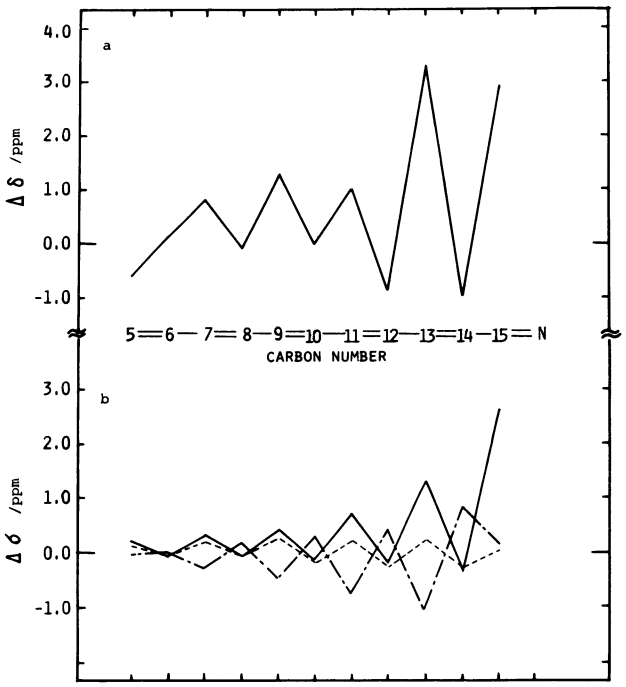


Fig. 5. Observed (a) and calculated (b) chemical shift differences of the polyene chain carbons going from the isolated Schiff base to each of the complexes as shown in Fig. 6.
(b) ——: Complex I, ——: complex II, ----: complex III.

important in a dilute solution, although a weak hydrogen-bond has been detected in more concentrated solutions (0.5 mol dm^{-3}) by IR spectra.[32] It is clear that in compounds **1** and **2** the peptide framework plays an essential role in the spatial arrangement of the tyrosine side chain to the Schiff base.

Theoretical Analysis of the UV and ^{13}C NMR Spectra of Retinylidenelysyl Peptides 1 and 2. CNDO/S calculations showed that the dipole moment of the unprotonated retinal Schiff base is directed perpendicular to the polyene axis on the polyene plane in the ground state. Bt upon excitation to the first excited state its direction becomes nearly parallel to the axis, and its magnitude increases from 2.7 to 3.7 D. Assming that a dipole-dipole type interaction is the main contributor to the spectral shift, this suggests that the π-π^* band of the Schiff base is effectively red-shifted by an electric dipole oriented antiparallel to the polyene axis. On the basis of CPK modeles of compounds **1** and **2**, three types of Tyr-Schiff base complexes were selected as association models. The geometrial configurations of these complexes are shown in Fig.6. Complexes I and II correspond to the models for a hydrogen-bond formation and stacking, respectively. In complex III, the C_1–O bond of the phenol ring is arranged parallel to the polyene axis. The phenol ring is coplanar to the polyene plane in complexes I and III.

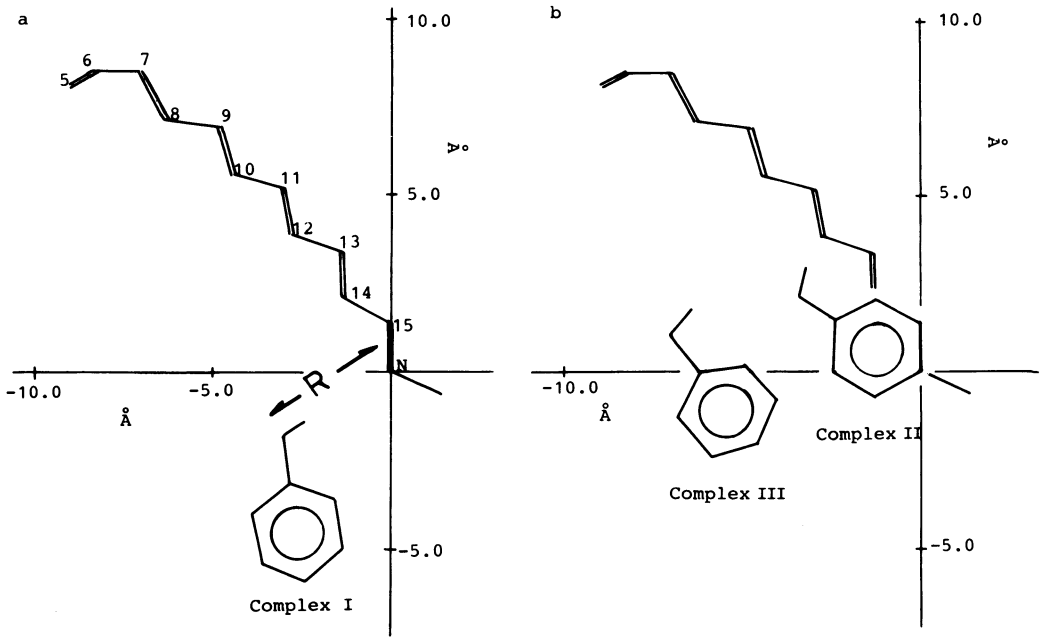


Fig. 6. The geometrical configurations of complexes between NUPM and the phenol ring assumed in the calculations.
(a) Complex I: hydrogen-bonding model. OH bond is placed on the extended line of the lone pair electron. (b) Complex II: stacking model. C_2, C_3, and C_4 of phenyl ring is placed 3 Å above C_{14}, C_{15} and N atoms, respectively. (c) Complex III: parallel model.
C_1–O bond of the phenol ring is arranged parallel to the polyene axis on the polyene plane.

Table 3. The Transition Energies, E_{tran}, of NUPM and the Complexes, and the Complex Formation Energies, ΔE_{compl}, in the Ground State

Compound	E_{tran}/eV(nm)	ΔE_{tran}/eV	ΔE_{compl}[c]/eV
NUPM	3.79(327.5)	0	—
Hydrogen bonding	3.73(331.6)	0.06	0.26
Stacking	3.69(335.7)	0.10	0.07
Parallel	3.78(328.3)	0.01	—
Compound **1**	3.31(375)[a]	0.09[b]	—

a) Observed in CCl_4 at room temperature. b) ΔE_{tran} was evaluated by subtracting E_{tran} of compound **1** from that of NRB. c) ΔE_{compl} means the energy change going from the isolated Tyr+isolated NUPM system to the complex. This was estimated by CNDO/2 method.

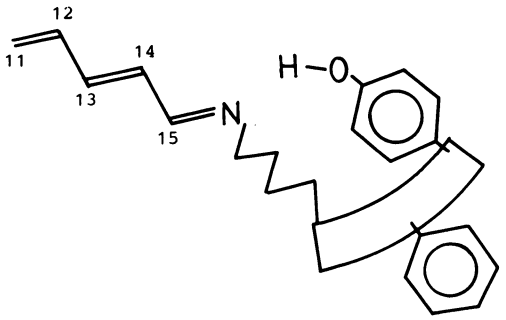


Fig. 7. Schematic representation of the overall conformation of compound **1** in low dielectric media based on CPK model considerations. The side chain of Tyr is oriented to the Schiff base. The steric hindrance about C_α–C_β bond of Phe is minimized.

Table 3 summarizes the transition energies (E_{tran}) of these complexes and NUPM, and also the complex formation energies (ΔE_{compl}) in the ground state. The complex formation energy is estimated as follows,

$$\Delta E_{compl} = E_s + E_t - E_{compl}$$

where E_s, E_t, and E_{compl} corresponds to the total energy of NUPM, the phenol ring and the respective complexes. The hydrogen bond length (R) and the O–H length were taken as 2.6 and 0.96 Å, respectively. The former value corresponds to the minimum energy structure in the ground state (determined by CNDO/2 calculations). In the other complexes, a van der Waals contact was assumed between the Schiff base and the phenol ring. The tyrosine+Schiff base system is stabilized by forming complex I or II. The transition energy decreases upon going from an isolated tyrosine (NUPM) to complex I or II. Figure 4-b shows the calculated chemical shift differences ($\Delta\delta$) going from NUPM to each of the complexes. The zigzag pattern (similar to Fig. 4-a) was reproduced in the formation of complex I. Consequently, the hydrogen-bonding model (complex I) is uniquely acceptable as an association model in compound **1**.

The hydrogen-bond length dependence was studied for the transition energy of complex I. Its values at R=2.8, 3.0 and 3.6 Å are 3.76, 3.77, and 3.79 eV, respectively. The transition energy rapidly approaches the value at an infinite bond length (3.79 eV). This explains the highly sensitive blue-shift of the absorption maxima of compounds **1** and **2** with a rise in temperature (Fig. 3). The second amino acid, Phe, of compound **1** is more bulky relative to Gly of compound **2**. The difference in the hydrogen-bond length, depending on the peptide framework, may induce a slight difference in the absorption maxima between these compounds.

NRB shows an absorption maximum at 376 nm in 2-chloroethanol,[33] which is a weaker hydrogen-bonding molecule (pK_a=14.3) relative to the tyrosine side chain (pK_a=11.1). Considering the case of NRB, a lower limit in the hydrogen-bond shift induced by a tyrosine side chain can be estimated as about 10 nm, according to the temperature dependence of the absorption maxima of compounds **1** and **2**.

Figure 7 shows a schematic representation of the overall conformation of compound **1** as predicted from the CPK model under the assumption of Tyr-Schiff base hydrogen bonding. The peptide backbone is folded to form the hydrogen bond. The side chain of Phe is exposed to the solvents.

The effect of a π-charge transfer interaction was examined in the above hydrogen-bonding system. A total charge transfer of 0.08 was found from the polyene part to the phenol ring in both the ground state and the first excited state, but the total π-charge density on the phenol ring was kept constant during hydrogen-bond formation. This means that the effect of a π-charge-transfer interaction is negligible in this system. This is in agreement with a report of Komatsu and Suzuki.[34] It seems that the hydrogen-bonding shift is chiefly induced by a dipole-dipole type of interaction between the Schiff base and Tyr.

Conformational Analysis of Retinylidenelysyl Peptide 1 by ^{1}H NMR. Table 4 summarizes the observed vicinal spin–spin coupling constants between α- and β-protons and the rotamer populations of compounds **1** and **1′**. Figure 8 shows the Newman projection of three staggered rotamers about the C_α–C_β bond of the side chain. Fractional populations P_I, P_{II}, and P_{III} were estimated by the following equations:

$$P_I = (J_{\alpha\beta A} - J_g)/(J_t - J_g) \quad (1)$$

$$P_{II} = (J_{\alpha\beta B} - J_g)/(J_t - J_g) \quad (2)$$

$$P_{III} = 1 - (P_I + P_{II}), \quad (3)$$

where $J_{\alpha\beta A(or\ B)}$ represents the observed coupling

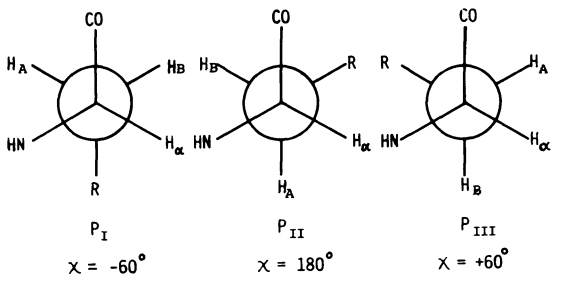


Fig. 8. Three stable rotamers about C_α–C_β bond and their notations.

Table 4. Proton Coupling Constants (Hz) and Rotamer Populations of Compounds **1** and **1′**

Compound	Residue	$J_{\alpha\beta A}$	$J_{\alpha\beta B}$	P_I	P_{II}	P_{III}
1′ a)	Phe	8.08	5.84	0.50	0.30	0.20
	Tyr	9.04	4.88	0.59	0.21	0.20
1′ b)	Phe	8.68	5.88	0.56	0.30	0.15
	Tyr	7.36	5.36	0.43	0.25	0.31
1 b)	Phe	6.60	6.00	0.37	0.31	0.33
	Tyr	8.04	5.88	0.50	0.30	0.21

a) Observed in DMSO-d_6. b) Observed in $CDCl_3$: DMSO-d_6=9 : 1.

constant between α- and βA (or βB)-protons, and J_t and J_g are the values of $^3J_{\alpha\beta}$ for the protons whose relative conformations are trans and gauche, respectively. The values of J_t and J_g are taken from Pachler's report:[35] J_t=13.56 Hz and J_g=2.60 Hz.

The relative rotamer populations of Phe in compound **1′** are almost independent of the solvent polarity. Accompanied by the formation of the Schiff base linkage, P_I decreases along with an increase in P_{III}, while P_{II} is kept constant. Consequently, three rotamers are equally populated in compound **1** in less polar solvents. This means that the side chain of Phe is randomly interconverted among the three rotamers during equilibrium. On the other hand, the rotamer population about the C_α-C_β bond of Tyr is non-random in compound **1**, with a prefered orientation of rotamer I. It seems that the conformational stability of each side chain based on a $^3J_{\alpha\beta}$ analysis is consistent with that predicted from the spatial arrangement of the side chains (Fig. 7).

In conclusion, a proximal tyrosine residue induces the red shift of the π-π^* band of an unprotonated retinal Schiff base through the hydrogen bond in a lower dielectric media. This hydrogen-bonding shift is estimated to be no less than 10 nm. The temperature coefficient, $d\lambda_{max}/dT$, was considerably large in the artificial pigments used. The hydrogen-bonding shift reached 20 nm at 5 °C. The absorption maximum of the M_{412} intermediate was shifted to the red by 47 nm compared with that of NRB in carbon tetrachloride at 30 °C. A significant part of the red shift in the M_{412} could be explained by the hydrogen-bond formation between the Schiff base and a proximal tyrosine.

The authors wish to express their thanks to Professor Isao Ando of Tokyo Institute of Technology for the use of the CNDO/2 program and his helpful discussions. We also would like to thank Dr. Shigetoshi Amiya of the Central Research Laboratories of Kuraray, Co. Ltd., Japan, for ^{1}H NMR spectra. We thank the Computer Center, Institute for Molecular Science, for the use of the HITAC M-200H computer.

References

1) W. Stoekenius and R. Bogomolni, *Annu. Rev. Biochem.*, **51**, 587 (1982).
2) H. D. Lemke and D. Oesterhelt, *FEBS Lett.*, **128**, 255 (1981).
3) H. Bayley, K. S. Huang, R. Radhakrishnan, A. H. Ross, Y. Takagi, and H. G. Khorana, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 2226 (1981).
4) M. A. Marcus, A. Lewis, *Science*, **195**, 1328 (1977).
5) A. Lewis, J. Spoonhower, R. A. Bogomolni, R. H. Lozier, and W. Stoeckenius, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 4462 (1974).
6) M. Braiman and R. Mathies, *Biochemistry*, **19**, 5412 (1980).
7) K. Bagley, G. Dollinger, L. Einstein, K. Singh, and L. Zimanyi, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 4972 (1982).
8) T. Konishi and L. Packer, *FEBS Lett.*, **92**, 1 (1978).
9) H. D. Lemke and D. Oesterhelt, *Eur. J. Biochem.*, **115**, 595 (1981).
10) T. Iwasa, T. Takeda, F. Tokunaga, P. S. Scherrer, and L. Packer, *Biosci, Rep.*, **2**, 949 (1982).
11) O. Kalisky, M. Ottolenghi, B. Honig, and R. Korenstein, *Biochemistry*, **17**, 1037 (1981).
12) V. Rosenbach, R, Goldberg, C. Gilon, and M. Ottolenghi, *Photochem. Photobiol.*, **36**, 197 (1982).
13) J. M. Fukumoto, J. H. Hanamoto, and M. A. EL-Sayed, *Photochem. Photobiol.*, **39**, 75 (1984).
14) J. H. Hanamoto, P. Dupus, and M. A. EL-Sayed, *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 7083(1984).
15) D. Engelman, R. Henderson , A. D. Maclachlan, and B. A. Wallace, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 2023 (1980).
16) P. K. Das, R. S. Becker, D. Hannck, and E. Bayer, *J. Am. Chem. Soc.*, **101**, 239 (1979).
17) M. Engelhard and B. Hess, Proc. 7th American Peptide Symp. Univ. Wisconsin, June 14 (**1981**).
18) E. K. S. Vijayakuman and P. Balaram, *Photochem. Photobiol.*, **39**, 667 (1984).
19) M. Karplus and J. A. Pople, *J. Chem. Phys.*, **38**, 2803 (1963).
20) J. D. Bene and H. H. Jaffé, *J. Chem. Phys.*, **48**, 1807 (1968).
21) M. Sakurai, I. Ando, Y. Inoue, and R. Chûjô, *Photochem. Photobiol.*, **34**, 367 (1981).
22) R. L. Ellis, G. K. Kuehnlenz, and H. H. Jaffé, *Theoret.*

Chim. Acta, **26**, 131 (1972).
23) P. Jacques, J. Faure, O. Chalvet, and H. H. Jaffé, *J. Phys. Chem.,* **85**, 473 (1981).
24) R. R. Birge, J. A. Bennett, L. M. Hubburd, H. L. Fang, B. M. Pierce, D. S. Kliger, and J. E. Leroi, *J. Am. Chem. Soc.,* **104**, 2519 (1982).
25) P. Tavan, K. Shulten, and D. Oesterhelt, *Biophys. J.,* **47**, 415 (1985).
26) T. Hamanaka and T. Mistui, the 12 th Meeting of the Biophysical Society of Japan, Tokyo, Japan, Preprints, **1973**, 81.
27) Y. Inoue, Y. Tokito, R. Chûjô, and Y. Miyoshi, *J. Am. Chem. Soc.,* **99**, 5592 (1977).
28) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York (**1972**), Chap. 11.
29) Y. Inoue, Y. Tokito, S. Tomonoh, and R. Chûjô, *Bull. Chem. Soc. Jpn.,* **52**, 265 (1979).
30) J. Kobayashi, U. Nagai, T. Higashijima, and T. Miyazawa, *Biochim. Biophys. Acta,* **577**, 195 (1980).
31) J. Kobayashi, T. Higashijima, U. Nagai, and T. Miyazawa, *Biochem. Biophys. Acta,* **621**, 190 (1980).
32) P. P. Rastogi and G. Zundel, *Biochem. Biophys. Res. Commun.,* **99**, 804 (1981).
33) T. Suzuki and Y. Kito, *Photochem. Phtobiol.,* **15**, 275 (1972).
34) T. Komatsu and H. Suzuki, *J. Phys. Soc.Jpn.,* **40**, 1725 (1976).
35) K. G. R. Pachler, *Spectrochm. Acta,* **20**, 581 (1964).