Models for Visual Pigments. The Effect of the Imidazolyl Group on the Absorption Maxima of the Retinal Schiff Base

Masato NANASAWA* and Hiroyoshi KAMOGAWA
Faculty of Engineering, Yamanashi University, Takeda, Kofu 400
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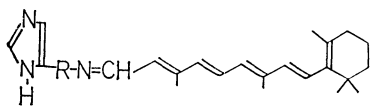
The title effect has been investigated with the imidazolyl group either externally added or intramolecularly combined. *N*-Retinylidenebutylamine was protonated with imidazole derivatives under neutral conditions and was absorbed at longer wavelengths compared with carboxylic acids. The absorption peak with the imidazolyl group intramolecularly combined was highly affected by the structure, protonated N^{α}-retinylidene-L-histidine octadecylamide having an absorption maximum at 494 nm caused by the inductive effect and the polar imidazolyl group.

The absorption maxima of visual pigments bound to opsin by a Schiff-base linkage are observed at 500 nm, although the protonated products of synthetic analogues absorb at approximately 450 nm. As regards this bathochromic shift, a large number of interesting observations and theories have been reported: the polarizability of the micro environment,[1] the electrostatic effect of polar or charged groups in the vicinity of the chromophore,[2] and the twist or distortion of the π-conjugated system.[3] However, there are still many unsolved problems. Recently two synthetic models were reported which absorb at longer wavelengths by means of protonation with trifluoroacetic acid[4] or in an aqueous hydrochloric acid solution.[5] Since native photochromic processes proceed under mild conditions, we examined the effects of amino acids as protonating agents.[6] In this paper we intend to deal especially with the imidazolyl group of the histidine residue. The imidazolyl group is known to be responsible for a buffering capacity;[7] hence, it may be able to protonate the Schiff-base linkage in a physiological pH range and to mediate a charge relay system between protonated Schiff-base nitrogens and counter anions. On the basis of these assumptions, we have examined the protonation of *N*-retinylidenebutylamine(Bu–SB) with the salt of N^{α}-benzyloxycarbonyl-L-histidine butylamide (**1**) plus *N*-benzyloxycarbonyl-L-alanine (Z–α-Ala), N^{α}-octadecanoyl-L-histidine (**2**), and 4,5-imidazoledicarboxylic acid monohexadecyl ester (**3**). As Schiff-base models with the imidazolyl group, the following were synthesized, and the absorption maxima of the protonated products were determined:



R = $-CH_2-$: **4**–SB. $-CH_2CH_2-$: **5**–SB.
$-CH_2\underset{|}{C}H-CONH-C_{18}H_{37}$: **6**–SB.
$-CH_2CH_2NHCOCH_2CH_2-$: **7**–SB.
$-CH_2CH_2-NHCO(CH_2)_5-$: **8**–SB.

Experimental

all-trans-Retinal was prepared by the oxidation of vitamin-A with MnO_2 and was recrystallized from petroleum ether;[8] mp 59—60 °C, λ_{max} 367.5, nm, E_1 % 1536 in petroleum ether. Isomerization to the *cis*-isomer was conducted by the irradiation of *all-trans*-retinal (1% in petroleum ether) containing iodine (1% to retinal), using a 150 W xenon lamp with a UV filter fitted as the light source;[9] λ_{max} 364.5 nm, $E_{1\%}$ 1259 in petroleum ether.

Preparation of N^{α}*-Benzyloxycarbonyl-L-histidinamide*: A mixture of N^{α},N^{im}-bis(benzyloxycarbonyl)-L-histidine(Z–His–Z 6.85 g, 15.5 mmol, the benzyloxycarbonyl group was abbreviated as Z) and dicyclohexylcarbodiimide(DCC 3.5 g, 17 mmol) in CH_2Cl_2 (50 ml) was stirred in an ice bath for 45 min, filtered, and washed with CH_2Cl_2. To the filtrate, alkylamine (31 mmol) in $CHCl_3$ (50 ml) was added, the mixture being stirred in an ice bath, allowed to stand overnight, and then refluxed for 30 min. While hot, the reaction mixture was filtered and washed with $CHCl_3$. The filtrate was dried over Na_2SO_4, concentrated to about 30 ml, and then cooled at −20 °C. The product thus precipitated was recrystallized from acetone or ethanol. **1**: yield, 62% mp; 143—145 °C. IR (KBr): 3300 (NH), 3150 (imidazole–NH), 1690 (carbamate C=O), 1650 cm^{-1} (amide C=O). ^{1}H-NMR ($CDCl_3$): 0.9—1.5 (m, 7H, $CH_3CH_2CH_2-$), 3.0 (m, 4H NH–$C\underline{H}_2-$ and imi–CH_2-), 4.3 (q, 1H, α-CH), 5.2 (s, 2H, phenyl–$C\underline{H}_2-$), 6.9, 7.8 (s, 2H, imidazole ring), 7.3 ppm (8H, phenyl, NH). Found: C, 62.68; H, 7.32; N, 16.01%. Calcd for $C_{18}H_{23}N_4O_3$: C, 62.97; H, 6.31; N, 16.33%. MS (*m/e*): 343 (M^+, 11%). N^{α}-Benzyloxycarbonyl-L-histidine octadecylamide: yield, 36%; mp 151—154 °C.

N^{α}-Octadecanoyl-L-histidine (**2**) was prepared with octadecanoyl chloride; yield, 45%; mp 165—180 °C.[10] The 4,5-imidazoledicarboxylic acid monohexadecyl ester (**3**) was obtained by condensation with 4,5-bis(chloroformyl)imidazole[11] and hexadecyl alcohol; yield, 71%; mp 167—170 °C. In the IR of the salt containing **1** and Z–α-Ala (mp 125—126 °C), the absorption at 1710 cm^{-1} (COOH) no longer exists, and the salt peak is observed at 2000—2750 cm^{-1}. ^{1}H-NMR and elemental analyses made it posisble to identify the structure. 4-(Aminomethyl)imidazole (**4**) was obtained starting with D-fructose *via* 4-(hydroxymethyl)imidazole, oxidation with HNO_3 to aldehyde, conversion to oxime, and reduction with H_2 (5% Pd on charcoal) in methanol saturated with HCl.[12]

L-Histidine Octadecylamide (6): To N^{α}-Z–histidine octadecylamide (2.35 g, 4.4 mmol) in anhydrous acetic acid (5.0 ml), a 30% solution of hydrogen bromide in acetic acid (5.7 ml) was added with vigorous stirring at room temperature. The reaction mixture was stirred for 1 h and then poured into anhydrous ether (100 ml). **6**·HBr was filtered, thoroughly washed with ether, and then dissolved in ethanol (10 ml). The solution was basified with saturated aqueous Na_2CO_3. The product was extracted with ethanol and recrystallized from ethanol–ether, **6**: yield, 42%; mp 110—115 °C. Found: C, 70.79; H, 11.53; N, 13.73%. Calcd for $C_{24}H_{40}N_4O$: C,

70.93; H, 11.33; N, 13.79%.

Preparation of N-*(ω-Aminoalkanoyl)histamine (7, 8)*: A mixture of Z-β-Ala (3.94 g, 17.7 mmol) and DCC (4 g, 19.5 mmol) in a 1 : 2 mixture of dimethylformamide–acetonitrile (20 ml) was stirred in an ice bath for 45 min; the reaction mixture was then filtered and washed with dimethylformamide–acetonitrile (30 ml). To the filtrate, histamine dihydrochloride (3 g, 16.1 mmol) and NaOH (0.7 g) in H_2O (15 ml) were added in one portion; the mixture was vigorously stirred for 5 h, and then filtered. The filtrate was concentrated to about 40 ml and poured into cold 10^{-1} M hydrochloric acid (250 ml, 1 M= 1 mol dm^{-3}). The insoluble by-product (DCC urea, Z-β-Ala–DCC) was filtered off. The filtrate was basified with saturated aqueous Na_2CO_3 and cooled in a refrigerator overnight. The product thus precipitated was filtered, washed with H_2O, and then recrystallized from ethanol–ethyl acetate. *N*-(*N*-Benzyloxycarbonyl-β-alanyl)histamine: yield, 62%; mp 137—139 °C. *N*-[ω-(Benzyloxyamino)hexanoyl]-histamine: yield, 82%; mp 111—114 °C. Deblocking of the ω-amino group were conducted in a manner similar to that used for **6**. **7**: yield, 52%; mp 67—68 °C, Found: C, 52.89; H, 8.38; N, 29.94%. Calcd for $C_8H_{14}N_4O$: C, 52.75; H, 7.69: N, 30.77%. **8**: yield, 67%; mp 44—46 °C. Found: C, 59.04; H, 9.50; N, 24.32%. Calcd for $C_{11}H_{20}N_4O$: C, 58.93; H, 8.93; N, 25.00%.

The Schiff base was prepared by mixing solution of retinal (10^{-2} M) and imidazole derivatives (10^{-2} M) in absolute ethanol containing 3A molecular sive 1/16 in. pellets at room temperature for 8 h in the dark. In the case of **4** and **6**, the reaction time was prolonged to 24 h. The absorption spectra were recorded on a Hitachi 200-10 spectrophotometer, using quartz cells 1 mm and 10 mm in path length. The pH of the protonating solution (90% aqueous ethanol) was measured on a Horiba M-7E pH meter. The calibration curve for the protonated *N*-retinylidenebutylamine (Bu–SBH$^+$) satisfied the Lambert Law over the concentration range from 1.4×10^{-5} to 2.2×10^{-4} M.

Results and Discussion

Effect of Imidazole Derivatives on the Protonation of N-*Retinylidenebutylamine (Bu–SB).* Figure 1 shows the degree of protonation plotted against the pH values of the solution, in which the pH values were controlled by the addition of Z-α-Ala (10^{-2} M) to **1** (2.5×10^{-3} M) in 90% aqueous ethanol. The basic nitrogen of imidazole ring (N^3, pK_a 14.5) is, of course, neutralized with Z-α-Ala in a polar solvent, and some association may take place between **1** and Z-α-Ala. However, the absorption peak at 440 nm appeared even under neutral conditions, which indicates that undissociated imine hydrogen

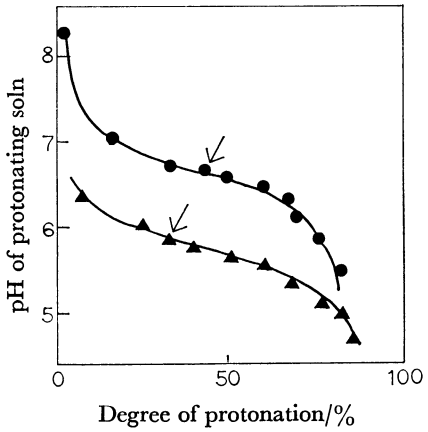


Fig. 1. Protonation of Bu–SB with amino acids.
●: With a mixture of **1** and Z-α-Ala, ▲: with Z-α-Ala only.
Bu–SB, 5×10^{-4} M. **1**, 2.5×10^{-3} M. Solvent, 90% aqueous ethanol. The arrows indicated the equimolar addition of Z-α-Ala to Bu–SB.

(N^1–H of imidazole ring) protonated the Schiff-base linkage.

In a nonpolar solvent such as chloroform, in which carboxylic acids with no steric hindrance at the α-position (Z-β-Ala, hexanoic acid) remain almost in undissociated or dimeric state, the extent of protonation increased upon the addition of **1** (5×10^{-3} M) to the

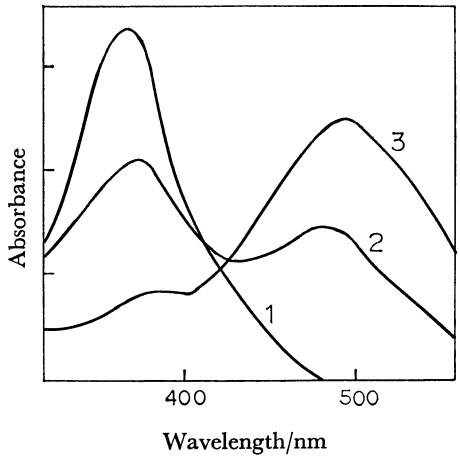


Fig. 2. Absorption spectra of **6**–SB
1: **6**–SB, 2: **6**–SBH$^+$, 3: **6**–SBH^{2+}. Concentration, 3.3×10^{-5} M; solvent, chloroform.

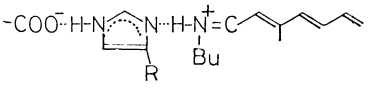
Table 1. Absorption maxima of imidazole derivatives

	λ_{max}/nm	SBH$^+$ a)			SBH$_2^{2+}$ b)		
		λ_{max}/nm	$\Delta\lambda$ c)/nm	Absorbance	λ_{max}/nm	$\Delta\lambda$ c)/nm	Absorbance
4–SB	368	468	100	0.458	482	114	0.695
5–SB	366	467.5	101.5	0.595	479	113	0.747
6–SB	370	482	112	0.305	494	124	0.651
7–SB	367	464	97	0.505	470.5	103.5	0.779
8–SB	367	464	97	0.577	466.5	99.5	0.790
Bu–SB	366	460	94	0.848			

a) Monoprotonated Schiff base. b) Diprotonated Schiff base. c) The magnitude of shift against the Schiff base. Concentration, 3.3×10^{-5}M; solvent, chloroform.

mixture of Bu–SB (5×10^{-4} M) and Z–β-Ala (2.5×10^{-3} M). It is possible that the proton transfer from undissociated or dimeric carboxylic acid to **1** took place and that the resulting positive charge at the imidazole ring promoted the formation of Bu–SBH$^+$.

The protonation with **1** did not appear in ethanol or even in its aqueous solution (N^1–H, pK_a 7.2), whereas in chloroform a small absorption appeared at about 470 nm. The hydrogen bond at N^3 with a solvent presumably aided proton release at the N^1 of the imidazole ring. With **2** and **3** existing as an ion pair in chloroform, the absorption peaks were slightly shifted to longer wavelengths (**2**, 455 nm, **3**, 458 nm) than that with Z–α-Ala (450 nm). The red shift might be explained by Scheme 1, in which electrostatic energy[13] between the counter anion and the positive charge on the nitrogen of the Schiff-base linkage is decreased by the charge-relay system.

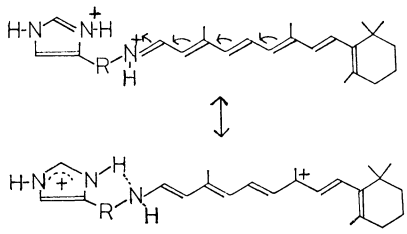


Scheme 1.

Absorption Maxima of the Protonated Schiff Base of Imidazole Derivatives. The 11-*cis*-retinal of rhodopsin is bound to an ε-amino group of a lynsine, and the bathochromic shift is caused by the placement of amino-acid residues near the protonated chromophore.[2] Schiff bases (**4**–SB—**8**–SB) with different distances between the Schiff-base linkage and the imidazole ring were synthesized in order to investigate the effect of the neighboring group on the absorption spectra. An attempt to synthesize a Schiff-base model with the least distance, such as a 4-aminoimidazole derivative, was unsuccessful because of the weak basicity of the amino group attached to the imidazole ring. It required 24 h to reach the constant absorption maxima of **4**–SB and **6**–SB after mixing, and their absorption peaks were slightly shifted to longer wavelengths compared with others; this shift was caused by the presence of an electron-withdrawing group at the α-position.[14] With one or two equivalents of Z–α-Ala, a protonated Schiff base did not indicate any absorption peaks, but only shoulders. With the intention of obtaining more obvious results, the protonation was conducted by the addition of an ethanolic solution containing one or two equivalents of hydrochloric acid to a Schiff base (2.5×10^{-2} M). Mono- and diprotonated Schiff bases were represented by SBH$^+$ and SBH^{2+} respectively; the pH values were 6.4 and 4.5 respectively in aqueous ethanol. The absorption maximum of the protonated *N*-retinylidene-L-alanine butylamide shifted to the red by approximately 106 nm, the magnitude of the shift against Bu–SBH$^+$ being large compared with that of the unprotonated product (SB: 371—366=5 nm, SBH$^+$: 477—460=17 nm). It seems reasonable to say that the carbonyl group at the α-position enhanced the electron-withdrawing character of the Schiff-base nitrogen.

The absorbance obtained by the addition of equimolar acid components was very small in the case of a Schiff base prepared with alkanediamines with short chains (ethylene, trimethylene) compared with that of imidazole derivatives. This denotes that the proton transfer from a Schiff-base linkage to primary amine might proceed, while in the latter case the transfer from the amphoteric imidazole ring is predominant. The red shifts caused by the neutral imidazolyl group (**4**–SBH$^+$, **5**–SBH$^+$) were approximately 7 nm compared with the absorption maximum of Bu–SBH$^+$, the magnitude of which was almost similar to that of Bu–SB with **2** and **3** externally added.

When the amount of acid was increased, the red shift was highly affected by the structure: shorter distances between two protonated sites might increase the repulsion between two cations (Scheme 2), thus bringing about an extrusion of the positive charge on the Schiff-base nitrogen to the ionane ring. The interaction of separated ions as observed in the absorption maxima was calculated over 8 Å on the basis of a molecular orbital theory.[15] The results of this calculation agree with those of our experiment, in which the maximum length affecting the red shift was six methylene chains (hexamethylenediamine–SBH$_2^{2+}$, 463 nm). The shifts caused by the protonated imidazole rings were larger than those of the corresponding model compounds, ethylenediamine–SBH$_2^{2+}$ (470 nm) and trimethylenediamine–SBH$_2^{2+}$ (468 nm).



Scheme 2.

The magnitude of shift against the absorption maximum of Bu–SBH$^+$ was 19 nm in terms of the influence of the protonated imidazole ring and 12 nm in terms of the inductive effect of the α-carbonyl group, the large shift of **6**–SBH^{2+} being explicable by adding the two values.

When a *cis*-isomerized retinal was bound to the imidazole derivatives, some interaction could be postulated between rings (β-ionane–imidazole). However, the absorption maxima of the protonated products were almost consistent with those of the *all-trans*-retinal. In actual visual pigments, the twist or distortion of the retinal moiety and a complicating structure of opsin might cause the red shift up to 580 nm. It is experimentally impossible to make a highly selective and restrictive model until the complete structure is known. We have, therefore, synthesized simple models simulating visual pigments, in which the absorption spectra shifted toward longer wavelengths close to that of rhodopsin.

References

1) C. S. Ivring, G. W. Bayers, and P. A. Leermarers, *J. Am. Chem. Soc.*, **91**, 2141 (1969).

2) B. Honig, A. D. Greenberg, U. Dinur, and T. G.

Ebrey, *Biochemistry*, **21**, 4593 (1976).
3) P. Blatz and P. Liebman, *Expt. Eye Res.*, **17**, 573 (1973).
4) P. K. Das, R. S. Becker, D. Hannak, and E. Bayer, *J. Am. Chem. Soc.*, **101**, 239 (1979).
5) I. Tabushi, Y. Kuroda, and K. Shimokawa, *J. Am. Chem. Soc.*, **101**, 4759 (1979).
6) M. Nanasawa and H. Kamogawa, *Bull. Chem. Soc. Jpn.*, **52**, 1727 (1979).
7) T. Wieland and G. Schneidel, *Ann. Chem.*, **580**, 159 (1953).
8) C. D. Robeson, W. P. Blum, J. M. Dietele, J. D. Cawley, and J. G. Baxter, *J. Am. Chem. Soc.*, **77**, 4120 (1955).
9) R. Hubbard, *J. Am. Chem. Soc.*, **78**, 4662 (1956).
10) G. Gilter, *J. Am. Chem. Soc.*, **90**, 5004 (1968).
11) For a related procedure, see L. F. Fieser and M. Fieser for Organic Synthesis I," John Wiley & Sons, New York (1967), p. 286.
12) R. A. Turner, *J. Am. Chem. Soc.*, **71**, 2801 (1949).
13) P. E. Blatz, J. H. Mohler, and H. V. Navangul, *Biochemistry*, **11**, 848 (1972).
14) B. Rosenberg and T. M. Krigas, *Photochem. Photobiol.*, **6**, 769 (1967).
15) H. Suzuki, T. Komatzu, and H. Kitajima, *J. Phys. Soc. Jpn.*, **37**, 177 (1974).