Preparation of Model Polymers for Visual Pigment and Spectral Changes

Masato NANASAWA* and Hiroyoshi KAMOGAWA
Faculty of Engineering, Yamanashi University, Takeda, Kofu 400
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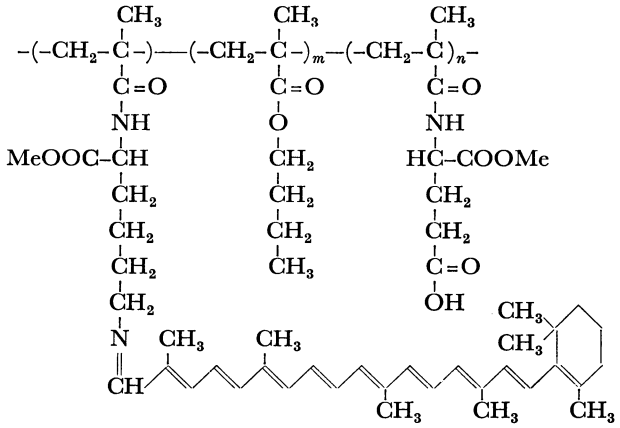
N-(8′-Apocarotene-8′-ylidene)butylamine (Bu-SB) has been protonated with amino acid derivatives in aqueous solution and the absorption maxima of the protonated products observed at 500 nm. Copolymers (from N^{α}-methacryloyl-L-lysine methyl ester and α-methyl *N*-methacryloyl-L-glutamate) bearing long conjugated double bonds as a side chain have been synthesized for the simulation of rhodopsin. The absorption spectra shifted to longer wavelengths, and the absorbances amounted to 4—8%, based on the maximum peaks.

The literature is extensive concerning the photochromic process in visual pigments and a number of interesting observations and results have been reported.[1] Nevertheless the mechanism remains unclarified. Absorption maxima (λ_{max}) of pigments bonded to opsin by Schiff base (SB) linkages are observed at approximately 500 nm, although the 11-*cis*-retinylidene analogue in solution absorbs in the ultraviolet. The bathochromic shift was thought due to the protonation of the SB linkage,[2] this hypothesis being qualitatively supported by indirect methods and theory.

The absorption spectra of long pendant conjugated chains attached to peptide backbones was reported to shift approximately 120 nm to longer wavelengths by the addition of acceptors with strong electron affinities and small molecular sizes.[3] It is difficult to produce an experimental model identical with rhodopsin in which the absorption peak shifts reversibly to the red region, but the following models have been prepared with the intention of simulating a visual pigment and examining spectral changes: amino acid derivatives have been employed as protonating agents for low molecular SB, since the acid components reported to date were mineral acids[4] and organic acids possessing a strong proton transfer constant such as trichloroacetic acid;[5] other models have been vinyl polymers bearing N^{ϵ}-(8′-apocarotene-8′-ylidene)-L-lysine methyl esters and α-methyl L-glutamates as side chains. The spectral changes of the amino acid derivatives have been investigated.



m: 0—4, *n*: 0—2.

The methacryloyl chain has been employed as the polymer backbone in place of peptides, since poly(amino acid)s are insoluble in organic solvents by denaturation during cleavage of the protecting groups and subsequent purification, bringing about low yields

Table 1. Poly(vinyl amino acid)s and SB

	Poly-Ly	Poly-Ly-1	Copoly-1	Copoly-2	Copoly-3
Composition[a]					
Vinyl-Z-Ly(OMe)	1	1	1	1	1
Vinyl-Glu-OBzl(OMe)	0	0	1	1	2
BMA	0	4	0	4	4
Yield %	54.0	73.1	63.8	78.8	68.4
$[\eta]$ dl/g[b]	0.235	0.392	0.182	0.180	0.295
Content mM/g[c]					
$-NH_2$	3.32 (75.9)	1.07 (85.6)	1.85 (87.2)	0.65 (66.6)	0.37 (77.9)
-COOH			1.77 (83.2)	0.70 (72.7)	0.66 (69.8)
Spectra of SB					
λ_{max}	428	430		415	416
$E_{1\%}$	208	127		26.3	8.3
Amount of SB[d] mol%	38.0	44.4		11.0	6.1

a) Composition of monomer for polymerization. b) In DMF at 25 °C. c) Content of ω-group in polymer measured by titration with 0.1 M NaOH or 0.1 M HCl in THF–H_2O, figures in parentheses indicate calculated values in %. d) Calculated values from the absorptivity of Bu-SB (λ_{max} 440 nm, $E_{1\%}$ 826) and amino residue in polymer.

of SB and difficulties in measurement of the shift magnitude in a heterogeneous system. N^{α}-Methacryloyl-N^{ϵ}-benzyloxycarbonyl-L-lysine methyl ester [Vinyl-Z-Ly(OMe)] has been prepared by the esterification of Z-Ly with thionyl chloride–methanol,[6] followed by amidation with methacryloyl chloride. Attempts to esterify γ-benzyl L-glutamate by conventional means were unsuccessful due to ready hydrolysis and ester exchange. Consequently γ-benzyl α-methyl N-methacryloyl-L-glutamate [Vinyl-Glu-OBzl(OMe)] has been prepared by the amidation of Glu-OBzl in cold alkaline solution with methacryloyl chloride, followed by the esterification with ethereal diazomethane solution. In order to examine the effect of neighboring groups and increase in solubility of the polymers, the vinyl amino acid derivatives have been copolymerized with butyl methacrylate (BMA) in dioxane by conventional free radical polymerization. The protecting groups at the amino acid ω-position have been cleaved with hydrogen bromide.

Table 1 gives the polymers and the SB linkages synthesized. The ω-group contents are seen to be slightly lower than the calculated values due to the preferential polymerization of BMA and the presence of protecting groups. The copolymer of Vinyl-Glu (OMe) and Vinyl-Ly(OMe) which consisted of 1: 1 or 2: 1 molar ratios did not bond with 8′-apocarotene-8′-al presumably due to salt formation of the amino groups with neighboring carboxyl groups in the polymer molecule. It was reported that only 11-*cis*- and 9-*cis*-retinal combine with opsin to form pigments and that only the 11-*cis* pigment has an absorption spectrum identical with that of rhodopsin.[7] In this study, however, the effect of the isomers of carotenal was not examined and 8′-apocarotene-8′-al, which was readily obtained by the potassium permanganate oxidation of β-carotene[8] and put up resistance to oxidation, was used as a pigment.

The absorption maxima (λ_{max}) of Poly-SB shifted to shorter wavelengths compared with Bu-SB (Table 1). This tendency increased after isolation and drying for Poly-SB (*e.g.* Poly-Ly-1: λ_{max}, 427 nm; $E_{1\%}$, 118), which may indicate that the delocalization of the π-electrons involved in the long pendant conjugated chain is affected by steric hindrance. The absorptivities were lower than the calculated values based on the amino group content in the polymer and the $E_{1\%}$ of Bu-SB, especially that of Copoly-SB being below 10. The measurements were conducted in the concentration range satisfying Lambert's Law, so that the low absorptivities indicate that SB formation is affected by the polymer backbones and the amount of amino group available for SB formation is decreased by salt formation in the polymer.

The spectrum of Bu-SB protonated with hydrochloric acid shifted 95 nm to longer wavelengths as previously reported.[9] For amino acid derivatives with low acidic dissociation constants (pK_a), Bu-SB remained unprotonated in non aqueous solvents but protonated in strong organic acids such as p-toluenesulfonic acid. In aqueous solvents, however, the spectrum of Bu-SB shifted to the red on mixing with amino acid derivatives,

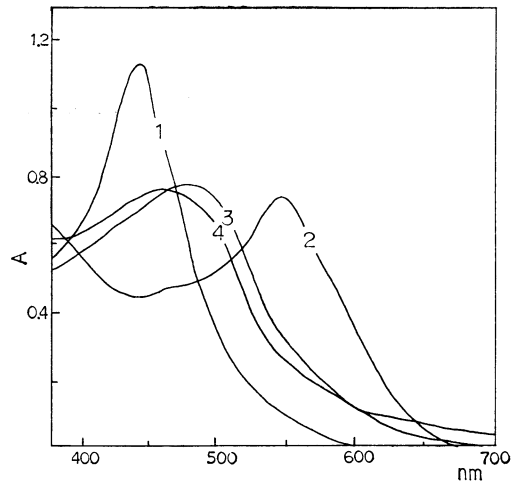


Fig. 1. Absorption spectra of Bu-SB with amino acids. 1; Bu-SB, 2; Bu-SB with HCl, 3; Bu-SB with Z-Glu, 4: Bu-SB with Poly-Glu(OMe). Solvent: 80% DMF.

although new peaks were observed at shorter wavelengths than that with hydrochloric acid (Fig. 1). The cause of this may be the difference in electrostatic energies of the counter ions.[10] The region of the maximum peaks are not clear with α- and γ-carboxy groups of amino acids. The absorptivity of Bu-SB protonated with poly(α-methyl N-methacryloyl-L-glutamate) [Poly-Glu(OMe)] was approximately half the value with low molecular amino acids, probably due to limited protonation by steric hindrance.

The spectra of Poly-Ly-SB protonated with amino acids were similar to those of Bu-SB, but the shifts were smaller. The amino acids as proton donors were in a large excess to the amino residue of Poly-Ly, and consequently neutralization was negligible. The positive charge on the nitrogen of the protonated lysine delocalized throughout the π-electron system is presumably interfered with the torsion of the conjugated chains and a small amount of acid components may be brought in

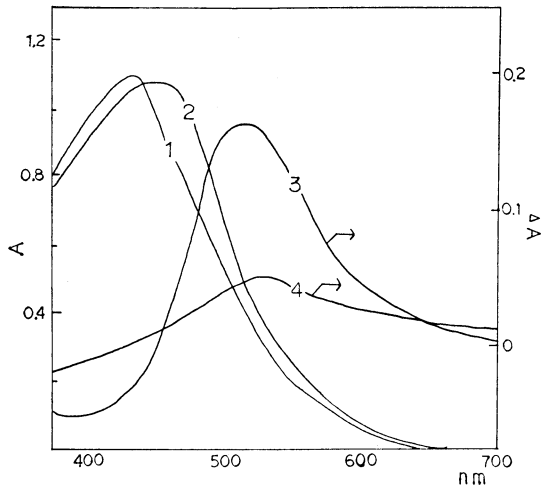


Fig. 2. Absorption spectra of Poly-SB and Copoly-SB 1: Poly-Ly-SB, 2: Poly-Ly-SB with Poly-Glu(OMe), 3: ΔA for Poly-Ly-SB with Z-Glu, 4: ΔA for Copoly-SB. Solvent: 80% DMF.

the proximity of the C=N bond due to steric hindrance of the polymer backbone or the long side chains. The low absorbance of Poly-Ly-SB with acids possessing large molecular sizes are analogous to those of Bu-SB and may be interpreted in terms of the steric hindrance discussed above. The bathochromic shift decreased in the order Z-Glu and Poly-Glu(OMe) (72, 47 nm respectively), indicating that the poorly dissociated carboxylic acid is incompletely bonded to the nitrogen in the hydrophobic region of the polymer matrix, thereby bringing about the poor delocalization of the positive charge throughout the conjugated double bonds.

The spectrum of Copoly-SB, which possesses an environment analogous to rhodopsin slightly shifted towards a longer wavelength by the addition of water. In order to obtain more obvious magnitude of the shift, the difference in absorbance (ΔA) plotted against the wavelength of Copoly-SB and Bu-SB in aqueous solution, the ΔA at the maximum peak being 4% of that for the λ_{max} of Copoly-SB (Fig. 2). This low absorbance appears to be due to there being fewer opportunities for collision between the SB linkage and the carboxylic acids fixed as side chains in the copolymer, since the addition of a lower molecular acid instead of an acid component as the side chain increases the absorbance (9%). The ΔA_{max} of Copoly-SB and with the addition of low molecular acids in aqueous solution was located at a slightly longer wavelength (5—10 nm) compared with Poly-SB and Bu-SB with the addition of amino acids. This suggests that the pendant polar groups such as the carboxylate anions and the salts in the vicinity of the chromophore pull the positive charge into the conjugate chain,[11] thus increasing the contribution to the resonance structure.

It is experimentally impossible to produce a highly selective and restrictive model which forms a SB in the aqueous phase and protonates quantitatively. It has been possible, however, to synthesize a simple polymer model simulating rhodopsin, in which the absorption spectrum shifted towards the longer wavelength *via* intramolecular protonation without the addition of external acid components. The magnitude of the shift, however, was small.

Experimental

The infrared, visible, ^{1}H-NMR and mass spectra were obtained using a Hitachi 215, a Hitachi 200-10, a JEOL-PXM60 and a Hitachi RMU-6MG spectrometer respectively. Elemental analyses were conducted using a Perkin-Elmer 250 instrument. 8′-Apocarotene-8′-al was prepared by the $KMnO_4$-oxidation of β-carotene and purified by aluminum oxide chromatography (Woelem-neutral), followed by partition separation.

Preparation of Vinyl-Z-Ly(OMe): Z-Ly(OMe) was prepared with Z-Ly and methanol–thionyl chloride.[6] To Z-Ly(OMe) HCl (10 g, 30.3 mM) in DMF–THF (2: 5, 70 ml) was added triethylamine (8.9 ml, 60 mM) and the Et_3N–HCl precipitated was filtered off. Methacryloyl chloride (3.5 ml) was added to the filtrate maintained below 5 °C. Stirring was maintained overnight in an ice bath, after which the mixture was filtered and washed with benzene. The filtrate, washed with dil.HCl, 10 % $NaHCO_3$ and water, was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The oily residue was purified by precipitation with a mixture of carbon tetrachloride and hexane. Yield 11.0 g. Found: C, 62.23; H, 7.08; N, 7.13%. Calcd for $C_{19}H_{26}N_2O_5$: C, 62.64; H, 7.14; N, 7.69%. IR (CCl_4): 3400 (NH), 1730 (ester C=O), 1700 (carbamate C=O), 1670 (amide C=O), 945 (C=C) cm^{-1}. ^{1}H-NMR (CCl_4), δ: 1.4—1.7 (m, 6H, lysine CH_2), 1.9 (s, 3H, methacryl CH_3), 3.1 (d, 2H, lysine δ-CH_2), 3.6 (s, 3H, ester CH_3), 4.3 (s, 1H, lysine α-CH), 5.1 (s, 2H, benzyl CH_2), 5.3, 5.6 (s, 2H, CH_2–C), 7.3 (s, 5H, phenyl) ppm. Mass (*m/e*): 364 (M^+, 5 % to CH_2=C–CH_3^+).

Preparation of Vinyl-Glu-OBzl(OMe): A finely powdered Glu–OBzl (7.13 g, 30 mM) was vigorously stirred for one minute with Et_3N (5 ml) in H_2O (50 ml) in an ice bath, followed by the immediate addition of methacryloyl chloride (3 ml) below 5 °C. The mixture was vigorously stirred for 15 min, after which the reaction mixture was extracted with two portions of chloroform (100 ml). The extract, washed with dil.HCl and water was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The Vinyl-Glu-OBzl thus obtained was esterified with an ethereal diazomethane solution and the solution washed with 10 % $NaHCO_3$ and water, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The reaction product was purified by precipitation with a mixture of carbon tetrachloride and hexane. Yield 4.7 g. Found: C, 63.34; H, 6.36; N, 5.23%. Calcd for $C_{17}H_{21}NO_5$: C, 63.95; H, 6.58; N, 4.39%. IR (CCl_4); 3400 (NH), 1740 (ester C=O), 1675 (amide C=O), 920 (C=C) cm^{-1}. ^{1}H-NMR (CCl_4), δ: 1.9 (s, 3H, methacryl CH_3), 2.2—2.4 (m, 4H, glutamate CH_2), 3.8 (s, 3H, ester CH_3), 4.6 (t, 1H, α-CH), 5.1 (s, 2H, benzyl CH_2), 5.3, 5.8 (s, 2H, CH_2=C), 7.4 (s, 5H, phenyl) ppm. Mass (*m/e*): 319 (M^+, 15 % to benzyl ion).

Preparation of Poly-SB: A portion of the monomer (5 g), the composition of which is listed in Table 1, the α,α'-azobisisobutyronitrile (50 mg) and dioxane (10 ml) were placed in a Pyrex glass ampoule which had been previously flushed with nitrogen, sealed, and heated at 75 °C for 48 h. The polymer thus obtained was precipitated by ether, washed with ether, and dried *in vacuo*. The polymer (1 g) was dissolved in trifluoroacetic acid (3 ml). Cleavage of the protecting groups was conducted with a 30 % solution of hydrobromic acid in acetic acid (3 ml) at 50 °C for 1 h. The polymer thus obtained was tranformed into the free state of the ω-position by the following procedure: Poly-Ly HBr in H_2O (10 ml) was basified with Et_3N, precipitated into acetone, and freeze-dried from H_2O; Poly-Glu(OMe) was dissolved in 5% Na_2CO_3 solution and acidified with dil.HCl to pH 2, the polymer thus precipitated was washed with water and dried *in vacuo*; the copolymer dissolved in a mixture of THF and H_2O was neutralized with 0.1 M NaOH to the isoelectric point[12] and stirred at this point for 10 h. The polymer thus precipitated was washed with water and freeze-dried from dioxane. IR (KBr) and ^{1}H-NMR (DMSO-d_6) indicated no absorption peak at 695 cm^{-1} and 7—8 ppm.

Poly-SB was prepared as follows: 0.5 mM of polymer (based on lysine residue) and 1 mM of 8′-apocarotene-8′-al were stirred in DMF (10 ml) with a molecular sieve (3A) under nitrogen and allowed to stand in the dark for several days. The reaction mixture was poured into ether, dissolved in DMF without drying, and the DMF solution extracted with petroleum ether using a Soxhlet liquid extractor to attain a sufficient removal of remaining 8′-apocarotene-8′-al. The spectra were measured in a 1 mm cell approximately 5 min after 10^{-3} M/l of SB (1 ml) and 10^{-3} M/l of amino acid derivatives (10 ml) in DMF were mixed.

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