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PREPARATION OF POLYPEPTIDE MODEL FOR VISUAL PIGMENTS

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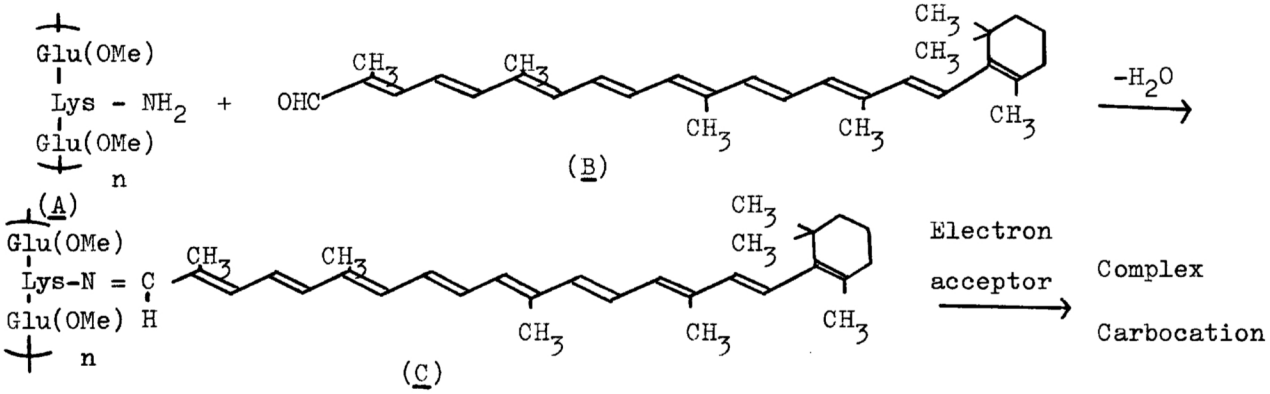
Schiff base(SB) of apocarotenal and copolypeptide(γ-methyl-L-glutamate and L-lysine) was prepared for the purpose of simulating visual pigments. The charge transfer(CT) complexes of conjugated side chains of the resulting polypeptide-SB were formed with various electron acceptors, bringing about a 120-nm shift of absorption band as recoginized in rhodopsin.

It is well known that rhodopsin is composed of the SB between cis-retinal and the lysine unit of a protein called opsin, and that the absorption maximum(λ_{max}) of the former(380 nm) is shifted to 498 nm in the matrix of opsin; this band disappeared by illumination. Many studies on spectral changes of carotene derivatives have been reported[1], but synthetic opsin models so far reported were limited only to low molecular weight compounds such as those from amino acids[2] and their trimer containing lysine units[3] except a modified polyaminostyrene in our previous report[4].

With intention of simulating this visual pigment, this paper will describe the preparation of the SB between a long chain copolypeptide and apocarotenal, together with the examinations of spectral changes of peptide-SB with various electron acceptors.



γ-Methyl-L-glutamate NCA(Glu(OMe)-NCA) and ε-N-benzyloxycarbonyl-L-lysine-NCA (Boc-Lys-NCA) were prepared with trichloromethyl chloroformate[5]. A 2:1 copolypeptide (A) (Glu(OMe)-NCA : Boc-Lys-NCA = 2:1) was prepared with tri-n-butylamine in dioxane

and the protected Boc-group cleaved with 3 molar excess HBr in AcOH. The HBr salt of copolypeptide thus precipitated was washed with acetone and neutralized with MeONa in DMF. A polypeptide, once isolated and dried, indicated very low solubility in any solvents, so that a slightly colloidal DMF solution without separating inorganic salts was used for the next SB formation step. Intrinsic viscosity (η) of HBr salt: 3.0 dl/g in H_2O at 25°C. Anal. Calcd for a structure-A: N,13.53; C,52.17; H,7.52%. Found: N,13.00; C,52.58; H,7.37%.

β-Apo-8'-carotenal(B) was prepared by the $KMnO_4$-oxidation of β-carotene, and purified by aluminum oxide chromatography(Woelm-neutral) followed by a partition separation: λ_{max} in petroleum ether(PE), 452 nm. Oxime, mp 171-174°C (lit.[6] 180°C). Anal. Calcd $C_{30}H_{47}NO$(B-oxime): N,3.20; C,82.30; H,10.70%. Found: N,3.47; C,83.17; H,9.41%. Peptide-SB(C) was prepared as follows: a 330 mg portion of B and 228 mg of A were stirred in 25 ml of DMF with molecular sieve 3A under N_2 stream with exclusion of light to protect B from photoisomerization. After several days, the reaction mixture was poured into acetone, and the resulting red solid washed with acetone until washings were colorless. The ir spectrum indicated an absorption attributable to the conjugated double bonds at 965 cm^{-1}. The λ_{max} in ethylenedichloride(EDC) is 435 nm. The SB formation amounted to 51.1 % by the caluculation from elemental analyses. Anal. Calcd for a structure-C: N,7.12; C,70.23; H,8.40%. Found: N,9.13; C,63.20; H,8.05%.

Peptide films composed of Glu(OMe)-Lys unit(5:1 in molar ratio; (η) of HBr salt, 2.4 dl/g in DMSO at 25°C) were prepared by drying 1 % solution of the HBr salt in dioxane-H_2O mixture at room temperature for several days. The films were then neutralized by immersing in 0.01 N aq. NaOH overnight and dried in vacuo after washing with H_2O. Peptide film-SBs were formed by immersing these films in a 5×10^{-2} M solution of B in EDC for 4 days in the dark place, followed by washing with PE to afford yellow films (λ_{max}, 430 nm).

It has been inferred that the bathochromic shift(about 120 nm) in opsin matrix is caused by the formation of CT complexes of cis-retinal with the Lewis acid portion of opsin, followed by conversion to carbocations[7]. Since the interaction between carotenal derivatives and π-electron acceptors as Lewis acid has not yet been reported, CT bands between low molecular weight compound such as butylamine-SB(Bu-SB)(λ_{max}, 448 nm; ϵ, 3.5×10^4 l/mole cm in EDC) and various electron acceptors were investigated prior to proceed to a treatment of polypeptide.

With trinitrofluorenone(TNF) and p-dinitrobenzene which possess relatively lower

electron affinity(EA), no CT-abs. peaks were observed. With benzoquinone(BQ) which possesses a lower EA than TNF but a smaller molecular size, however, CT-abs. peak was observed at 480 nm. CT-abs. peaks with other acceptors: 2,5-dichlorobenzoquinone(DBQ), chloranil(CA), tetracyanoethylene(TCNE) and I_2 were observed as shown in Table. With tetracyanoquinodimethane unobvious peaks were observed above 600 nm, CT-formations with BQ, DBQ and CA are slower than those with TCNE and I_2, requiring about 20 hr to reach constant values of absorbance.

The results that Benesi-Hildebrand plottings[8] were linear in the concentrations of 10^{-4}-5×10^{-3}M of Bu-SB and 5×10^{-5}M of acceptors (TCNE, I_2), and that an approximately linear relationship exists between the EA of π-acceptor[9] and CT energy($h\nu$) demonstrate the formation of CT complexes(Table).

Table CT-abs. peaks of butylamine-SB

	BQ	DBQ	CA	TCNE	I_2
λ max (nm)	480	520	540	560	590
$h\nu$ (eV)	2.56	2.38	2.30	2.21	
$\varepsilon \times10^3$ (l/M cm)	1.20	1.68	2.17	3.20	16.2
E A (eV)	2.02	2.41	2.59	2.89	

Colour reactions of peptide-SB were carried out either by mixing a suspension of C in EDC and 3 molar excess acceptor or by immersing the 5:1 peptide film-SB in a 5×10^{-3}M solution of acceptor in EDC. Typical examples are shown Fig-1 and -2. The λ_{max} of C(435 nm) is shifted about 10 nm toward shorter wavelengths as compared with that of Bu-SB, which may indicate that the delocalization of π-electron of the pendant long conjugated chain of C is influenced by some steric hindrances as well as polar effects exerted by the peptide backbone.

The absorptivities of CT bands against 435-nm bands(TCNE and I_2) were smaller than that of Bu-SB. No CT-abs. peaks were observed with BQ and CA, but the absorbance at 435 nm tended to decrease about 0.1 unit with slight shoulder peaks appearing at 480-500 nm. These findings may be due to the heterogeneity of reaction medium and steric hindrance in polypeptide chain. CT-abs. peaks appearing immediately after mixings with TCNE(550 nm) and I_2(560 nm) were gradually shifted toward shorter wavelengths and attained equilibria at 530 nm and 535 nm after 5 hr. These hypochromic shifts are presumably caused by the ionizations of complexes under the influence of peptide polarity. Bathochromic shifts are observed in film states, on the other hand, as generally known for CT complexes in solid state[10]. The ir spectrum of C-TCNE showed a 2200 cm^{-1} band attributable to C≡N and the result of elemental analyses indicated that complex contained 0.61 mole of TCNE based on one mole of C.

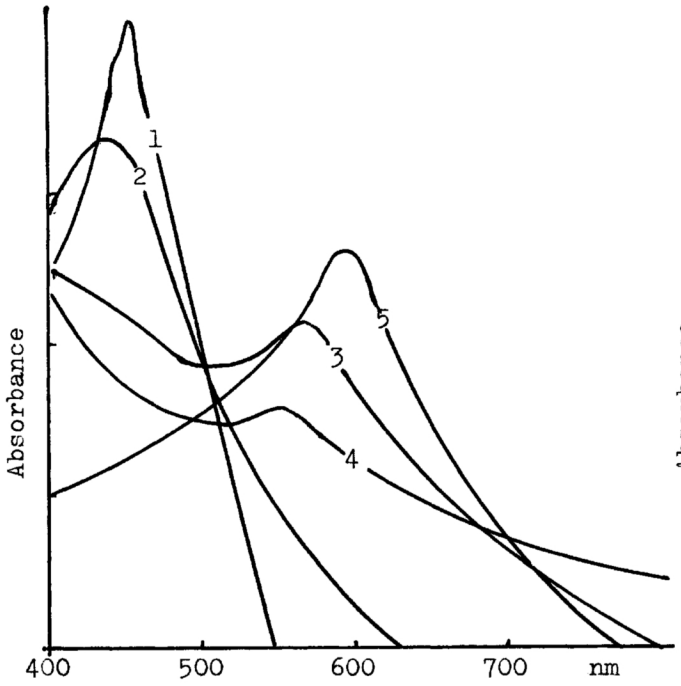


Fig-1 Spectra changes of peptide-SBs with acceptors in EDC

1. Butylamine-SB
2. Peptide-SB
3. Peptide-SB + I_2
4. Peptide-SB + TCNE
5. Butylamine-SB + I_2

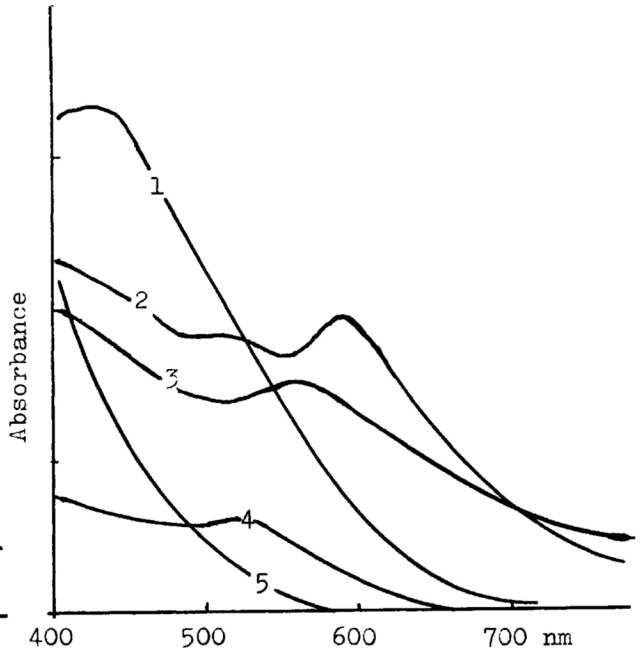


Fig-2 Spectra changes of peptide film-SBs with acceptors

1. Peptide film-SB
2. Peptide film-SB + TCNE
3. Peptide film-SB + I_2
4. Peptide film + I_2
5. Peptide film + TCNE

It is recoginized from the results described above that the spectrum of a pendant long conjugated chain attached to a polypeptide backbone, is changed about 120 nm with acceptors having strong EA and small molecular sizes, which is analogous to rhodpsin. In order to promote the simulation of visual pigments, formations of intramolecular complexes in such polymers and their photoeffects will be studied next.

References

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