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Retinal - Amino Acid Schiff Bases In Reverse Micellar Matrix

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Abstract *: The Schiff bases of retinal with natural amino acids can conveniently be prepared in sodium bis(2-ethylhexyl) sulphosuccinate reversed micelle-solubilized water pool (w = 8) in n-heptane. The micelle -constituted Schiff bases remain stable in protonated form in an environment that compares well with the reaction center of rhodopsins as far as polar and nonpolar domains of the active site is concerned.*

Ever since the discovery of retinal-lysine Schiff bases as chromophores of rhodopsins[1] numerous attempts have been made to prepare retinylidene Schiff bases as models of these light-sensitive membrane bound proteins. Further, new domains of interest in retinal Schiff bases have recently come in light because of the possible uses of retinylidene Schiff bases as nonlinear optical materials[2], and bacteriorhodopsin in semisynthetic biotechnical systems[3]. Most of the synthetic retinal Schiff bases as rhodopsin models have been prepared using amines which are soluble in organic solvents. There are only a few examples where retinylidene Schiff bases containing natural amino acids have been prepared. The main problem arises from the relative insolubility of amino acids and their salts in organic solvents in which retinal is soluble. In view of the above fact, and that the retinylidene-lysine Schiff base is formed, getsprotonatedand remains stable in the native proteins, it was thought desirable to attempt preparation of retinylidene Schiff bases with natural amino acids.

Retinal Schiff bases are commonly prepared by allowing retinal to react with amine in some organic solvent in the presence of dehydrating agent, usually anhydrous sodium sulphate, potassium carbonate or molecular sieves[4]. A few attempts have also been made to prepare retinal Schiff bases with amines insoluble in organic solvents. Thus, Schiff bases of L-lysine and poly-L-lysine have been prepared by allowing a methanolic solution of retinal to react with aqueous solution of L-lysine hydrochloride in presence of triethylamine, and aqueous poly-L-lysine in presence of sodium hydroxide respectively[5]. This method has recently been used with slight modification for the preparation of water soluble retinal-poly-L-lysine Schiff base[6]. Attempts have been made to overcome the main problem of relative insolubility of amino acids and their salts in organic solvents by using hydrophobic anions[7]. Thus, Schiff base models of rhodopsins have been prepared by allowing retinal to react with simple organic amino acids in presence of trifluoroacetate or 10-camphorsulphonate in chloroform or methanol. Glycine has been reacted with retinal derivatives in presence of trifluoroethanol[8]. Attempts have also been made to constitute retinal-lysine Schiff base in reverse micellar matrices[9]. However, rapid decomposition of the Schiff base due to high amino acid concentration and inadequate solubilization were encountered in preparing suitable models for rhodopsins. In order to improve the existing models of retinal-protein Schiff bases a novel and new methodology has been developed wherein Schiff bases of retinal with natural amino acids can be obtained and protonated in a relatively polar environment that surrounds the protonated Schiff base chromophore in the native protein. In a typical procedure, L-amino acid (2, 0.2 mmol) was added to a solution of sodium bis(2-ethylhexyl) sulphosuccinate (AOT, 1.0×10^{-2} M, 10 ml) in n-heptane containing 0.08 M water. The suspension

was vigorously stirred (teflon, 45 min) and filtered to obtain a clear solution. To this solution was added all-trans-retinal (1, 1.0×10^{-5}M) and powdered sodium hydroxide (20 mg). The mixture was briefly stirred (teflon, 5 min) and the resulting suspension was centrifuged. The supernatent was left at room temperature and the progress of the Schiff base (3) formation was monitored by uv-vis absorption spectroscopy.

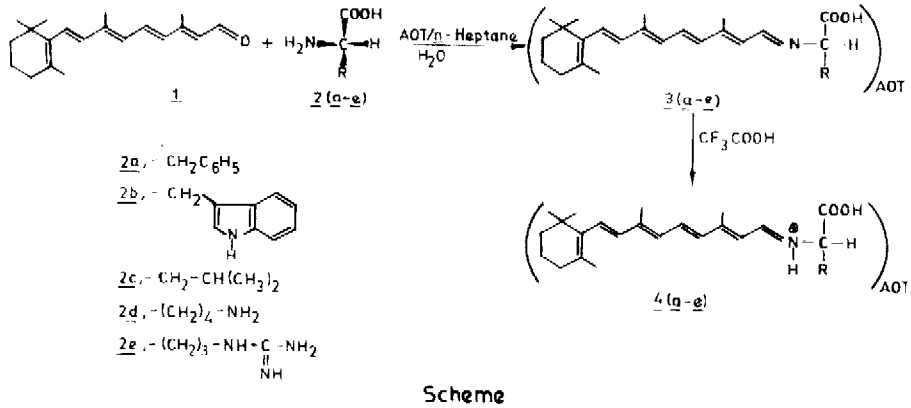
The Schiff base of retinal with various amino acids (Scheme) were formed within 15 minutes of mixing the retinal. The Schiff bases showed a slightly blue shifted absorption band in 356-360 nm (Table) range in comparison to retinal (368 nm). Addition of trifluoroacetic acid caused the expected red shift in the absorption band of Schiff bases as represented in the figure for non-protonated (3e) and protonated (4e) Schiff bases of arginine. The protonated Schiff bases were found to be stable upto 24-36 hr in the micellar system. No precipitate, gelification or unusal darkening of the solution were observed. There was no reaction between retinal and amino acids when suspended in n-heptane. Thus, the reaction in AOT matrix is caused by solubilization of amino acids in the water pool of reversed micelles. Judging from the hydrophobicity of the retinylidene and the amino acid part of the Schiff base, the Schiff base should be interfacially located with amino acid part towards the water-pool. The polar-nonpolar micro environment and the intimate encounter of retinal and amino acid in the restricted field provided by the reversed micelles are responsible for the enhanced formation of Schiff bases. The stability of the Schiff bases of amino acids and retinal in relatively hydrophilic environment may be due to the protection of labile R-CH=NH-C- in the interfacial region wherein the immonium nitrogen can engage water molecules in hydrogen bonding and get stabilized. The side chains of amino acids are found to influence the absorption spectrum of protonated Schiff base (4a-4e) much more than non - protonated Schiff base (3a-3c) as evidenced by $\Delta\lambda$ values shown in the Table.

Table: UV-vis absorption data for retinaldehyde-amino acid Schiff bases (SB) and their protonated forms (PSB) in AOT reversed micelle.

Amino Acid		λ_{max}(nm)		$\Delta\lambda$(nm)
		SB	PSB[a]	
L-Phenyl alanine	(2a)	358	464	106
L-Tryptophan	(2b)	360	447	87
L-Leucine	(2c)	359	437	78
L-Lysine	(2d)	356	441	85
L-Arginine	(2e)	359	440	81

(a) protonation by CF_3COOH.

The acid-base characteristic of the present micellar systems were so adjusted that self protonation of the Schiff base was not observed. Addition of trifluoroacetic acid caused the protonation of the imine nitrogen as evidenced by the red-shift in the absorption spectrum of the protonated Schiff base. In acidic medium, α-carboxyl will be in -COOH form and side chain nitrogens will be protonated. All these groups are expected to undergo fine interactions with the



Scheme

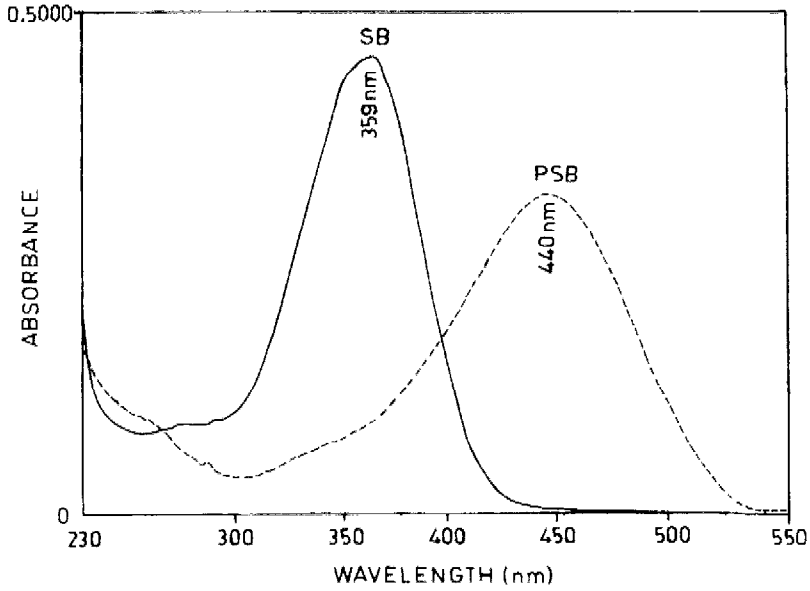


Fig. : UV-vis absorption spectra of all-trans-N-retinylidene-arginine Schiff base(SB, 3e) and its protonated form (PSB, 4e) in 1.0×10^{-2} M AOT in n-heptane ($\omega = 8$).

micellar matrix. Under acidic conditions, the positively charged nitrogen of the amino acid side chains appear to undergo repulsive electrostatic interaction with the imine nitrogen. It is known that the retinylidene chromophore may be influenced by adjacent ionizable groups[10]. More recently it has been shown that the presence of ionic species in the vicinity of retinylidene Schiff base can cause dramatic changes in the pKa[6]. Hence, the protonation behaviour is expected to be influenced by the microenvironment that surrounds the protonated Schiff base. It is believed that the water

molecules also play important role in protonation of the Schiff base chromophore. It may be noted that for the chromophore of bacteriorhodopsin relatively polar microenvironment for stabilization through hydrogen bonding to water has been invoked earlier[11,12]. Presence of a few water molecules near the retinal Schiff base chromophore in bacteriorhodopsin has recently been confirmed by a pioneering work[13]. It is most significant that retinylidene-amino acid Schiff base remain stable even in the presence of water and AOT. Apparently, Schiff base formation, protonation and stability require fine tuned interactions and a delicate balance between the right amount and right kind of water molecules. These requirements are eminently fulfilled in the protein and appear to be well mimicked in surfactant solubilized water pools in apolar solvents[14].

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