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FACILE SCHIFF BASE FORMATION BETWEEN PYRIDOXAL AND AMINO ACID IN REVERSED MICELLES

Hiroki KONDO, Hiroyuki YOSHINAGA, and Junzo SUNAMOTO*

Department of Industrial Chemistry, Faculty of Engineering, Nagasaki University, Nagasaki 852

Schiff base formation between pyridoxal and alanine, arginine or methionine is facilitated by a factor of 10-100 in 0.10 M AOT/ 0.33 M H_2O/1.5 mM NaOH/CCl_4 reversed micelles compared with that in aqueous media. There are two species of the Schiff base present in the system, one being the ionized form (SB 1) and the other un-ionized form (SB 2) with respect to the azomethin and phenolic hydroxyl groups. The former is predominant for alanine and arginine, while the latter is favored by methionine.

Pyridoxal phosphate is a coenzyme playing an essential role in the metabolism of amino acids.[1] The mechanism of its action was investigated intensively especially in model systems and one of the basics has been elucidated to be the formation of Schiff base between pyridoxal phosphate (or its analogues) and amino acid.[2,3] In addition, the importance of general acid-base catalysis in pyridoxal-mediated reactions of amino acids was proposed[4,5] but much remains to be clarified as to the actual mechanism by which the reactions are so tremendously catalyzed in a very specific fashion. A clue may be obtained by exploring the hydrophobic field effect on the reactions, because some of the model reactions were facilitated significantly in apolar media of organic solvents[6] or aqueous micelles.[7] This is in part due to the favorable formation of Schiff bases which is a requisite for the subsequent transformations of substrate.[2,3] In this context, reversed micelles appear to be a suitable system, since they provide a highly restricted field, which is a less polar microdomain surrounded by the hydrophobic environment of bulk apolar solvents.[8,9] In this communication, we would like to show that Schiff base formation between pyridoxal and amino acids is greatly enhanced in the anionic sodium 1,2-bis(2-ethylhexyloxycarbonyl)-1-ethanesulfonate (AOT)/CCl_4 reversed micelles.

When pyridoxal and amino acid (alanine, arginine, or methionine) were solubilized in carbon tetrachloride containing 0.10 M AOT, 0.33 M water, and 1.5 mM sodium hydroxide, there appeared new absorption bands at 335 and 419 nm (Fig. 1). They are characteristic of pyridoxylideneamino acids.[10] It should be emphasized that the Schiff base formation in reversed micelles is readily detectable in small excess (1-10 fold) of the amino acid concentrations over pyridoxal. It is clear that Schiff base formation is favored greatly in the reversed micelles, in sharp contrast to that in aqueous media.[11] Another interesting finding is that there are two species in

equilibrium. They differ in the site of protonation, as shown in Scheme 1. The ionized form with respect to the azomethin moiety and phenolic hydroxy group (SB 1) is normally found in aqueous media and absorbs light at around 420 nm.[11] The unionized form (SB 2) is observed, on the other hand, only in a hydrophobic environment such as in an organic solvent,[10] in aqueous micelles,[12] or with the use of alkylamines bearing a long hydrocarbon chain.[13,14] That both forms of Schiff base are present in the reversed micelles indicates that the polarity around the Schiff base is intermediate of water and an organic solvent. Hence, it is quite conceivable that the molar ratio of SB 1 to SB 2 varies with the nature of substituent (R) of amino acid. In order to evaluate the equilibrium constant for Schiff base formation (K) and the molar ratio of SB 1 to SB 2 (α), measurements were made at several concentrations of amino acid. The data are analyzed in the following way.

Eqs. 1 and 2 represent material balances for pyridoxal and amino acid, respectively.

$$[PL] + [SB\ 1] + [SB\ 2] = T_P \quad (1)$$

$$[AA] + [SB\ 1] + [SB\ 2] = T_A \quad (2)$$

[PL], free pyridoxal concentration; T_P, total pyridoxal concentration
[AA], free amino acid concentration; T_A, total amino acid concentration

$$\alpha = \frac{[SB\ 1]}{[SB\ 2]} \quad (3)$$

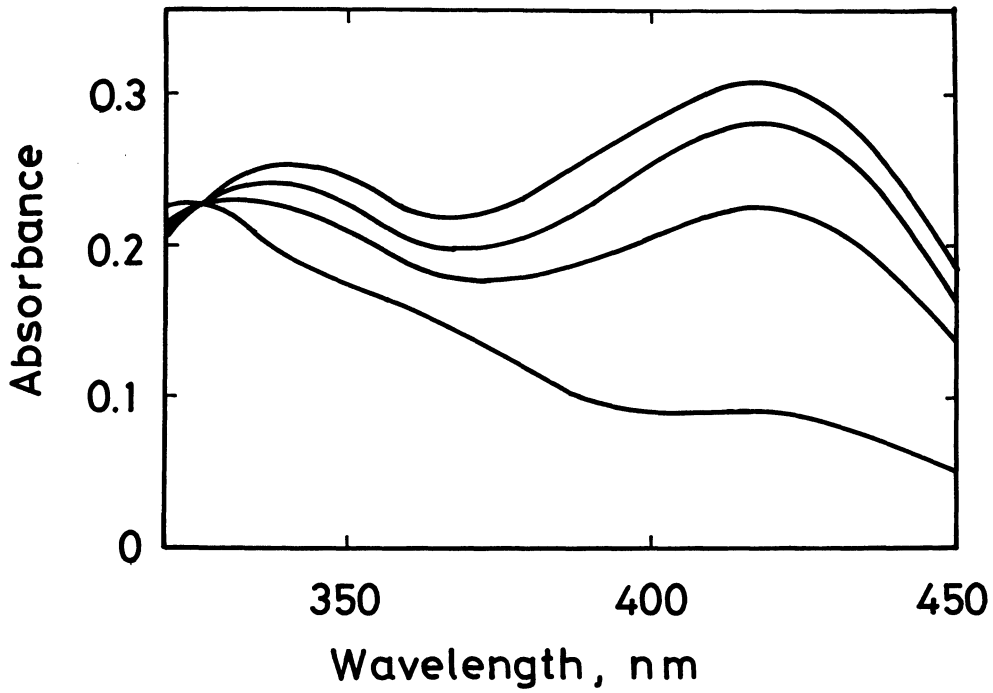
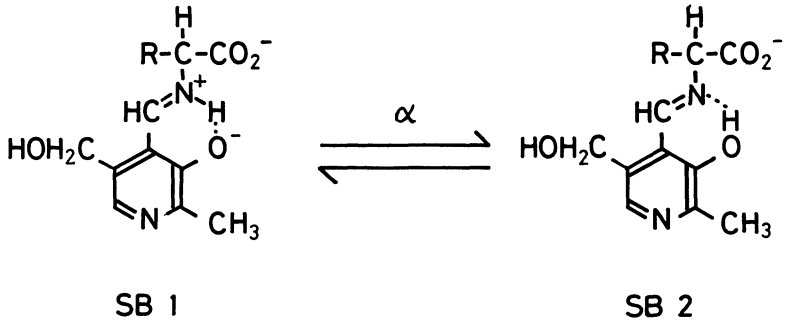


Fig. 1. Electronic absorption spectra of Schiff base formed between pyridoxal (0.99×10^{-4} M) and arginine in 0.10 M AOT/0.33 M H_2O/1.5 mM NaOH/CCl_4 reversed micelles at 25.0 °C. Amino acid concentrations are 0.99, 2.97, 4.95, and 9.90×10^{-4} M from bottom to top.



SB 1 SB 2

Scheme 1

$$[SB] = [SB\ 1] + [SB\ 2] \quad (4)$$

The equilibrium constant for the Schiff base formation is defined by Eq. 4.[15]

$$K = \frac{[SB]}{[PL][AA]} \quad (5)$$

Since SB 2 does not have significant absorption at 419 nm, the observed absorbance (A_{419}) is simply the sum of absorption of pyridoxal and SB 1 (Eq. 5). At 335 nm, pyridoxal and SB 2 contribute to the observed absorbance (A_{335}). The extinction coefficient of SB 2 at 335 nm is half that of SB 1 at 419 nm,[14] and hence the relationship may be formulated as in Eq. 6.[16]

$$A_{419} = 100[PL] + \varepsilon_1[SB\ 1] \quad (6)$$

$$A_{335} = 3200[PL] + 0.5\varepsilon_1[SB\ 2] \quad (7)$$

By combining Eqs. 1-6, Eq. 7 is derived.

$$2(32A_{419} - A_{335}) = \frac{64\alpha - 1}{\alpha + 1}\varepsilon_1[SB] \quad (8)$$

$$[SB] = \frac{K(T_P + T_A) + 1}{2K} - \sqrt{\left\{\frac{K(T_P + T_A) + 1}{2K}\right\}^2 - T_P T_A} \quad (9)$$

The best correlation was searched over a wide range of K by iterative computation.[17] The final results thus obtained are summarized in Table I. The equilibrium constants (K) for the Schiff base formation in the reversed micelles lie in the range of 2×10^3

Table I. Several Numerical Parameters for the Schiff Base Formation between Pyridoxal and Amino Acids in 0.10 M AOT/ 0.33 M H_2O/1.5 mM NaOH/CCl_4 Reversed Micelles at 25.0 °C

	K (M^{-1})	α
Alanine	2×10^3	4.8
Arginine	1×10^4	1.3
Methionine	2×10^4	0.59

- 2×10^4 M^{-1}. This corresponds to 10-100 fold enhancement compared to that in normal aqueous media.[18,19] The constant varies to some extent among the amino acids employed. The SB 1 species is predominant for alanine and arginine, while the SB 2 species is favored by methionine. This is controlled primarily by the nature of substituent R. Thus, methionine bearing the least polar methylthioethyl group yields the un-ionized form of Schiff base most. Since pyridoxal, amino acids, and Schiff bases all are essentially insoluble in the bulk apolar phase, they are mostly incorporated into the interior water pool of reversed micelles. Judging from its hydrophobicity, however, the Schiff base of methionine should be somewhat distributed in a more apolar environment. This leads to the more formation of the un-ionized species of Schiff bases.

In conclusion, we believe that both the less polar reaction field and the intimate encounter of pyridoxal and amino acid in the restricted field provided by the reversed micelles are responsible for the enhanced formation of Schiff bases. The increase in the amount of Schiff base affects the subsequent reaction of amino acids. Thus, the Schiff base of histidine shows a slow spectral change with time under these mild conditions.[20] Taken together, reversed micelles can provide a nice reaction field just like an apoenzyme does in the holoenzyme system.

References and Notes

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15) Since Schiff base formation is a dehydration process, the water concentration term should be included in the numerator of Eq. 4 in a rigorous sense. This is, however, neglected for the sake of simplicity, because the amount of water produced upon Schiff base formation does not alter the total water concentration in the medium (0.33 M) significantly.
16) The numbers 100 (Eq. 5) and 3200 (Eq. 6) are the molar extinction coefficients of pyridoxal in M^{-1} cm^{-1} at 419 nm and 335 nm, respectively, under these conditions.
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