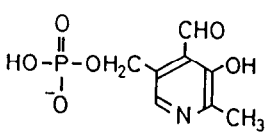
Tetrahedron Letters,Vol.24,No.23,pp 2403-2406,1983 0040-4039/83 $3.00 + .00
Printed in Creat Britain

FACILE TRANSAMINATION OF ORDINARY AMINO ACIDS BY A PYRIDOXAL MODEL INCORPORATED INTO A CATIONIC MICELLE

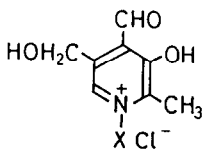
Hiroki Kondo*, Jun-ichi Kikuchi, and Junzo Sunamoto
Department of Industrial Chemistry, Faculty of Engineering,
Nagasaki University, Nagasaki 852, Japan

Summary: *N*-Laurylpyridoxal (**2a**) forms a Schiff base with amino acids in CTACl micelles with a large equilibrium constant and the Schiff base undergoes transamination in the absence of metal ions.

Pyridoxal 5'-phosphate (PLP, **1**) is a coenzyme playing an important role in the metabolic reactions of amino acids such as transamination and racemization.[1] Most of these reactions can be mimicked in nonenzymatic systems under rather unphysiological conditions; high temperature, extreme pH[2] and use of anomalously high substrate (amino acid) concentrations.[3] Furthermore, metal ions are often needed to effect such reactions in model systems but they have never been found involved in enzymatic reactions.[2] Hence, factors responsible for the more efficient catalysis of PLP in enzymatic systems remain to be elucidated. In view of a fact that PLP serves principally as an electron sink in its catalysis, and that the reactions of a Schiff base derived from PLP and amino acid (*e.g.* proton abstraction from the α position) are subject to general base catalysis,[3] we have devised a model system in which both aspects of PLP catalysis, *i.e.* strong(er) electron-withdrawing ability and base catalysis, can be met. We would like to show that **2a** carrying a dodecyl group on the pyridine nitrogen is capable of undergoing transamination with ordinary amino acids in aqueous hexadecyltrimethylammonium chloride (CTACl) micelles under near-physiological conditions.



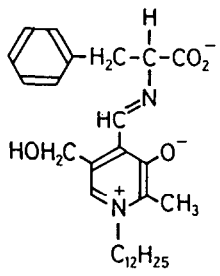
1



2 a ($X=C_{12}H_{25}$)
b ($X=CH_3$)

Compound **2a** was prepared by reaction of pyridoxal monomethylacetal with lauryl iodide, followed by anion exchange with freshly prepared silver chloride. The pK_a value of the 3-hydroxyl group of **2a** in 3 mM CTACl micelle was determined by spectroscopic titrations as 2.6 at 30.0 °C. The absorption maxima of the protonated and ionized forms were 296 and 330.5 nm, respectively. Compound **2a** forms a Schiff base with amino acids such as L-phenylalanine over a broad pH range (~5-11) in the aqueous CTACl micelle. The Schiff base possesses an absorption maximum at 394 nm

and formula **3** is assigned to this species. The equilibrium constant for the Schiff base formation K_{pH}, defined by eq. 1 (T denotes sums of all the ionic species of each compound), was determined



3

$$K_{pH} = \frac{[\text{Schiff base}]_T}{[\textbf{2a} \text{ or } \textbf{2b}]_T \cdot [\text{Amino acid}]_T} \qquad (1)$$

spectroscopically at pH 8.0, 30.0 °C.[4,5] The results summarized in Table 1 reveal that the Schiff base formation in the present system is enhanced ~100-fold compared with that for a pyridoxal or **2b** system, as the equilibrium constant for the latter usually lies somewhere around 10 M^{-1}.[5,6] Furthermore, the Schiff base formation in the micelle is highly dependent on the side chain structure of amino acid, *i.e.* the more hydrophobic,[7] the greater is the formation constant: L-tryptophan > L-phenylalanine > L-leucine > L-alanine. This indicates that the main driving force of the enhanced Schiff base formation is the hydrophobic interaction of CTACl micelle and an

Table 1. Equilibrium and Rate Constants for the Reaction of 0.10 mM **2a** with 10 mM Amino Acids in 10 mM Phosphate Buffer, pH 8.0 at 30.0 °C in the Presence of 3 mM CTACl and 0.10 mM EDTA

Amino acid	K_{pH}/M^{-1}	$k_{obs} \times 10^5/s^{-1}$	$k \times 10^5/s^{-1}$
L-Trp	2600	1.6	1.7
L-Phe	1500	1.5	1.6
L-Leu	300	0.61	0.81
L-Ala	14	~0.1 [1)]	~1

1) The rate was determined by the "coupled assay", in which pyruvate formed was allowed to react with an excess of NADH in the presence of lactate dehydrogenase and the remaining NADH was evaluated by fluorescimetry.

apolar side chain of amino acid. This idea is supported by the observation that the K_{pH} value of such amino acids as glutamic acid and *O*-phosphoserine carrying an ionic side chain does not vary significantly between the **2a**-CTACl and **2b** systems (data not shown). In addition, a red shift of the Schiff base absorption from 374 nm to 394 nm in going from the **2a**-CTACl to **2b** system may be taken as evidence for the location of the Schiff base of **2a** in the micellar pseudo-phase.

The Schiff base of **2a** and amino acid undergoes a slow transamination reaction in 3 mM CTACl at pH 8.0 and 30.0 °C. This was deduced from a decay of the Schiff base absorption with a concomitant build-up of a new absorption band at 335 nm (Fig. 1).[8] This spectral change was accompanied by an increase in the fluorescence intensity at ~438 nm, attributable to the pyridoxamine form of **2a**. Another product, phenylpyruvate, was identified qualitatively after conversion to its 2,4-dinitrophenylhydrazone. Thus, the whole reaction sequence may be illlustrated as in the following scheme:

$$\textbf{2a} + \text{Amino acid} \underset{}{\overset{K}{\rightleftharpoons}} \text{Schiff base } (\textbf{3}) \xrightarrow{k} \textbf{4a} + \text{keto acid} \qquad (2)$$

4a: pyridoxamine form of **2a**

All kinetic runs were carried out in the presence of 0.10 mM EDTA to mask the effect of traces of contaminating metal ions, because some metal ions are a powerful activator of the transamination even at a μM level. The kinetic data are analyzed in terms of a pseudo first-order rate law (eq. 3). In a system where the Schiff base formation is not quantitative, k_{obs} was converted into specific rate constant k with the aid of eq. 4, where 0 stands for the initial concentration.

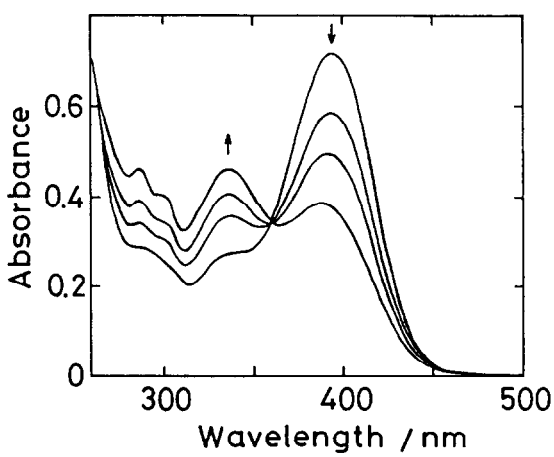


Fig. 1. Transamination of the Schiff base of **2a** (0.10 mM) and phenylalanine (10 mM) in 10 mM phosphate buffer (pH 8.0) containing 3 mM CTACl and 0.10 mM EDTA at 30.0 °C. Spectra were taken at 0, 5, 10 and 20 h after mixing of **2a** with phenylalanine.

$$-\frac{d[\mathbf{3}]}{dt} = k_{obs}[\mathbf{3}] \qquad (3) \qquad k_{obs} = \frac{k \cdot K[\text{Amino acid}]_0}{K[\text{Amino acid}]_0 + 1} \qquad (4)$$

$$\frac{d[\mathbf{2a}]}{dt} + \frac{d[\mathbf{3}]}{dt} + \frac{d[\mathbf{4a}]}{dt} = 0 \qquad (5)$$

Equation 4 is derived by combining eq. 1 and the law of mass balance (eq. 5). The results of this analysis are compiled in Table 1.

The rate constant k for the transamination of Schiff base does not vary much among the amino acids examined, but the apparent rate k_{obs} does differ over 10-fold between tryptophan and alanine. The difference stems mainly from the binding ability of amino acid to **2a** in the CTACl micelle. In this respect, the present system specifically promotes transamination of hydrophobic amino acids by virtue of their selective incorporation into a micelle. In addition, the α-hydrogen of amino acid is made labile by the positive charge on the pyridine ring and the abstraction of the α-hydrogen is facilitated by hydroxide ions concentrated by the cationic micelle.[9,10] This mode of catalysis is reminiscent of the catalysis of PLP-dependent enzymes in which a proton is donated to the 1-N of coenzyme and the α-hydrogen is abstracted by the lysine amino group of the apoenzyme that binds PLP in the absence of a substrate.[11]

Acknowledgment We are grateful to Messrs. S. Uchida and T. Kitamikado for their expert technical assistance. This work was supported by a Grant-in-Aid for Special Project Research 57102007 from the Ministry of Education, Science and Culture.

References

1) E. E. Snell and S. J. DiMari, "The Enzymes," 3rd ed., P. D. Boyer Ed., Academic Press, New York, 1970, Vol. II, Chap. 7.
2) D. E. Metzler, M. I. Ikawa, and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 648 (1954).
3) T. C. Bruice and R. M. Topping, *J. Am. Chem. Soc.*, **85**, 1480 (1963).
4) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949).
5) D. E. Metzler, *J. Am. Chem. Soc.*, **79**, 485 (1957).
6) C. M. Metzler, A. Cahill, and D. E. Metzler, *J. Am. Chem. Soc.*, **102**, 6075 (1980).
7) Y. Nozaki and C. J. Tanford, *J. Biol. Chem.*, **246**, 2211 (1971).
8) J. R. Maley and T. C. Bruice, *Arch. Biochem. Biophys.*, **136**, 187 (1970).
9) J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems," Academic Press, New York, 1975, Chap. 4.
10) Y. Murakami and H. Kondo, *Bull. Chem. Soc. Jpn.*, **48**, 541 (1975).
11) Y. Morino, A. M. Osman, and M. J. Okamoto, *J. Biol. Chem.*, **249**, 6684 (1974).

(Received in Japan 7 March 1983)