CHEMISTRY LETTERS, pp. 31-34, 1982. 

# STRUCTURE OF THE METAL SCHIFF BASE COMPLEX OF PYRIDOXAL AND HISTIDINE IN SOLUTION

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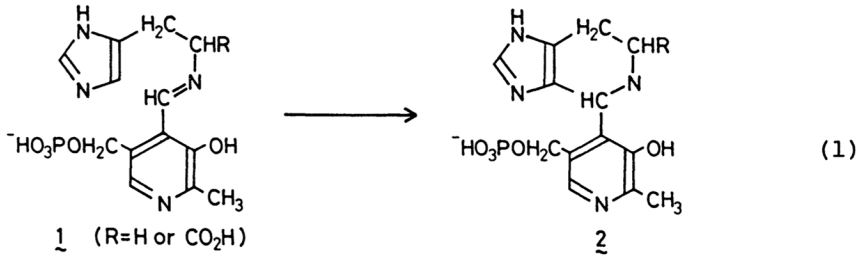
The cyclization of pyridoxal Schiff base of histidine or histamine is prohibited by metal ions such as $Cu^{2+}$ and $Zn^{2+}$. On the basis of a similar extent of metal inhibition for histidine and histamine, it is concluded that an identical Schiff base complex with the imidazole group coordinating to the metal is responsible for the inertness of both substrates.

Pyridoxal phosphate (PLP) is a cofactor playing a crucial role in the metabolism of amino acids in biological systems. Such metabolic reactions of amino acids involve transamination, racemization, and decarboxylation, and all these reactions proceed through the Schiff base of PLP and amino acid.[1] PLP-catalyzed reactions of amino acids can be duplicated in part in model systems mostly with the aid of certain metal ions. There is, however, a notable difference, among others, between enzymic and nonenzymic reactions. In the latter system some amino acids undergo unusual reactions which have no enzymic counterparts. One of the examples is the cyclization of histidine Schiff base (Eq. 1). The reaction has been known for over




1 (R=H or $CO_2H$) 2 (1)

three decades,[2] and it is classified as the intramolecular Mannich reaction.[3] This paper is concerned with the inhibition of this cyclization reaction by metal ions in a hope of gaining a clue to the reaction mechanism with a special emphasis on the structure of metal Schiff base complex involved in the reaction.

An equimolar mixture of pyridoxal (PL) and histidine or histamine in methanol containing 0.50 mM sodium hydroxide was allowed to react at 25.0 °C. The progress of reaction was followed spectrophotometrically as shown in Fig. 1. The initial

absorption of PL or the Schiff base at ~400 nm disappeared with time and a new absorption maximum was established at 285 nm, which is compatible with the cyclized product of PL and histidine or histamine.[3,4] The rate of cyclization was determined in the presence of 10-fold excess histidine or histamine over PL: $k_{obs}$ = $4.97 \times 10^{-4}$ $s^{-1}$ and $5.95 \times 10^{-3}$ $s^{-1}$ for histidine and histamine, respectively.

The cyclization is suppressed drastically by the addition of metal ions such as $Cu^{2+}$ and $Zn^{2+}$.[5] Even $Mg^{2+}$ gave out a significant inhibitory effect under the present conditions, *i.e.*, in methanol. The inhibitory potency of metal ions is $Cu^{2+}$, $Zn^{2+}$, and $Mg^{2+}$ in the decreasing order (Table 1). When PL, histidine, and metal ion are present in equal quantities ($1.0 \times 10^{-4}$ M) the cyclization is negligibly slow and the absorption maximum is obtained at 380 nm. A virtually identical band location is observed for the metal complex of alanine Schiff base.[5,6] This, however, does not necessarily mean that the structure of all these Schiff base complexes is exactly identical (see below).

The metal complex of alanine Schiff base assumes a structure like *3*, while that of histamine Schiff base may be *4* with the imidazole moiety coordinating to the metal. This would best explain the metal inhibition of cyclization reaction. By reference to the knowledge about the reaction mechanism in the absence of a metal ion, the cyclization begins with the attack of 5'-carbon of imidazole on the

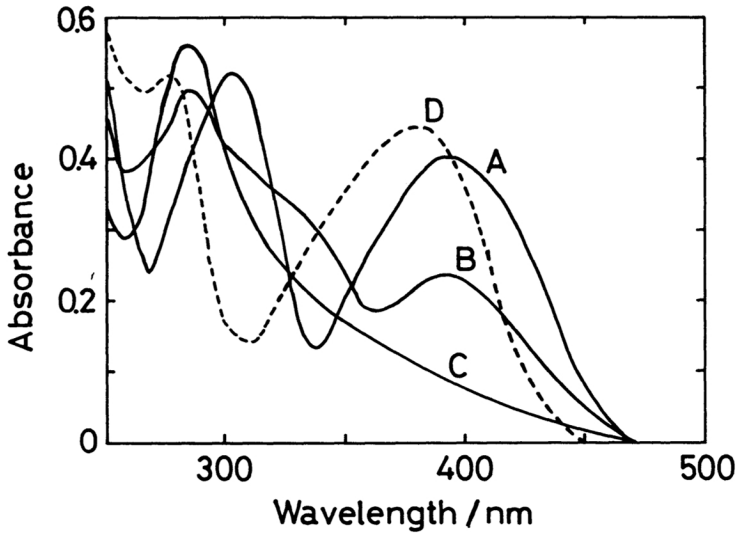
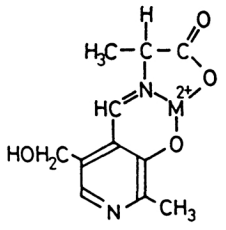



Fig. 1. Electronic absorption spectra of pyridoxal Schiff base of histamine and its zinc(II) complex in methanol containing 0.50 mM sodium hydroxide at 25.0 °C. A, pyridoxal alone ($1.0 \times 10^{-4}$ M). B and C are those determined 30 min and 3 h after addition of pyridoxal to histamine ($1.0 \times 10^{-4}$ M), respectively. D containes zinc chloride ($1.0 \times 10^{-4}$ M) in addition to pyridoxal and histamine.

Table 1. Inhibitory Effect of Metal Ions on the Cyclization of Histidine or Histamine Schiff Base of Pyridoxal in Methanol Containing 50 mM Sodium Hydroxide at 25.0 °C[a)]

| Metal Ion | $k_{obs}$, $s^{-1}$ | |
|---|---|---|
| | Histidine | Histamine |
| ——— | $4.97 \times 10^{-4}$ | $5.95 \times 10^{-3}$ |
| $Cu^{2+}$ | ~0 | ~0 |
| $Zn^{2+}$ | $2.42 \times 10^{-6}$ | $1.68 \times 10^{-5}$ |
| $Mg^{2+}$ | $3.37 \times 10^{-4}$ | $2.51 \times 10^{-3}$ |

a) [PL·HCl] = $1.0 \times 10^{-4}$ M, [Histidine or Histamine] = $1.0 \times 10^{-3}$ M, [Metal Ion] = $1.0 \times 10^{-3}$ M



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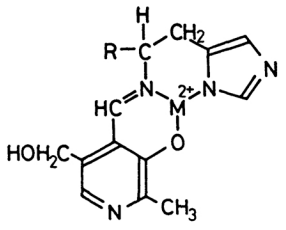
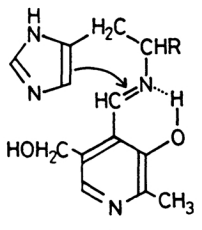


4 (R=H or $CO_2H$)

azomethine carbon (*5*).[3)] Imidazole coordination to the metal makes this attack impossible, thus completely suppresses the cyclization. This appears to be the case for histidine. As shown in Table 1, the extent of metal inhibition is almost the same for histamine and histidine, suggesting strongly that both substrates are subject to an identical inhibition mechanism. In other words, the imidazole moiety of histidine, rather than the carboxylate group, may be coordinating to the metal (*4*).



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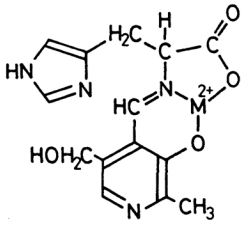

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If the carboxylate group coordinated to the metal (*6*), the azomethine carbon would become more electrophilic, rendering the cyclization facile. An alternative

explanation would be that in the Schiff base complex *6*, the approach of imidazole moiety to the azomethin carbon is inhibited for steric reasons.[5] A CPK model building suggests some steric constraints around the reaction site but they do not appear to be severe enough to suppress completely the cyclization. Therefore, we conclude that it is complex *4* that is responsible for the inertness in the cyclization of histidine Schiff base. This cautions us that the carboxylate group of pyridoxal Schiff base is not always the third ligand, when another potential ligating group is present in the amino acid. The structure of metal Schiff base complexes of pyridoxal and amino acid in solution has been deduced mainly from $^{1}H$ NMR data,[7-9] which do not give good enough information about the coordination of carboxylate group. On the other hand, $^{13}C$ NMR spectroscopy provides more direct evidence for or against the carboxylate coordination. Research is being conducted along this line in this laboratory.

This work was supported by a Grant-in-Aid (No. 521325) from the Ministry of Education, Science, and Culture.

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(Received October 22, 1981)