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Species Absorbing in the 500-nm Region in Pyridoxal Catalysis. V.[1] Divalent Metal Chelates

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A transient species with an absorption at around 500 nm was formed on addition of Mn(II), Cu(II), Co(II), Zn(II), and Ni(II) ions to a methanol solution of the aldimine of of pyridoxal N-methochloride and ethyl alaninate. It is concluded that the species is the metal chelate of a quinoid intermediate in the metal ion mediated isomerization of the aldimine and the ketimine. The formation of the quinoid metal chelate was completed within a few seconds after the chelation of the aldimine and its rate was not greatly dependent on the metal ions. The rate of the disappearance of the quinoid metal chelate was affected by the properties and the concentrations of the metal ions and ,in the case of Cu(II), by the presence of the other chelating ligand. The life time of the 1:1 Cu-quinoid chelate was several seconds, whereas that of the 1:2 chelate and of the ternary complex formed from Cu(II), the quinoid, and a bidentate or tridentate ligand were longer than several hours. The phenomenon was explained on the assumption that the latter complexes are distorted octahedral and the weak coordination between Cu(II) and the carbonyl oxygen retards the protonation of the quinoid.

Keywords——enzyme model; pyridoxal catalysis; Schiff base; pyridoxal N-methochloride; ternary complex; Cu(II) complex; quinoid intermediate; divalent metal chelates; transamination

The key step in most enzymatic pyridoxal catalysis is the proton abstraction from the α-carbon of an amino acid in the Schiff base (aldimine) of pyridoxal phosphate.[3] The deprotonated intermediate has a quinoid structure and absorbs in the 500-nm region of the spectrum.[4]

In the previous paper of this series,[5] we reported a metastable species with an absorption maximum at 488 nm, which was formed on addition of Al(III) to a methanol solution of pyridoxal and the ester of an amino acid. This species was identified as the Al(III) chelate of a quinoid intermediate, the ester of pyridoxylideneamino acid deprotonated at the α-carbon, and, hence, it serves as a model for the enzymatic intermediate. Similar species were formed with Ga(III), In(III), and trivalent lanthanides, but not with the divalent transition metals.

The divalent metals were found to form short-lived species absorbing in the 500-nm region, when pyridoxal was replaced by pyridoxal N-methochloride in the reaction. The present paper is concerned mainly with the divalent metal chelates of the 500-nm species.

Experimental

Experimental procedures are essentially the same as described in the previous paper.[5] A Union-Giken Model RA-1300 stopped-flow rapid-scan analyzer was used for the measurement of rapid reactions.

1) Part III and IV: Y. Karube and Y. Matsushima, *J. Am. Chem. Soc.*, **98**, 3725 (1976); **99**, 7356 (1977).
2) Location: *Maidashi, Fukuoka, 812, Japan*; *a*) Author for correspondence.
3) For reviews, see L. Davis and D.E. Metzler, "The Enzymes," 3 rd ed. Vol. 7, P.D. Boyer Ed., Academic Press, New York, N.Y., 1972, Chapter 2; A.E. Braunstein, "The Enzymes," 3 rd ed. Vol. 9, P.D. Boyer Ed., Academic Press, New York, N.Y., 1973, Chapter 10.
4) Y. Morino and E.E. Snell, *J. Biol. Chem.*, **242**, 2800 (1967); see also the literature cited in ref. 3 and 5.
5) Part II: S. Matsumoto and Y. Matsushima, *J. Am. Chem. Soc.*, **96**, 5228 (1974).

Results

The 500-nm Species

Neutral methanol solutions of pyridoxal N-methochloride (1×10^{-4} M) and ethyl alaninate (1×10^{-3} M) were mixed and allowed to stand for 3 hr at room temperature. The spectrum showed that the Schiff base (aldimine) was formed in the solution. To the solution, a methanol solution of metal perchlorate (5×10^{-4} M) was added. The concentrations are those in the final mixture.

The absorption at the 500-nm region appeared instantly on the addition of metal ion, reached its maximum, and then, disappeared. The absorbance in the maximum was 1.0—3.5 under these conditions. Spectra after the disappearance had an absorption band at around 335 nm, which can be assigned to the metal chelate of the ketimine from N^1-methylpyridoxamine. Then, overall reaction is the metal ion mediated isomerization of the aldimine to the ketimine as shown in Chart 1 and the 500-nm species is the intermediate with a quinoid structure.

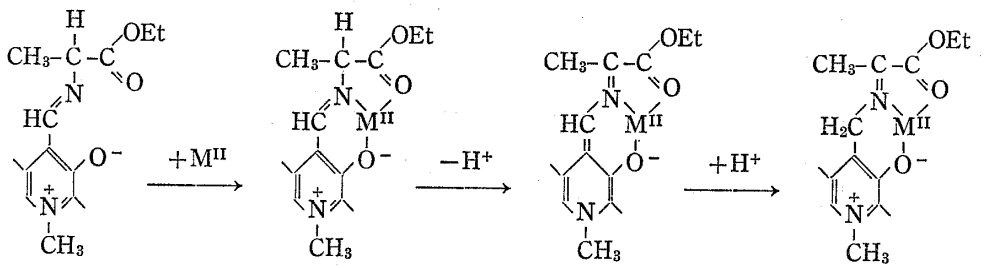

Chart 1

Spectra of the 500-nm species were recorded on a rapid-scan spectrometer and the rates of its appearance and disappearance were measured by the stopped-flow techniques. After the addition of the metal ion, the 500-nm absorption reached a maximum within 4 sec. As for the time to reach the maximum, there was no large difference among the divalent metals examined. The disappearance followed the first-order kinetics. The results are summarized in Table I. The absorption peaks did not significantly shift on addition of acid or alkali.

Table I. The 500-nm Species

Metal ion	Mn(II)	Cu(II)	Co(II)	Zn(II)	Ni(II)	Al(III)
λ_{max} (nm)	528	508	526	515	521	500
t_{max} (sec)[a]	0.2	0.35	0.42	1.5	3.3	60
k_{obs} (sec^{-1})[b]	4.0	0.15	0.12	0.047	0.03	3.8×10^{-4}

a) Time when the absorbance reached a maximum after the addition of the metal ion.
b) The observed first order rate constant for the disappearance of the species under the conditions described in the text.

In the pyridoxal N-methochloride-ethyl alaninate-Al(III) reaction, the appearance and the disappearance of the 500-nm species were much slower than in the reaction of the transition metals. Rate data are included in Table I.

Cu(II) Complex

In the reaction of pyridoxal N-methochloride (1×10^{-4} M), ethyl alaninate (1×10^{-3} M), and Cu(II) perchlorate, the rate of the disappearance of the 508-nm absorption was dependent on the concentration of the Cu(II) salt. The results of the stopped-flow measurement are shown in Fig. 1.

The rate of the appearance was unaffected, when Cu(II) was present in more than an equimolar amount to the Schiff base. The rate of the disappearance increased with an increase of the Cu(II) concentration. In the presence of more than 1×10^{-3} M Cu(II), the 508-nm species was hardly observable, probably because the species did not accumulate due to the rapid disappearance. Under the concentrated Cu(II), the predominant Cu(II) chelate must be the 1:1 complex of the quinoid.

When the Cu(II) concentration was smaller than that of the Schiff base, the life time of the 508-nm species became prolonged. At 5×10^{-5} M Cu(II) (a half molar amount to the Schiff base), the visible absorption was observed for several hours. The absorbance in its maximum was nearly equal to that under more concentrated Cu(II). This indicates the same amount of the quinoid was formed, since the absorption is ascribed to the π-π^* transition of the quinoid moiety.[6] Thus the 1:2 Cu(II) quinoid complex must be formed under these conditions.

In the solutions containing Cu(II) in less than a half molar amount to the Schiff base, the life time of the 508-nm absorption was also several hours, but the absorbance in its maximum was low. The 1:2 complex must be predominant under these low Cu(II) conditions.

The results suggest that the life time is longer in the 1:2 complex than in the 1:1 complex.

During the 508-nm band was observable, appeared another intense absorption at 298 nm. The absorbance of the both bands changed synchronously. The 298-nm band may be ascribed to the π-π^* band of the quinoid structure. The corresponding absorption was at 292 nm in the Al(III) catalyzed reaction.

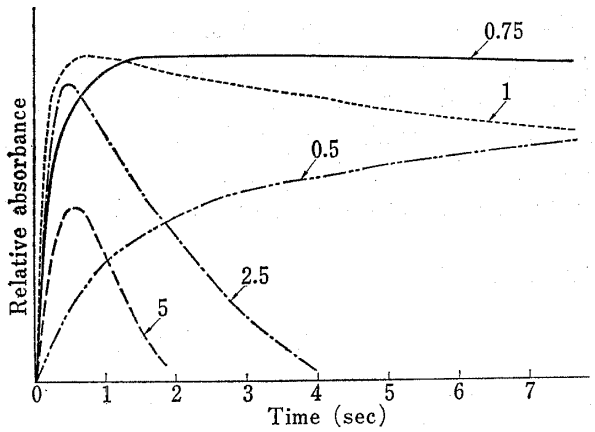

Fig. 1. The Stopped-flow Measurement of the 508-nm Absorption in the Reaction of Pyridoxal N-Methochloride (1×10^{-4}M), Ethyl Alaninate (1×10^{-3}M), and Cu(II) Perchlorate. The concentration of Cu(II) are indicated besides the curves in 10^{-4} M unit.

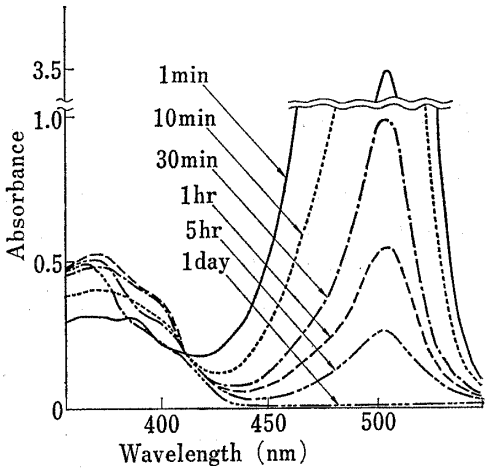

Fig. 2. Spectral Change Accompanying the Reaction of Pyridoxal N-Methochloride (1×10^{-4}M), Ethyl Alaninate (1×10^{-3}M), Cu(II) Perchlorate (5×10^{-4}M), and Ethylenediamine (7.5×10^{-4}M). Times after initiating the reaction are indicated besides the spectral curves.

Effect of Other Ligands

The marked difference in the life times of Cu(II)-quinoid complexes described above might be caused by their geometry. The 1:1 complex should be square planar with a tridentate quinoid and a solvent molecule. The 1:2 complex may be either square planar or distorted octahedral. The two quinoid molecules should be bidentate in square planar

6) Y. Karube, Y. Ono, Y. Matsushima, and Y. Ueda, *Chem. Pharm. Bull.* (Tokyo), in press.

complex, while tridentate in octahedral complex. In order to obtain further information, the effect of the other chelating ligand on the life time of the 508-nm absorption was examined.

In the presence of varying amounts of ethylenediamine (en), the reaction of pyridoxal N-methochloride (1×10^{-4} M), ethyl alaninate (1×10^{-3} M), and Cu(II) (5×10^{-4} M) was followed. Without added en, the 508-nm absorption disappeared in several seconds. In the region [en]$=2.5-5.0\times10^{-4}$ M, the duration of the absorption was several minutes. At [en]$=7.5\times10^{-4}$ M (1.5 equimolar to Cu(II)), the absorption was intense and stable for about one day. Fig. 2 is the spectral change accompanying the reaction.

When $Cu(en)_2(ClO_4)_2$ was used ([en]$=1\times10^{-3}$ M), the intensity and the stability of the absorption were slightly lowered than at [en]$=7.5\times10^{-4}$ M. With an increase in [en] over 1×10^{-3} M, decreased the absorbance and the life time of the 508-nm band.

Similar results were obtained by the addition of other bidentate ligands such as 8-quinolinol, α-alanine, β-alanine, α-methyl-α-alanine, anthranilic acid, succinic acid, maleic acid, and phthalic acid. The duration of the absorption was in the order of the stability constants of Cu-ligand chelates[7] in the same ligand concentration. The disappearance was slowest in the presence of the bidentate ligands in 1.5—2.0 equimolar amount to Cu(II).

The tridentate ligands such as diethylenetriamine (dien), α,α',α''-tripyridyl (tripy), and glycylglycine showed the same effect in the presence of an equimolar amount. These results indicate that the life time of the quinoid elongated in a ternary complex composed of a six-coordinate Cu(II), a quinoid molecule, and one or two molecules of the ligand.

The addition of $Cu(en)_2(ClO_4)_2$ to the Schiff base formed the 508-nm species as mentioned above. One nitrogen atom must have dissociated before the coordination of the Schiff base. Unlike Cu(II) complexes, ligands are inert to exchange in Co(III) complexes. Addition of *cis* and *trans* forms of $[Co(en)_2Cl_2]Cl$ to the Schiff base did not form the quinoid absorption. On the other hand, $[Co(NH_3)_3Cl_2H_2O]Cl$, $Co(dien)Cl_3$, and $Co(tripy)Cl_3$ formed species absorbing at 513 nm. These clearly indicate that the Schiff base is tridentate and converted to the quinoid on complexing with metals.

With Zn(II) the life time of the 500-nm species was also varied by other chelating ligands, but the effect was not so prominent as with Cu(II). With Ni(II), Co(II), and Mn(II), the similar effect was hardly observed and the disappearance of the absorption was only slightly accelerated in the presence of excess metal ions.

Discussion

In the overall reaction shown in Chart 1, the chelation of the Schiff base with transition metals should be a rapid step. The other two steps proceeded in measurable rates. The results show that the deprotonation of the Schiff base chelate was faster under the conditions described above and its rate was not greatly affected by the metal ions. The poor dependence on the properties of the metals leads us to assume that the deprotonation may take place readily when the coplanarity from the aromatic ring to the carbonyl group of the ester is achieved by the metal chelation. The positive charge on the quaternized pyridine nitrogen may attract the electrons and promote the deprotonation. The role of the positive charge can be understood in the comparison with the reaction of pyridoxal. Al(III) formed the 500-nm species more slowly and the divalent metals did not produce the species in the reaction with the Schiff base of pyridoxal.[5]

Unlike the deprotonation, the rates of the reprotonation of the quinoid were greatly dependent on the metal ions and on the properties of the complexes. The extremely slow rate in the Al(III) chelate and the rapid rates in the transition metal chelates suggest that

7) L.G. Sillén and A.E. Martell, "Stability Constants of Metal-Ion Complexes," The Chemical Society, London, 1964.

the strong coordination to the quinoid molecule accelerates the reprotonation. The fact that the life time was longer in the 1:2 Cu(II) complex than in the 1:1 complex and the effect of the other chelating ligands can be explained in terms of the nature of the coordination.

The structure of the ternary complex formed form Cu(II), the quinoid molecule, and the chelating ligand must be distorted octahedral. The planar quinoid molecule should coordinate to Cu(II) with the phenolate oxygen, imine nitrogen and carbonyl oxygen atoms. Since the coordination of the chelating ligand may be stronger than that of the quinoid, the ternary complex can be assumed as shown in Chart 2. Similar distorted octrahedron can be assumed for the 1:2 Cu-quinoid complex. The structure of the 1:1 complex must be square planar with a tridentate quinoid and a solvent molecule.

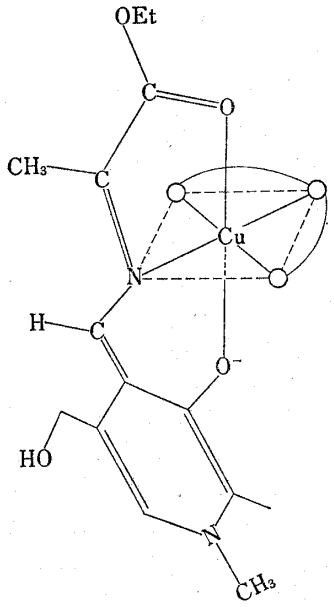

Chart 2

The bond between the carbonyl oxygen and Cu(II) is longer and weaker in the 1:2 and ternary complexes than in the 1:1 complex. If we assume that the weak coordination results in the retardation of the protonation of the quinoid molecule, the observed phenomena will be reasonably understood. The assumption is borne out by the facts that the dependence of the life time on the metal ion concentration and on the presence of the chelating ligand was prominent only with Cu(II) and that the stronger was the chelating ligand, the longer was the life time of the species.

When alanine was used in place of its ester in the reaction, the 500-nm species was either unobservable or more rapidly disappeared than in the reaction of the ester.[8] As the carboxylate oxygen coordinates more strongly than the ester oxygen, this fact also support the idea. The validity of the assumption would be verified by the isolation and the characterization of the complex.

In the previous paper,[5] we report that the 500-nm band in the reaction of pyridoxal, ethyl alaninate, and Al(III) split into two peaks under certain conditions. The shorter absorption peak was at 452 nm and was observable under slightly alkaline conditions. The other peak was at 500 nm and was intensified under acidic conditions. The former absorption was assigned to the quinoid species in which the pyridine nitrogen was not protonated, while the latter to the quinoid with the protonated pyridine nitrogen. In the reaction of pyridoxal N-methochloride, a similar split of the absorption never took place and the absorption was at longer wavelength than 500 nm. The fact supports the previous assignments.

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8) Y. Karube and Y. Matsushima, unpublished results.