Species Absorbing in the 500-nm Region in Pyridoxal Catalysis. II.[1] Trivalent Metal Chelates in Methanol[2]

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Abstract: Addition of aluminum nitrate to a mixture of pyridoxamine and ethyl pyruvate in methanol produced a species with an absorption maximum at 488 nm. This species disappeared in a few hours at room temperature with the formation of a transaminated species, the Al(III) chelate of the aldimine (ethyl pyridoxylidenealaninate). The substance absorbing at 488 nm is concluded to be a metastable intermediate in the Al(III)-catalyzed interconversion of ketimine (ethyl pyridoxyliminopyruvate) and aldimine. The metastable species absorbing in the 500-nm region of the spectrum was also observed on addition of trivalent metal ion of the aluminum subgroup or lanthanide elements to a mixture of pyridoxal and ethyl alaninate in methanol. Replacement of pyridoxal by 3-hydroxy-4-(or 2-) formylpyridine and of ethyl alaninate by ester, amide, or dipeptide of α-amino acids, but not by ethyl α-aminoisobutyrate, resulted in the formation of a similar species. On the basis of structural requirements of the reactants and on other grounds, the absorption in the 500-nm region is ascribed to the metal chelates of a carbanion, which is formulated either as a ketimine that lacks one of the protons from the 4-methylene carbon of the pyridoxamine moiety or as an aldimine deprotonated at the α carbon of the amino acid ester. In solutions containing pyridoxal, Al(III) ion, and excess ethyl alaninate, a split of the 500-nm band into two peaks was observed. This phenomenon was tentatively explained in terms of the presence of species protonated and nonprotonated on the pyridine nitrogen.

Vitamin B_6 is an essential cofactor to many enzymes which catalyze amino acid reactions. The catalytic activities have been shown to be duplicated by the cofactor in the absence of specific apoprotein. Studies on these nonenzymatic model reactions have greatly helped in understanding its catalytic role.[3] It has been accepted that both enzymatic and model systems operate according to the general mechanism proposed independently by Braunstein and Shemyakin[4] and by Metzler, Ikawa, and Snell.[5]

According to this theory, transamination between α-amino acid and α-keto acid proceeds by formation and isomerization of Schiff bases, *i.e.*, aldimine and ketimine.

$$\text{pyridoxal} + \text{RCH(NH}_2\text{)COOH} \underset{+H_2O}{\overset{-H_2O}{\rightleftharpoons}} \text{aldimine} \rightleftharpoons$$

$$\text{ketimine} \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} \text{pyridoxamine} + \text{RCOCOOH}$$

In most enzymatic and model systems, isomerization between an aldimine and a ketimine was rate determining and was, therefore, the subject of kinetic studies. This isomerization step necessarily involves an intermediate in which the α carbon of an amino acid in an aldimine is deprotonated. This intermediate was first postulated in the classical paper by Metzler, Ikawa, and Snell.[5] In model systems, kinetic evidence for the intermediate was obtained by Matsushima and Martell[6] and by Auld and Bruice.[7] In these studies, the intermediate was in low steady state and, therefore, was not directly observed as a discrete chemical species.

Jenkins[8] found that *erythro*-β-hydroxy-L-aspartate reacted with an aspartate aminotransferase to form a complex absorbing in the 500-nm region of the spectrum and suggested that the deprotonated aldimine structure was responsible for the absorption. Morino and Snell[9] observed enzyme–substrate and –pseudosubstrate complexes of the same spectral character in tryptophanase. They showed that the α hydrogen of L-alanine, a pseudosubstrate, was labilized along with the formation of the complex and supported the assignment of the absorption to the deprotonated aldimine structure.

In a model system, Schirch and Slotter[10] observed a species with an absorption peak at 480 nm in an ethanol solution of pyridoxal *N*-methochloride and diethyl aminomalonate. Maley and Bruice[11] reported a similar species in the reaction of 1-methyl-4-formylpyridinium iodide with α-amino acids in water. In the previous communication,[1] we reported a species absorbing at 488 nm in nonenzymatic transamination system containing pyridoxamine, ethyl pyruvate, and Al(III) ion in methanol. This was the first observed intermediate with a visible absorption in the metal ion mediated pyridoxal catalysis. In this work, the intermediate was observed with an unmodified vitamin B_6 compound, pyridoxamine. The preceding workers failed to find the species with compounds lacking a quaternized pyridine nitrogen.

Quite recently, Abbott and Martell[12] studied nmr spectra of a D_2O solution containing pyridoxal, amino

(1) Part I: S. Matsumoto and Y. Matsushima, *J. Amer. Chem. Soc.*, **94**, 7211 (1972).

(2) This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education of Japan.

(3) See, for example, (a) E. E. Snell, P. M. Fasella, A. E. Braunstein, and A. Rossi-Fanelli, Ed., "Chemical and Biological Aspects of Pyridoxal Catalysis," Macmillan, New York, N. Y., 1963; (b) E. E. Snell, A. E. Braunstein, E. S. Severin, and Yu. M. Torchinsky, Ed., "Pyridoxal Catalysis: Enzymes and Model Systems," Interscience, New York, N. Y., 1968.

(4) A. E. Braunstein and M. M. Shemyakin, *Biokhimiya*, **18**, 393 (1953).

(5) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Amer. Chem. Soc.*, **76**, 648 (1954).

(6) Y. Matsushima and A. E. Martell, *J. Amer. Chem. Soc.*, **89**, 1331 (1967).

(7) D. S. Auld and T. C. Bruice, *J. Amer. Chem. Soc.*, **89**, 2098 (1967).

(8) W. T. Jenkins, *J. Biol. Chem.*, **239**, 1742 (1964).

(9) Y. Morino and E. E. Snell, *J. Biol. Chem.*, **242**, 2800 (1967).

(10) L. Schirch and R. A. Slotter, *Biochemistry*, **5**, 3175 (1966).

(11) J. R. Maley and T. C. Bruice, *J. Amer. Chem. Soc.*, **90**, 2843 (1968).

(12) E. H. Abbott and A. E. Martell, *J. Amer. Chem. Soc.*, **95**, 5014 (1973).

acid, and aluminum ion and reported the presence of an intermediate with a structure of aluminum chelate of a deprotonated aldimine. Since the D_2O solution was colorless, the intermediate they studied may not be the same as that in the previous studies.[1,10,11] Abbott and Bobrik[13] reported the isolation of a 1,4-dihydropyridine tautomer of a Schiff base from pyridoxal and diethyl aminomalonate. This may be a compound closely related to the species with a visible absorption.

The present paper describes a species absorbing in the 500-nm region observed in systems containing pyridoxal, amino acid derivatives, and trivalent metal ions in methanol with the details of the previous communication.[1]

Experimental Section

Experimental Procedures. A typical experimental procedure was as follows. Solutions of pyridoxal and ethyl alaninate were prepared by dissolving their hydrochlorides in methanol with an equimolar amount of KOH. These solutions were prepared immediately before the measurements in order to minimize reactions with the solvent.

The neutral methanol solutions of pyridoxal and ethyl alaninate were mixed in a volumetric flask. The mixture showed a gradual spectral change, which was completed in 3 hr. The final spectrum had strong bands at 338 and 254 nm and a weak band at 420 nm, a combination indicating the formation of an aldimine.[14] To the aldimine solution, a methanolic aluminum nitrate and, then, methanol were added to a definite volume. The moment of the addition of aluminum ion was taken as the initiation of the reaction and the concentrations of the reactants were expressed as those in the final mixture. An aliquot was transferred to a glass-stoppered 10-mm silica cell and submitted to absorption measurements. The absorption spectra were recorded with pure methanol as a reference. Spectrophotometers used were Hitachi Model EPS-3T and Shimadzu Model MPS-50. The preparation and mixing of the solutions and the spectral measurements were carried out at room temperature.

Materials. Spectrograde methanol was used directly as a solvent. Pyridoxal hydrochloride, pyridoxamine dihydrochloride, salicylaldehyde, 3-hydroxypyridine, *o*-hydroxybenzylamine, methyl histidinate hydrochloride, glycylglycine, alanylglycine, methyl glycylglycinate, glycinamide hydrochloride, ethyl pyruvate, ethyl phenylglyoxylate, and sodium pyruvate were obtained from commercial sources. They were purified by recrystallization or by distillation prior to use. Inorganic substances were certified reagent grade chemicals and were used without further purification. Ethyl α-ketobutyrate,[15] 3-hydroxy-4-formylpyridine,[16] 3-hydroxy-2-formylpyridine,[16] 3-methylpyridoxal,[17] and alaninamide[18] were synthesized according to the references cited. 4-Aminomethylpyridine and 3-hydroxy-4-aminomethylpyridine were prepared by reduction of oximes of 4-formylpyridine and 3-hydroxy-4-formylpyridine, respectively, with a Pd/C catalyst. Hydrochlorides of amino acid esters were prepared by bubbling dry HCl through ethanolic suspensions of amino acids.

Results

I. Reactions from Pyridoxamine. Reaction of Pyridoxamine, Ethyl Pyruvate, and Aluminum Ion. When a methanolic aluminum nitrate was added to a preequilibrated mixture of pyridoxamine and ethyl pyruvate, an absorption peak appeared at 488 nm with a shoulder at around 460 nm. Its intensity reached a maximum 15–30 min after the addition of Al(III) ion. The absorption band was almost superimposable on that reported in the enzyme systems.[8,9] The 488-nm band decreased gradually with an increase of the intensity of a newly formed absorption at around 360 nm. In several hours, the visible absorption disappeared and absorption bands at 365 and 270 nm were observed in the final spectrum.[19] These bands can be ascribed to the Al(III) chelate of the aldimine (ethyl pyridoxylidenealaninate), as it is well established that the metal chelates of pyridoxylideneamino acids have π bands in these regions of the spectrum.[14b,20]

The spectral change observed clearly indicates that the species assignable to the 488-nm band is a metastable intermediate in the Al(III)-catalyzed isomerization of the ketimine and the aldimine and is closely related to the enzyme–substrate complexes of the same spectral character.[8,9]

For the generation of the 488-nm species, all three reactants, pyridoxamine, ethyl pyruvate, and Al(III) ion, were essential. As the use of aluminum perchlorate or chloride in place of aluminum nitrate gave the same results, the anions do not participate in the formation of the intermediate. The 488-nm absorption disappeared instantly on addition of chelating agents such as tetrasodium ethylenediaminetetraacetate and acetylacetone. This shows that the Al(III) is involved in the species.

When the three reactants were mixed simultaneously, only a very slight absorption was observed in the visible region. In solutions 1×10^{-4} *M* in pyridoxamine and 1×10^{-2} *M* in ethyl pyruvate, the intensity of the 488-nm band increased with an increase of the Al(III) concentration and leveled off at 5×10^{-5} *M*. This suggests the ratio of pyridoxamine to Al(III) ion in the species is 2:1.[21] No signal was observed in an esr measurement of a solution containing the 488-nm species.[22] This fact shows that the species is not a radical.

Replacement Studies. In order to know the structural features of the reactants for the formation of a similar absorption band, one of the three reactants was replaced by analogous compounds.

Replacement of pyridoxamine by 3-hydroxy-4-aminomethylpyridine produced a less intense 488-nm band. *o*-Hydroxybenzylamine, 4-aminomethylpyridine, and 3-hydroxypyridine did not form a similar band. These facts indicate that, in addition to aminomethyl group necessary for ketimine formation, *o*-hydroxy group and pyridine nitrogen are required for the 488-nm species.

Neither acidic nor carboxylate forms of pyruvic acid could replace its ester. Ethyl esters of glyoxylic acid, α-ketobutyric acid, and phenylglyoxylic acid formed a similar but less intense band.

With Ga(III) ion, the appearance and the disappearance of the visible absorption were faster and the intensity was greater than with Al(III) ion. With In(III) ion,

(13) E. H. Abbott and M. A. Bobrik, *Biochemistry*, **12**, 846 (1973).
(14) (a) D. E. Metzler, *J. Amer. Chem. Soc.*, **79**, 485 (1957); (b) Y. Matsushima and A. E. Martell, *ibid.*, **89**, 1322 (1967); (c) Y. Matsushima, *Chem. Pharm. Bull.*, **16**, 2046 (1968).
(15) E. Vogel and H. Schinz, *Helv. Chim. Acta*, **43**, 125 (1950).
(16) D. Heinert and A. E. Martell, *J. Amer. Chem. Soc.*, **81**, 3933 (1959).
(17) D. Heyl, E. Luz, S. A. Harris, and K. Folkers, *J. Amer. Chem. Soc.*, **73**, 3430 (1951).
(18) P. S. Yang and M. M. Rising, *J. Amer. Chem. Soc.*, **53**, 3183 (1931).
(19) This spectral change was presented in Figure 1 of the previous communication, ref 1.
(20) (a) L. Davis, F. Roddy, and D. E. Metzler, *J. Amer. Chem. Soc.*, **83**, 127 (1961); (b) Y. Matsushima, *Chem. Pharm. Bull.*, **16**, 2143 (1968).
(21) For more exact determination of the ratio, rates of appearance and disappearance of the 488-nm species may be considered. Kinetic studies on the reactions discussed in this paper are in progress and will be reported.
(22) The esr measurement was carried out by Drs. S. Kida and Y. Nonaka of the Department of Chemistry, Kyushu University, to whom the authors are grateful.

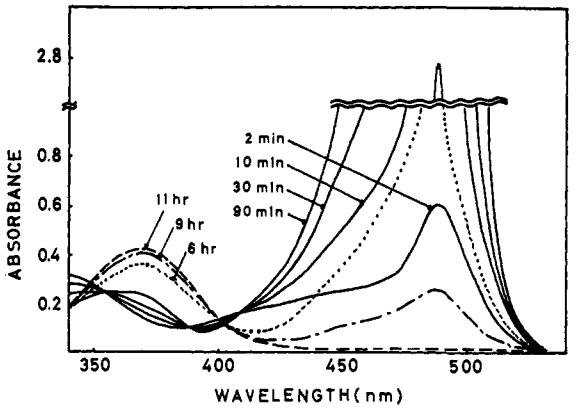


Figure 1. Spectral-time study of the reaction of 1×10^{-4} M pyridoxal, 1×10^{-4} M ethyl alaninate, and 1×10^{-4} M aluminum nitrate in methanol. Times after initiating the reaction are indicated beside the spectral curves.

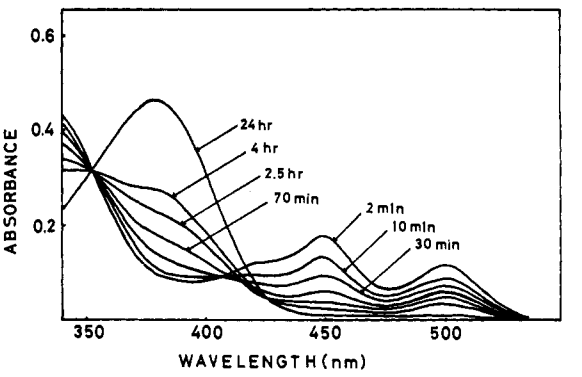


Figure 2. Spectral-time study of the reaction of 1×10^{-4} M pyridoxal, 1×10^{-3} M ethyl alaninate, and 2×10^{-4} M aluminum nitrate in methanol. Times after initiating the reaction are indicated beside the spectral curves.

a small and broad absorption was found in the 500-nm region. Though Zn(II), Ni(II), and Cu(II) ions catalyze the isomerization of the ketimine to the aldimine smoothly in the pyridoxamine–ethyl pyruvate–metal ion system,[23] an intermediate with the visible absorption was not observed. No visible absorption was produced by Mg(II), Ca(II), Mn(II), Fe(III), Cd(II), Sn(II), La(III), Gd(III), and Lu(III) ions.

Effects of Solvents. Reaction of pyridoxamine, ethyl pyruvate, and Al(III) ion in water did not produce a similar species to that in methanol. This is probably caused by a decreased formation of Schiff bases in aqueous media. Measurements in solvents other than water and alcohols were impossible, as not all of the three reactants are soluble in these solvents. Then, an equal amount of pure solvent was added to methanol solution containing 488-nm species. Addition of water, pyridine, ethanolamine, *n*-propylamine, triethylamine, and methanol containing KOH destroyed the species instantly. On the other hand, addition of ethanol, diethyl ether, dioxane, cyclohexane, petroleum ether, chloroform, carbon tetrachloride, carbon disulfide, ethyl acetate, acetone, dimethylformamide, and dimethyl sulfoxide did not change the spectrum and the half-life of the species significantly.

II. Reactions from Pyridoxal. Reaction of Pyridoxal, Ethyl Alaninate, and Al(III) Ion. Figure 1 shows spectral changes when Al(III) ion was added to the aldimine formed from pyridoxal and ethyl alaninate in methanol. Concentrations of the three reactants were 1×10^{-4} M. An intense absorption band began to appear at 488 nm immediately after the addition of Al(III) ion. The absorbance at 488 nm reached its maximum 90 min after the addition and was stable for about an hour before it decreased gradually and disappeared in about 10 hr. There were two broad absorptions at around 370 nm and at 280 nm in the spectrum after the disappearance of the band at 488 nm.

The absorbance of the 488-nm band was 2.8 in a 10-mm cell at its maximum. Such a strong intensity can only be expected with π–π^* electronic transitions of an organic ligand.

For the full generation of the 488-nm band, the formation of the aldimine of pyridoxal and ethyl alaninate was necessary before the addition of Al(III) ion. The addition of pyridoxal to a mixture of ethyl alaninate and Al(III) ion or that of ethyl alaninate to a mixture of pyridoxal and Al(III) ion produced a slight absorption in the visible region.

In a solution 1×10^{-4} M in pyridoxal and 1×10^{-3} M in ethyl alaninate and in Al(III) ion, the absorbance and the shape of the band did not differ significantly from those shown in Figure 1, though the appearance and the disappearance were faster. The concentration of Al(III) ion was varied in order to investigate its effect on the spectra in the visible region in solutions 1×10^{-4} M in pyridoxal and 1×10^{-3} M in ethyl alaninate. The spectra of solutions 5×10^{-4} M and 1×10^{-3} M in Al(III) ion were essentially identical. In a 3.5×10^{-4} M solution, the absorption peak shifted to 499 nm with a decreased intensity. In a 3×10^{-4} M solution, two distinct absorption peaks were observed at 452 and 500 nm. The peak at 500 nm was higher than that at 452 nm. In a 2×10^{-4} M solution, the peak at 452 nm was higher and the absorbance and the half-life of the visible absorption decreased, as shown in Figure 2. Solutions 1×10^{-4} M or lower concentrations in Al(III) ion showed only a slight absorption in the 500-nm region. Thus, in an excess of ethyl alaninate, the 488-nm absorption decreased in intensity and split into two peaks.

Replacement Studies. The solutions used in these studies were 1×10^{-4} M in pyridoxal and its analogs, and 1×10^{-3} M in derivatives of amino acids and metal ions, unless otherwise noted. 3-Hydroxy-4-formylpyridine and 3-hydroxy-2-formylpyridine could replace pyridoxal, while 3-*O*-methylpyridoxal, 4-formylpyridine, and salicylaldehyde could not, under the same conditions. Thus, the essential parts of pyridoxal required for absorption in the 500-nm region are formyl group, the pyridine nitrogen atom, and the *o*-hydroxy group, characteristics associated with species involved in the generally accepted mechanism of pyridoxal catalysis.[3-5]

For the observed phenomena, ethyl alaninate could be replaced by esters of α-amino acids but not by α-amino acids or simple amines. Absorption peak at 474 nm formed by ethyl glycinate disappeared in 30 min. On the other hand, similar absorption bands formed by esters of phenylalanine, histidine, and tryptophan were stable for more than 20 hr.

(23) Y. Karube, S. Matsumoto, and Y. Matsushima, unpublished results.

Amides and dipeptides of α-amino acids also replaced ethyl alaninate for the formation of the visible absorption. Glycinamide, glycylglycine, and methyl glycylglycinate formed a rather short-lived intermediate as ethyl glycinate. The absorption bands formed by alaninamide and alanylglycine had a peak at 504 and 510 nm, respectively.

The long-wavelength band was not observed with ethyl esters of the following amino acids: α-aminoisobutyric acid, which lacks an α hydrogen, β-alanine, which is a β-amino acid, and sarcosine, which possesses a secondary amino group.

Trivalent metal ions of an aluminum subgroup and most of the lanthanide elements produced a similar band in the 500-nm region in the pyridoxal–ethyl alaninate–metal ion system. With transition metal ions such as Fe(II), Fe(III), Ni(II), Cu(II), and Zn(II) ions, the band was not observed.

Ga(III) ion formed a similar intense band at 488 nm. As in the case of Al(III) ion, a decrease in concentration of Ga(III) ion in an excess of ethyl alaninate resulted in a decrease in intensity and a split of the band into two peaks at 452 and 498 nm.

Figure 3 shows a spectral change observed on addition of Gd(III) ion to an aldimine solution. The visible bands formed by the trivalent ions except Al(III) and Ga(III) ions were less intense, as in the case of Gd(III) ion. The band did not have a shoulder at the short wavelength side and the disappearance was faster.

The wavelengths of the band seem to be correlated with the ionic radii of the metal ions (Table I). With

Table I. Absorption Maxima of the 500-nm Band in the Pyridoxal–Ethyl Alaninate–Metal Ion System

Metal ion	λ_{max}, nm	Ionic radius,[a] Å	Metal ion	λ_{max}, nm	Ionic radius,[a] Å
Al(III)	488	0.51	Gd(III)	533	0.97
Ga(III)	488	0.62	Sm(III)	534	1.00
In(III)	504	0.81	Nd(III)	534	1.04
Lu(III)	527	0.85	Pr(III)	536	1.06
Ho(III)	530	0.91	Ce(III)		1.07
Y(III)	530	0.92	La(III)		1.14

[a] R. C. West, Ed., "Handbook of Chemistry and Physics," The Chemical Rubber Co., Cleveland, Ohio, 1965.

lanthanide ions of larger ionic radii, the 500-nm bands were observed only in the presence of excess metal ions. A similar band was not observed with La(III) and Ce(III) ions.

Effect of Acid and Base Concentrations. Effect of HCl and KOH concentrations on the formation of the 488-nm band was studied in solutions 1×10^{-4} *M* in pyridoxal and 1×10^{-3} *M* in ethyl alaninate and in Al(III) ion. The desired acid and base concentrations were obtained in aldimine solutions by adding standard methanol solutions of HCl or KOH.

In solutions 5×10^{-4} *M* or higher concentrations in KOH, the visible absorption was not formed. In a 2.5×10^{-4} *M* KOH solution, a small absorption appeared. The absorbance of the band increased with an increase of HCl concentration in the range of 0–1 $\times$ 10^{-3} *M*. Under the more concentrated HCl, the aldimine was not formed and, therefore, the visible band was not observed.

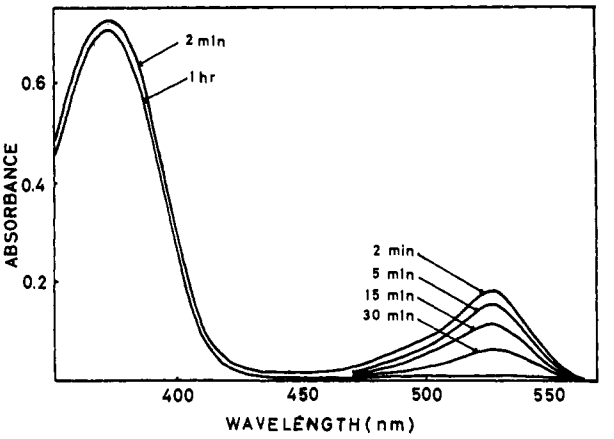


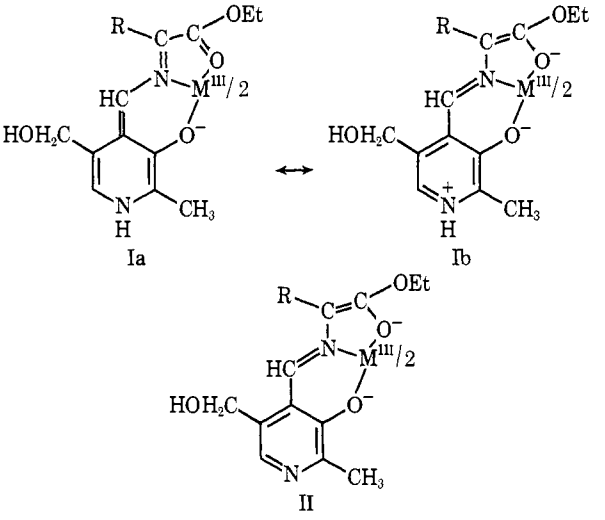
Figure 3. Spectral-time study of the reaction of 1×10^{-4} *M* pyridoxal, 1×10^{-3} *M* ethyl alaninate, and 1×10^{-3} *M* gadolinium chloride in methanol. Times after initiating the reaction are indicated beside the spectral curves.

To a solution having two absorption peaks at 452 and at 500 nm (Figure 2), a small amount of a methanolic HCl was added to make the solution 3×10^{-4} *M* in HCl. The 500-nm peak increased, while the 452-nm peak decreased in intensity. When a small amount of a methanolic KOH was added, the 500-nm peak disappeared instantly, but the 452-nm peak did not show an immediate decrease.

Discussion

The visible absorption bands observed in the pyridoxamine–ethyl pyruvate–Al(III) ion system and in the pyridoxal–ethyl alaninate–Al(III) ion system were the same in wavelength and in the band shape and, hence, are assigned to the same species. The species is concluded to be the Al(III) chelate of a carbanion, which may be formulated either as a ketimine that lacks one of the protons from the 4-methylene carbon of the pyridoxamine moiety or as an aldimine deprotonated at the α carbon of the amino acid ester. The metal chelate of the carbanion may be expressed as Ia and Ib (Scheme I).

Scheme I



The fact that the species was observed with esters of α-keto acids and of α-amino acids and not with carboxylate forms indicates large contribution of the structure Ib to the stability of the species. Large delocalization

of π electrons from the aromatic ring to the carbonyl oxygen can well explain the absorption in the long wavelength region.

This assignment is supported especially by the fact that ethyl α-aminoisobutyrate, lacking an α hydrogen, did not form the visible band. Glycine derivatives form a short-lived species, while species formed by esters of aromatic amino acids were stable for about 20 hr. This shows a considerable contribution of the amino acid side chains, R, to the stability of the carbanion.

The fact that two absorption peaks were formed in an excess of ethyl alaninate in the pyridoxal–ethyl alaninate–Al(III) ion system (Figure 2) suggests the presence of two different species. We tentatively assign these two absorption peaks to species protonated, I, and nonprotonated, II, on the pyridine nitrogen. It is well established[14,24] that protonation of pyridine nitrogen causes a red shift of 1–2 kcm^{-1} in a π band of pyridoxal and related substances. In the presence of sufficient Al(III) ion, an intense absorption of the protonated species, I, at a longer wavelength side hides that of the nonprotonated species, II. The latter is, then, observed as a shoulder on the short wavelength side of the band. In the presence of excess ethyl alaninate, however, it complexes Al(III) ion and lowers the concentration of the Al(III) chelate of the carbanion species. Then, the peak at 452 nm becomes observable with a decreased absorption of the main peak. Effects of the addition of methanolic HCl and KOH provided a further evidence to the assignments.

There may be several other explanations for this phenomenon. In an aqueous solution, bisaluminum complexes of an aldimine of pyridoxal and α-amino acids were demonstrated to exist as three diastereoisomers.[25] In a methanol solution of the Al(III) chelate of the carbanion, the species may also exist as several stereoisomers. Formation of mixed ligand complexes as Al(III)–carbanion–ethyl alaninate and that of polynuclear complexes are also probable. However, wavelengths of the visible band are not greatly different in these complexes, as the band has its origin in π–π^* electronic transition of the organic moiety. Therefore, the presence of these various complexes is unlikely to cause the split of the band. Further investigations are in progress in this laboratory to elucidate the phenomenon.

In the pyridoxal–ethyl alaninate–Al(III) ion system, the spectrum after the disappearance of the 488-nm band had very broad absorption bands at 370 and 280 nm. The former band can be assigned to the Al(III) chelate of the aldimine.[14,20] The latter band may be produced by the superposition of the second π band of the aldimine chelate at 270 nm and the band assignable to the Al(III) chelate of the ketimine at around 300 nm. When both ethyl alaninate and Al(III) ion were in excess, the absorbance of the 370-nm band decreased, while that of the 280-nm band increased with a slight red shift, in the final spectrum. These facts support the assignments of the band. However, even with a large excess of ethyl alaninate and Al(III) ion, the 370-nm band was still observed, indicating the presence of the aldimine chelate in the final equilibrium.

In the pyridoxamine–ethyl pyruvate–Al(III) ion system, conversion of the ketimine to the aldimine was almost complete with an excess of ethyl pyruvate. In reactions both from pyridoxamine and from pyridoxal, the final equilibria of the isomerization seem to be displaced to the formation of aldimine.

Isomerization of a ketimine from pyridoxamine and ethyl pyruvate to an aldimine in methanol was catalyzed by many ions including Zn(II), Ni(II), Cu(II), Al(III), and Ga(III) ions.[23] Trivalent lanthanide ions were very poor catalysts of the isomerization. The aldimine chelates were not formed significantly even after 3 days. This might be the reason why the 500-nm absorption was not observable in the pyridoxamine–ethyl pyruvate–lanthanide ion system. In the pyridoxal–ethyl alaninate–lanthanide ion system, intensities of the visible bands were small and the disappearance was fast. The spectra after the disappearance, having strong absorption bands at 380 and 270 nm, show the presence of a large amount of the aldimine chelate and of the ketimine chelate in a very small amount, if any.

There may be an optimum ionic radius for the 500-nm metal chelates. An increase of ionic radius in lanthanide ions decreased the half-lives of the chelate. Thus, La(III) and Ce(III) ions did not form the species.

Maley and Bruice observed a transient visible absorption in nonenzymatic transamination of 1-methyl-4-formylpyridinium iodide by amino acids in water.[11] However, they failed to observe a similar spectrum with 1-methyl-3-hydroxy-4-formylpyridinium chloride,[26] which has a structure more closely related to that of vitamin B_6. Metzler and coworkers[27] reported the appearance of a small absorption at 485 nm on addition of glutamate to a mixture of *O*-methylpyridoxal phosphate and apoaspartate aminotransferase. Corresponding absorption was not observed with analogs of pyridoxal phosphate having a 3-phenolic group. These facts suggest that a free phenolic or a phenolate group prevents the appearance of the visible absorption. In the present model systems, this effect would be diminished by the strong coordination of trivalent metal ions to phenolate oxygen. Coordination of metal ions to the carbonyl oxygen of the ester group would stabilize the carbanion in the chelate form.

Following implications to enzyme chemistry may be possible from the present results. The 500-nm absorption in enzymes is ascribed to the carbanion structure. The carbanion is stabilized by apoprotein in enzymes and by chelation to trivalent metal ions in the present enzyme model. The phenolate of the coenzyme and carboxylate of substrate may interact strongly with positively charged groups of apoprotein, as the phenolate and carboxylate groups seem to instabilize the carbanion. For the species absorbing in the 500-nm region coplanarity from the aromatic ring to the α carbon of amino acid has been assumed. However, it is concluded that the coplanar conjugated system extends from the aromatic ring to the carbonyl oxygen.

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