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The Models for the Active Site of Pyridoxal Enzymes. Calculations of π-π^* Transition Energies of Electronic Absorption[1]

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The theoretical energies and oscillator strengths of the π-π^* transitions were calculated by the Pariser-Parr-Pople type ASMO-SCF-LCAO-CI-MO method for the spectroscopic models for the active site of pyridoxal enzymes. The calculated structures are eight molecular species distinguished by the state of the protonation at the phenolate and the pyridine and azomethine nitrogens in the aldimine of 3-hydroxy-4-formylpyridine (**1**) with methylamine, species of the quinoid intermediate in which the α-carbon is deprotonated in the aldimine of **1** or 1-methyl-3-hydroxy-4-formylpyridinium (**6**) with alanine methyl ester, species of the aldimine of **6** and α-aminoacrylate anion, and species of the quinoid intermediate derived from **6** and 2-amino-3-butenoate anion. The calculated transition energies agreed satisfactorily with the observed ones and gave support for the band assignments made previously.

Keywords——ASMO-SCF-LCAO-CI-MO method; pyridoxal enzymes; enzyme model; Schiff base; quinoid intermediate; *o*-hydroxyaromatic aldehyde; α-aminoacrylic acid; 2-amino-3-butenoic acid; π-π^* transition energy

It has been established in pyridoxal enzymes that the coenzyme is in the forms of Schiff base and the three basic groups, *i.e.*, the phenolate, -O$^-$, the pyridine and azomethine nitrogens, are either protonated or deprotonated according to the environment. Hence, eitht molecular species can arise from a Schiff base. The diversities of the spectral properties of the enzymes are attributable to these structural factors of the chromophore.[3]

The correct interpretation of the spectra of the Schiff bases of pyridoxal phosphate (PLP) and related substances is essential for the understanding of the structure of the active site. In the previous paper of this series,[1a] spectral band assignments for the molecular species of the aldimines[4] derived from 3-hydroxy-4-formylpyridine (**1**), pyridoxal (PL), PLP, and related *o*-hydroxyaldehydes were established from the analyses of the spectra under various conditions. In agreement with the results that the longest band assigned to the aldimine species was 426 nm, none of the enzyme has an absorption peak above 430 nm.[3]

It has been found, however, that in the presence of substrates some enzymes exhibit transient absorption bands at the longer wavelength region and these bands have been ascribed to metastable intermediates in the catalysis.[5,6] The spectra of the enzymatic intermediates

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[1] Part II of molecular species of Schiff bases derived from *o*-hydroxyaromatic aldehydes. *a*) Part I: Y. Karube and Y. Matsushima, *Chem. Pharm. Bull.* (Tokyo), 25, 2568 (1977).

[2] Location: *Maidashi, Higashi-ku, Fukuoka, 812, Japan*; *a*) Author for correspondence.

[3] *a*) E.E Snell and S.J. DiMari, "The Enzymes," 3rd Ed., II., ed. by P.D. Boyer, Academic Press, New York, N.Y., 1970, Chapter 7; *b*) R.J. Johnson and D.E. Metzler, "Method in Enzymology," Vol. 18, Pt. A, ed. by D.B. McCormick and L.D. Wright, Academic Press, New York, N.Y., 1970, pp. 433—471; and the references therein.

[4] In this paper, the term "aldimine" indicates the condensation products of the aromatic aldehydes with simple primary amines and amino acids, and the term "Schiff base" is used to represent the aldimine and the intermediate species described below.

[5] W.T. Jenkins, *J. Biol. Chem.*, **239**, 1742 (1964); Y. Morino and E.E. Snell, *ibid.*, **242**, 2800 (1967).

[6] M.E. Goldberg and R.L. Baldwin, *Biochemistry*, **6**, 2113 (1967); M.A. Becker, N.M. Kredich, and G.M. Tomkins, *J. Biol. Chem.*, **244**, 2418 (1969); S. Guggenheim and M. Flavin, *ibid.*, **246**, 3562 (1971); M. Tokushige and A. Nakazawa, *J. Biochem.*, 72, 713 (1972).

were proved to be reproducible in nonenzymatic model reactions.[7-9] The structures of the intermediates and the regions of the spectrum they absorb have been proposed as follows: the quinoid intermediate, in which the α-carbon of the amino acid in the aldimine is deprotonated, absorbs at ~500 nm[7,8]; the aldimine of α,β-unsaturated amino acid at ~470 nm[9a]; the quinoid intermediate derived from β,γ-unsaturated amino acid at ~550 nm.[9b]

From the intensities and the mode of the wavelength shift in various media, the spectral bands are ascribable to the π-π^* transitions. The n-π^* bands are supposed to be hidden by the strong π-π^* bands.

With a view to obtaining further evidence for the band assignments to the molecular species of the Schiff bases,[1a,8,9] we calculated the theoretical energies and oscillator strengths of the π-π^* transitions by the Pariser-Parr-Pople[10] type ASMO-SCF-LCAO-CI-MO method, in which the two-center repulsion integrals were calculated by the Mataga-Nishimoto approximation.[11]

Experimental

Values for the one-center Coulomb repulsion integral, the valence state ionization potential, and the effective nuclear charge used in the calculation were those reported by Kwiatkowski.[12] The same values were employed for both azomethine and pyridine nitrogen atoms. Methyl group was treated as a hyperconjugation model.[13] The CI calculations for electronic transitions were performed including the total thirty-six singly excited configurations which correspond to all one-electron excitations between the highest six occupied orbitals and the lowest six vacant orbitals.

The Schiff bases were regarded as planar molecules. Values for atomic distances and bond angles in benzene[14] and pyridine[15] rings were taken from the cited literature. The geometries in the azomethine and amino acid regions were assumed as follows; the bond angles in the conjugated system were 120° and the aromatic and the amino acid moieties were at *trans* position; atomic distances were standard values reported.[16]

Calculations were carried out on a FACOM 230-60 computer at the Computation Center of Kyushu University.

Experiments on the spectral properties of the intermediates were performed according to the procedure described in the previous papers.[8,9]

Results and Discussion

Aldimine

Comparison of the spectra of the aldimines derived from **1**, PL, and PLP showed that the substituents at the 2 and 5 positions of PL and PLP have no profound effect on the spectral properties of the species and the corresponding species of the aldimines of the aldehydes have quite similar properties. MO calculations are planned to include the eight structures, A_1I—

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7) L. Schirch and R.A. Slotter, *Biochemistry*, **5**, 3175 (1966); E.H. Abbott and M.A. Bobrik, *ibid.*, **12**, 846 (1973); J.R. Maley and T.C. Bruice, *J. Am. Chem. Soc.*, **90**, 2843 (1968).

8) S. Matsumoto and Y. Matsushima, *J. Am. Chem. Soc.*, **94**, 7211 (1972); **96**, 5228 (1974).

9) *a*) Y. Karube and Y. Matsushima, *J. Am. Chem. Soc.*, **98**, 3725 (1976); *b*) *Idem*, *ibid.*, **99**, 7356 (1977).

10) R. Pariser and R.G. Parr, *J. Chem. Phys.*, **21**, 466, 767 (1953); J.A. Pople, *Trans. Faraday Soc.*, **49**, 1375 (1953).

11) N. Mataga and K. Nishimoto, *Z. Physik. Chem.* (Frankfurt), **13**, 140 (1957).

12) J.S. Kwiatkowski, *Theoret. Chim. Acta*, **10**, 47 (1968); **11**, 167 (1968); M. Berndt and J.S. Kwiatkowski, *ibid.*, **17**, 35 (1970).

13) T. Morita, *Bull. Chem. Soc. Jpn.*, **33**, 1486 (1960).

14) L.E. Sutton (ed.) "Tables of Interatomic Distances and Configuration in Molecules and Ions," The Chemical Society, London, 1958 and 1965.

15) K.K. Innes, J.P. Byrne, and I.G. Ross, *J. Mol. Spectr.*, **22**, 125 (1967).

16) J.A. Pople and D.L. Beveridge, "Approximate Molecular Orbital Theory," McGraw-Hill, New York, N.Y., 1970.

A$_1$VIII17) (Chart 1), that are involved in the acid-base equilibria of the aldimine of **1**. As the spectra are almost identical in the aldimine of **1** with butylamine and methylamine, the calculations are carried out on the latter aldimine, whereas the spectral analyses were made on the former.1a)

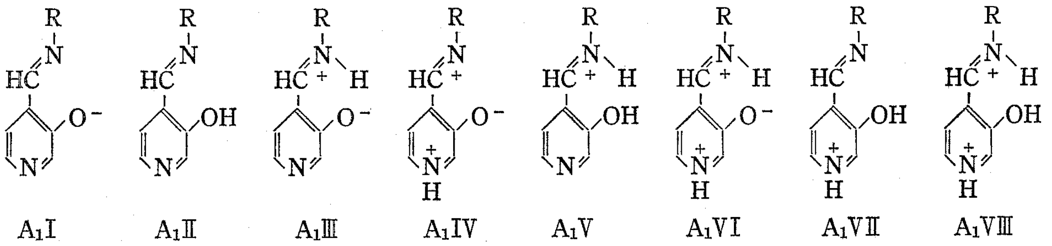
A$_1$I A$_1$II A$_1$III A$_1$IV A$_1$V A$_1$VI A$_1$VII A$_1$VIII

Chart 117)

The parameter κ18) was determined as 0.4214 so that the result fit in the observed energy of the lowest π-π^* band of the anionic species, A$_1$I, the assignment of which is unequivocal. With this value, the π-π^* transition energies were calculated for benzene, pyridine, and their mono- and disubstituted derivatives with hydroxy, methoxy, and formyl groups. The results were consistent with the reported data.3b,19)

Table I lists the results of the calculation for A$_1$I—A$_1$VIII with the observed data.1a) The agreement of the transition energy of A$_1$I comes from the determination of the parameter κ. Good agreements in the structures A$_1$II—A$_1$VII lend further support for the previous assignments.1a) For A$_1$VIII the calculated energy was somewhat lower than the observed one. Probably, the extremely short life time of the species and the overlapping of the band of A$_1$V misled the spectral assignment.

Table I. π-π^* Transitions of Aldimine, A$_1$

Species	Obs.a,b)		Calcd.a)	
A$_1$I	3.40(6700)		3.40(0.223)	4.73(0.150)
A$_1$II	3.85(3300)	5.06(8400)	3.91(0.156)	4.83(0.366)
A$_1$III	2.98 (900)		3.14(0.297)	4.72(0.259)
A$_1$IV	3.21		3.14(0.302)	4.66(0.277)
A$_1$V	3.40		3.38(0.214)	4.66(0.393)
A$_1$VI	2.97(3500)	4.73(4700)	2.86(0.395)	4.58(0.213)
A$_1$VII	3.79(2100)	4.86(5300)	3.63(0.298)	4.81(0.430)
A$_1$VIII	3.40		3.06(0.318)	4.67(0.317)

a) Observed and calculated transition energies are expressed in eV unit. Numbers in parentheses are either observed extinction coefficient or calculated oscillator strength.
b) Observed values are taken from ref. 1*a*.

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17) Throughout this paper, the following symbols are used to represent the species or the resonance structures. The kind of the Schiff base is indicated by the letters: A, aldimine; B, the quinoid intermediate; C, the aldimine of α-aminoacrylic acid; D, the quinoid intermediate derived from β,γ-unsaturated amino acid. The aldehyde from which the Schiff base is derived is indicated, where necessary, by the subscript of Arabic numerals to the letters. Roman numerals indicate the molecular species distinguished by the state of the protonation on the three basic groups as in the previous paper (ref. 1*a*). Lower case is used in the distinction of resonance structures.

18) The parameter κ is defined by the equation, $\beta_{rs}=-\kappa S_{rs}(I_r+I_s)$, where β_{rs} and S_{rs} are the resonance and overlap integrals, respectively, for r and s neighbors and I is the valence state ionization potential.

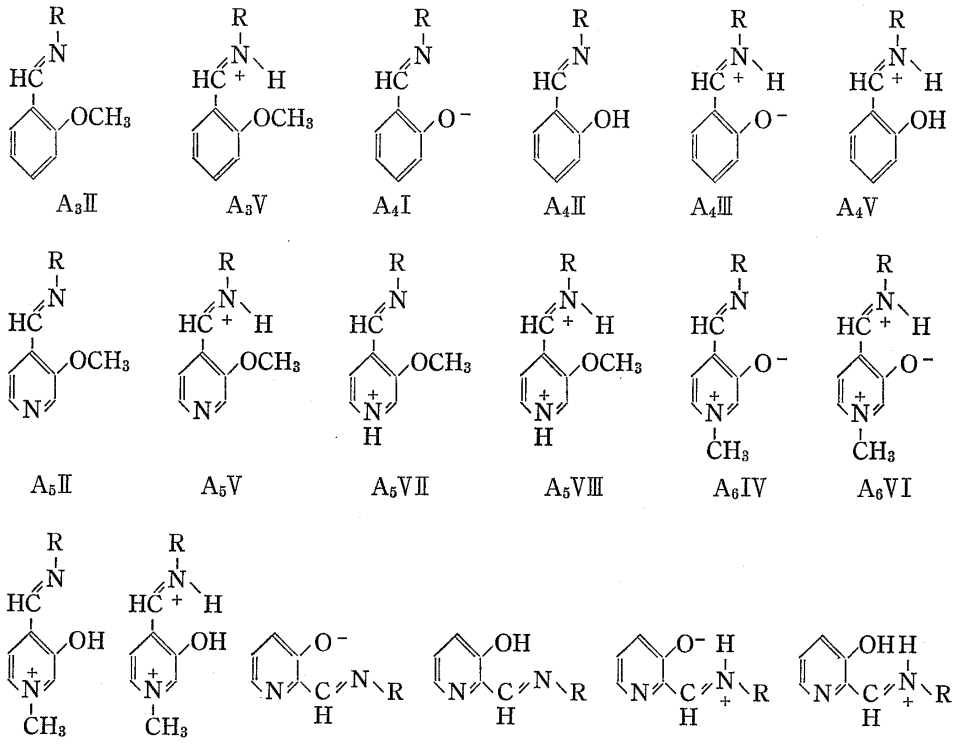
19) *a*) R.M. Silverstein and G.C. Bassler, 'Spectrometric Identification of Organic Compounds," 2nd Ed., J. Wiley & Sons, New York, N.Y., 1967; *b*) K. Nakamoto and A.E. Martell, *J. Am. Chem. Soc.*, **81**, 5857, 5863 (1959); *c*) D. Heinert and A.E. Martell, *ibid.*, **85**, 138 (1963); *d*) S.F. Mason, *J. Chem. Soc.*, **1959**, 1253.

MO's were calculated for all possible structures of the aldimines of methylamine with anisaldehyde (**3**), salicylaldehyde (**4**), 3-methoxy-4-formylpyridine (**5**), and 1-methyl-3-hydroxy-4-formylpyridinium salt (**6**). The four structures of that with 3-hydroxy-2-formylpyridine (**9**) (A_9I, A_9II, A_9III, and A_9V) were also calculated (Chart 2). The results are listed in Table II. The agreements between the observed and the calculated value are satisfactory, with the exception of A_4III. Thus the spectral assignments for these aldimine made previously are proved to be reasonable.

TABLE II. π-π^* Transitions of Aldimines, A_3, A_4, A_5, A_6, and A_9

Species	Obs.[a)]		Calcd.[a)]	
A_3II	4.02 (6400)	4.94 (15000)	4.07 (0.154)	4.71 (0.242)
A_3V	3.56 (6100)	4.49 (19500)	3.52 (0.220)	4.50 (0.329)
A_4I	3.50 (6600)	4.80 (10300)	3.61 (0.234)	4.72 (0.110)
A_4II	3.91 (3300)	4.88 (10800)	4.13 (0.154)	4.77 (0.319)
A_4III	3.08 (2000)	4.48 (5000)	3.41 (0.272)	4.69 (0.303)
A_4V	3.52 (6500)	4.51 (23000)	3.66 (0.177)	4.56 (0.423)
A_5II	4.00 (4800)	5.14 (9800)	3.91 (0.158)	4.83 (0.358)
A_5V	3.44 (750)	4.33 (460)	3.37 (0.224)	4.64 (0.388)
A_5VII	3.87 (850)	4.73 (3500)	3.62 (0.302)	4.80 (0.423)
A_5VIII			3.04 (0.333)	4.64 (0.309)
A_6IV	3.25 (8700)		3.14 (0.308)	4.65 (0.283)
A_6VI	2.94 (5600)	4.66 (5200)	2.87 (0.400)	4.58 (0.220)
A_6VII	3.79 (2300)	4.80 (5900)	3.64 (0.304)	4.79 (0.447)
A_6VIII			3.07 (0.324)	4.67 (0.331)
A_9I	3.47 (9000)	4.88 (10300)	3.37 (0.294)	4.74 (0.013)
A_9II	3.92 (6600)	5.10 (5900)	3.90 (0.279)	4.91 (0.094)
A_9III	3.13 (3300)	4.70 (2800)	3.24 (0.356)	4.74 (0.090)
A_9V	3.53 (11700)	4.82 (8600)	3.54 (0.301)	4.69 (0.220)

a) See footnotes, a, b in Table I.



A_3II A_3V A_4I A_4II A_4III A_4V

A_5II A_5V A_5VII A_5VIII A_6IV A_6VI

A_6VII A_6VIII A_9I A_9II A_9III A_9V

Chart 2

The results of the calculation also confirm some empirical rules on the band shift in this series of the compounds[1a,19b–19d]; The protonation of the azomethine and the proton dissociation of the phenol caused a large bathochromic shift, whereas the protonation of the pyridine nitrogen results in a small red shift[20]; the replacement of the phenol by the methoxy group and that of the pyridinium hydrogen by the methyl group do not significantly affect the spectral properties.

The Quinoid Intermediate

The appearance of an intense absorption at the 500-nm region (~2.47 eV) has been reported in some enzymes in the presence of substrates or pseudosubstrates, and the absorption has been ascribed to the intermediate structures in which α-carbon atom in the aldimine of the coenzyme is deprotonated.[5] The quinoid structure with an extended conjugate system may be the cause of the large bathochromic shift.

The model reaction system giving rise to the spectra similar to the enzymatic intermediate was described previously.[8] It consisted of methanol solutions of the three reactants; pyridoxal, amino acid derivatives, and Al(III). The replacement of PL in the system by its analogs gave analogous results. The observed spectra for the species with **1** and **6** are listed in Table III.

Table III. Absorption Spectra and Band Assignment of the Quinoid Intermediate

Aldehyde	Amino acid	Spectra[a]		Assignment
3-Hydroxy-4-formylpyridine (**1**)	Methyl alaninate	2.51 (5800)	2.64^{s} (2900)	Al-chelate, pyridinium species
		2.82 (10500)	2.99^{s} (6400)	Al-chelate, pyridine species
1-Methyl-3-hydroxy-4-formylpyridinium (**6**)	Alanine	2.46 (4500)	2.62^{s} (1400)	Al-chelate, pyridinium species
6	Methyl alaninate	2.47 (30000)	2.64^{s} (13000)	Al-chelate, pyridinium species

a) Wavelengths are expressed in eV unit. Numbers in parentheses are the absorbance divided by the concentration of the aldehyde measured under the conditions described previously.[8]

Since the model system contained Al (III), the absorption bands were ascribed to the AI (III) chelates of the intermediates.[21] Enzymatic intermediate is not in the form of the metal chelate. Apoenzyme is believed to arrange the active site planar and thus facilitate the deprotonation at the α-carbon atom. In the model system, both the intermediate species protonated and unprotonated at the pyridine nitrogen (pyridinium and pyridine species, respectively) were well distinguished.[8] The latter has not been reported in enzymatic systems.

MO's were calculated for the species involved in acid-base equilibria of the unchelated intermediate as in the case of the aldimine. Spectral assignments for all species have not been established in this intermediate. Structures used for the calculation are shown in Chart 3 and the results are summarized in Table IV. In the calculation based on the quinoid-type resonance structure, $\mathrm{B_a}$, which is commonly assumed for this intermediate, the result on $\mathrm{B_a}$VII gave best fit to the absorption in the enzymes[5] and the model systems.[8] The transition energies are less affected in $\mathrm{B_a}$ than in A by the state of the protonation on the phenolate and the imine nitrogen. The large oscillator strength of the first π-π* band calculated to $\mathrm{B_a}$IV,

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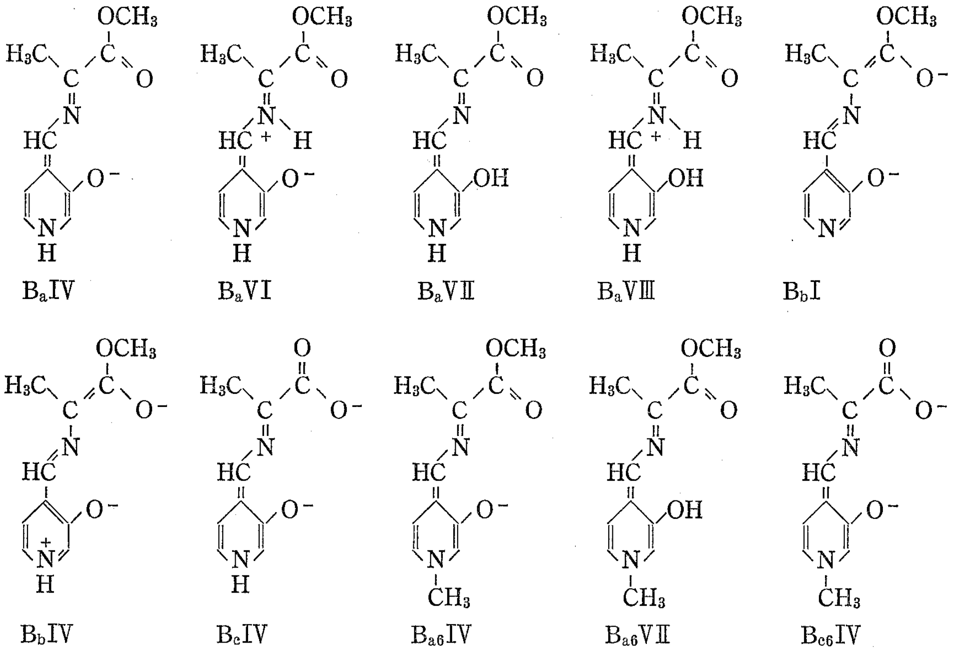
20) According to the calculation, the shift is large in the cases of the protonation of the azomethine to the phenol species (II→V, VII→VIII) and of the dissociation of the phenol to the species with unprotonated azomethine (II→I, VII→IV). In the other cases (I→III, IV→VI; V→III, VII→VI), the magnitude of the shift is comparable to that in the protonation of the pyridine nitrogen.

21) Recently, model systems were found which do not contain metal ion and give rise to the intermediate spectra. The results, along with those of other workers (ref. 7), indicate that the wavelengths of the species were not significantly affected by the chelation with Al(III). Details will be reported.



B_aIV B_aVI B_aVII B_aVIII B_bI

B_bIV B_cIV $B_{a6}IV$ $B_{a6}VII$ $B_{c6}IV$

Chart 3[17]

TABLE IV. Transitions calculated for the Quinoid Structures and the Schiff Bases of Unsaturated Amino Acids

Structure	Transitions[a]		Structure	Transitions[a]	
B_aIV	2.39(1.21)	2.81(0.14)	$B_{a6}VII$	2.51(1.17)	2.64(0.32)
B_aVI	2.31(1.47)	2.69(0.19)	$B_{c6}IV$	2.49(1.23)	2.77(0.25)
B_aVII	2.51(1.15)	2.63(0.33)	CIV	3.04(0.38)	3.95(0.35)
B_aVIII	2.37(1.36)	2.52(0.37)	CVI	2.58(0.42)	3.05(0.21)
B_bI	2.86(0.48)	3.76(0.13)	CVII	3.53(0.40)	3.90(0.41)
B_bIV	2.62(0.49)	3.45(0.28)	CVIII	2.61(0.36)	3.14(0.26)
B_cIV	2.49(1.22)	2.77(0.25)	DIV	2.30(1.59)	2.65(0.14)
$B_{a6}IV$	2.39(1.21)	2.82(0.14)	DVIII	2.13(1.76)	2.41(0.05)

[a] Transition energies are expressed in eV unit. Numbers in parentheses are calculated oscillator strengths.

VI, VII, VIII structures may well reflect the intenseness of the band recognized in the enzymatic[5] and model systems.[8]

One of the characteristic features of the absorption spectra is the presence of a shoulder at high energy side.[5,8] The first and the second π-π^* bands locate so closely in the calculation on B_a that the shoulder can be assigned to the second π-π^* band of the same species.

As the quinoid-type representation for the pyridine species is impossible, the calculation was carried out on the structure B_bI. The calculated energy of the first π-π^* transition was in excellent agreement with the observed energy, though that of the second transition did not correspond to the observed shoulder at the higher energy side.[8] The calculation for the pyridinium species based on the same resonance structure, B_bIV, gave slightly higher energy than the observed one.

In Table IV are included the results of the calculation based on the carboxylate forms of the amino acid, B_cIV, and the quinoid structures derived from **6**, *i.e.* $B_{a6}IV$, $B_{a6}VII$, and $B_{c6}IV$. Calculated energies are in reasonable agreement with the observed ones.

Schiff Base of *α*-Aminoacrylic Acid

In a few pyridoxal enzymes which catalyze *α*,*β*-elimination or *β*-replacement reactions of amino acids, a transient species absorbing at 455—470 nm (2.63—2.72 eV) have been reported[6] and suggested to be the aldimine with α,β-unsaturated amino acid. The spectral model absorbing at 467 nm (2.65 eV) was demonstrated in a system containing **6**, tryptophan, and Al (III).[9a] The absorption is ascribable to the Al (III) chelate of the aldimine of **6** with α-aminoacrylate anion. Pyridine species of the intermediate is unknown.

MO's were calculated on the structures CIV, CVI, CVII, and CVIII (Chart 4) and the results are included in Table IV. Calculations on the structures derived from **1** gave essentially the same results as the corresponding structures of **6**. According to the calculation, the bathochromic shift accompanying the azomethine protonation is large, whereas that accompanying the dissociation of the phenol is less eminent in this intermediate.

Unlike the aldimine and the quinoid intermediate, the calculated energy of the anionic species, CVI, did not agree with the experimental data. The results on the species with protonated azomethine, CVI, CVIII, agree with the observed value of the Al (III) chelate.[9a]

The Quinoid Intermediate with β,γ-Unsaturated Amino Acid

In the pyridoxal catalyzed β,γ-elimination and γ-replacement reactions of amino acids, a quinoid intermediate of β,γ-unsaturated amino acid was postulated and was predicted to absorb above 500 nm (<2.47 eV).[22]

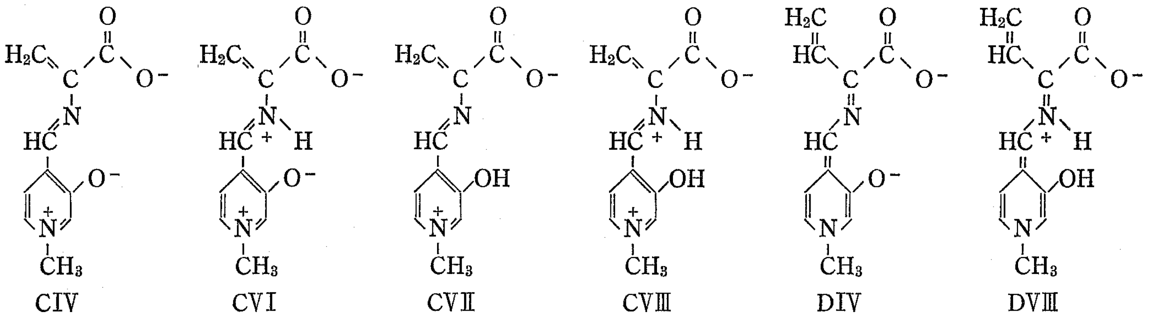


Chart 4[17]

The reliable spectrum of this enzymatic intermediate has never been appeared in the literature. Quite recently, a model for this intermediate was found.[9b] A species absorbing 550 nm (2.25 eV) was formed in the reaction of pyridoxal N-methochloride, 2-amino-3-butenoic acid, and Al (III), and in that of pyridoxal N-methochloride, methionine, Al (III), and 2-mercaptoethanol. Absorption peak was at 540 nm (2.29 eV), when pyridoxal N-methochloride was replaced by **6**.

The structure was proposed to be the Al (III) chelate of the quinoid intermediate derived from **6** and 2-amino-3-butenoate anion, D. The results of the calculation on DIV and DVIII shown in Table IV can well account for the proposal.

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[22] L. Davis and D.E. Metzler, "The Enzymes," 3rd ed., Vol. 7, ed. by P.D. Boyer, Academic Press, New York, N.Y., 1972, Chapter 2.