Oxidative Deamination of Amino Acids by Molecular Oxygen with Pyridoxal Derivatives and Metal Ions as Catalysts

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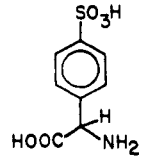
Abstract: The oxidative deamination of (*p*-sulfophenyl)glycine by Cu^{2+} and vitamin B_6 coenzymes pyridoxal 5'-phosphate or 5'-deoxypyridoxal to give (*p*-sulfophenyl)glyoxylic acid is described. The reaction conditions for kinetic measurements and catalysis were selected on the basis of previous equilibrium measurements of complex species present in these reaction systems. The reaction occurs in two modes, stoichiometric and catalytic. The stoichiometric route involves the reaction of the amino acid Schiff base of pyridoxal 5'-phosphate or 5'-deoxypyridoxal to form the oxime of the coenzyme and the keto acid. The stoichiometric reactions, which occur moderately rapidly at room temperature in aqueous solution, become part of an overall catalytic system in which the amino acid and dioxygen are converted to keto acid and ammonia in the presence of low concentrations of coenzyme and metal ion. Spectrophotometric studies demonstrate the relatively rapid conversion of the Schiff base metal chelate to the oxime complex, which is slowly converted to additional Schiff base complex by reaction with additional amino acid. Hydroxylamine, which is presumably formed in the displacement reaction, is converted to ammonia under the reaction conditions employed. The concentration of the intermediate oxime complex remains relatively constant when the reaction is run in the catalytic mode. Conversion to the final products was followed by isolation of the ammonia and keto acid formed, and by the quantitative estimation of ammonia. A dioxygen-18 tracer study demonstrated that dioxygen is the source of the oxime oxygen, while the oxygen of the keto acid comes from the solvent. Mechanisms previously proposed for vitamin B_6 and metal ion catalyzed amino acid oxidation are discussed, and a selection is made on the basis of $^{18}O_2$ labeling of the hydroxylation product.

Introduction

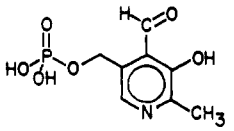
Biological oxidative deamination reactions have received considerable attention since their discovery[1] because they are part of the metabolic pathway for endogenous amino compounds.[2] The majority of enzymes mediating the reactions termed as amine oxidases are flavoproteins, but there is a small class of amine oxidases that require Cu(II) for activity.[3,4] In synthetic organic chemistry, this reaction may serve as a convenient pathway for converting amino compounds to analogous carbonyl compounds. Preparation of α-keto acids is sometimes complicated by facile decarboxylation reactions.[5] Inorganic oxidants such as Fermy's salt have been shown[6-8] to carry out the conversion of α-amino acids to α-keto acids.

Earlier studies[9-13] have indicated that vitamin B_6 may catalyze the conversion of amino acids to keto acids in the presence and absence of metal ions by transamination. It has also been observed that, in the presence of dioxygen, the amino acid to keto acid conversion can occur via an oxidative deamination process in which the reduced form of the coenzyme is reoxidized at an intermediate stage.[14-18] This reaction has been observed in model systems only in the presence of transition-metal ions.

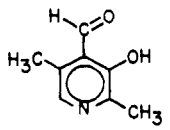
This paper describes an investigation of the oxidation of (*p*-sulfophenyl)glycine, **1**, by dioxygen in the presence of Cu(II) and the vitamin B_6 coenzyme pyridoxal 5'-phosphate, **2**, and a synthetic analogue, 5'-deoxypyridoxal, **3**. The reaction was carried out in aqueous solutions rather than methanol, as was done previously[18] because of indications that solvent oxidation accompanies the oxidation of substrate when methanol was used as solvent, although it is well known[19] that Schiff base intermediates are more completely formed in alcohol solvents than in water. This investigation has resulted in identification and isolation of a new type of intermediate in the oxidative deamination of amino acids.



1, (*p*-sulfophenyl)glycine, SPG



2, pyridoxal 5'-phosphate, PLP



3, 5'-deoxypyridoxal, DPL

Experimental Section

Materials. Pyridoxal 5'-phosphate was obtained from ICN Biomedical Co., and the extent of hydration was determined by the potentiometric method.[20] D(-)C-phenylglycine (from Sigma) was recrystallized from an ethanol-water mixture at pH 4.6. 5'-Deoxypyridoxal, (4-methoxyphenyl)glycine, and (4-sulfophenyl)glycine were synthesized by the methods described previously.[21] The disodium salt of dihydrogen EDTA and nitrate salts of Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) were of

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(1) Zeller, E. A. *Helv. Chim. Acta* **1938**, *21*, 880.
(2) Malmstrom, B. G.; Andreasson, L.-E. In *The Enzymes* (*Vol. VIIIB*), 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1981; p 507.
(3) Mondovi, B.; Riccio, P. *Adv. Inorg. Biochem.* **1984**, *6*, 225.
(4) Yamada, H.; Yasunobu, K. T. *J. Biol. Chem.* **1962**, *237*, 3077.
(5) Tanaka, M.; Kobayashi, T.; Sakakura, T. *Angew, Chem., Int. Ed. Engl.* **1984**, *23*, 518.
(6) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.
(7) Morey, J. Dzielendziak, A.; Saa, J. M. *Chem. Lett.* **1985**, 263.
(8) Garcia-Raso, A.; Deya, P. M.; Saa, J. M. *J. Org. Chem.* **1986**, *51*, 4285.
(9) Martell, A. E. In *Advances in Enzymology*; Meister, A., Ed.; Wiley: New York, 1982; Vol. 53, p 163.
(10) Braunstein, A. E.; Shemyakin, M. M. *Biokhimiya* **1953**, *18*, 393.
(11) Christen, P., Metzler, D. E., Eds. *Transaminases*; John Wiley: New York, 1985.
(12) Snell, E. E., Fassella, P. M., Braunstein, A. E., Rossi-Fannelli, A., Eds.; *Chemical and Biological Aspects of Pyridoxal Catalysis*; Pergamon: Oxford, 1968; p 90.
(13) Martell, A. E. In *Chemical and Biological Aspects of Pyridoxal Catalysis Part A*; Evangelopoulous, A. E., Ed.; Alan R. Liss: New York, 1984; p 68.
(14) Metzler, D. E.; Snell, E. E. *J. Biol. Chem.* **1952**, *198*, 353.
(15) Ikawa, M.; Snell, E. E. *J. Am. Chem. Soc.* **1954**, *76*, 4900.
(16) Hill, J. M.; Mann, P. J. G. *Biochem. J.* **1966**, *99*, 454.
(17) Hamilton, G. A.; Revesz, A. *J. Am. Chem. Soc.* **1966**, *88*, 2069.
(18) Tatsumoto, K.; Haruta, M.; Martell, A. E. *Inorg. Chim. Acta* **1987**, *138*, 231.
(19) Heinert, D.; Martell, A. E. *J. Am. Chem. Soc.* **1963**, *85*, 188.
(20) Motekaitis, R. J.; Martell, A. E. *Determination and Use of Stability Constants*; VCH: New York, 1989.
(21) Shanbhag, V. M.; Martell, A. E. *Inorg. Chem.* **1990**, *29*, 1023.

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certified analytical reagent grade, and potassium hydrogen phthalate was of acidimetric standard grade. Carbonate-free concentrate of KOH ("Dilut-it") was obtained from J.T. Baker. Deuterated reagents D_2O (99.8% D), 40% solution of KOD (99% D), and 37% solution of DCl(99% D) were purchased from Aldrich Chemical Co. Commercial nitrogen and argon gases were purified by bubbling through a solution of 3% alkaline pyrogallol. All other chemicals utilized were of reagent grade. Oxygen-18 gas was obtained from Cambridge Isotope Laboratories, Woburn, MA, in the form of 0.10-L samples of 97–98% isotopic purity.

Preparation of Solutions. Metal ion solutions (0.010 M) were prepared from their nitrate salts and were standardized by complexometric titrations[22] against a standard solution of Na_2H_2EDTA. The ionic strength of these solutions was adjusted by the addition of KCl as supporting electrolyte. The EDTA solution was standardized[22] by titration with a standard solution of Zn(II) prepared by dissolving a known amount of pure zinc metal filings in HNO_3.

Equilibrium Measurements. Protonation constants of PLP, DPL, PG, SPG, and MPG, stability constants for the formation of Schiff bases between amino acids and pyridoxal derivatives, and equilibrium constants for the formation of metal complexes of each of the ligands identified above as well as Schiff bases were determined by potentiometric equilibrium measurements and are described in a previous paper.[21] The distribution of species as a function of pH was evaluated by computer-assisted calculations[20,23] carried out with the stability constants previously measured.[21]

Spectrophotometric Measurements. UV-visible absorption spectra of experimental solutions at μ = 0.100 M (KCl) were recorded with a Perkin-Elmer Model 553 fast-scan spectrophotometer interfaced with a Perkin-Elmer R-1000 recorder. A matched pair of quartz cells with 1.000-cm path length was used for the routine measurements. For kinetic studies, the sample cell was replaced by a flow cell of the same path length. The temperature of the sample cell holder was maintained by the circulation of water at the desired temperature.

Organic compounds used in this study were characterized by ^1H and ^{13}C NMR measurements with Varian EM-390 and Varian XL-200 (dual probe) NMR spectrometers. Mass spectra (EI and FAB) of the reaction products and their derivatives were obtained with a VG Analytical 70S high-resolution mass spectrometer interfaced with a VG Analytical 11/250s data system and a HP-1890a gas chromatograph, operated by Dr. Thomas R. Sharp of the Departmental Mass Spectrometry Center.

Kinetic Measurements. Reaction mixtures containing 2.5×10^{-3} M (*p*-sulfophenyl)glycine, 2.5×10^{-4} M pyridoxal derivative, 2.5×10^{-4} M metal ion, and an appropriate amount of KCl to adjust the ionic strength to 0.100 M were placed in a thermostated cell and sealed from the external atmosphere. The cell was fitted with glass and calomel electrodes and a Gilmont piston buret containing 1.00 M KOH solution. The oxygen gas was bubbled through the solution at a steady rate. The reaction mixture was continuously circulated through the spectrophotometric flow cell with the aid of a peristaltic pump. The pH of the solution was raised to a desired value, and the spectra were recorded in the overlay mode at desired intervals of time. Spectral measurements were discontinued when there were no further significant changes in the measured spectra. The A_∞ was recorded for each experimental solution after an interval of about 15 days.

Individual kinetic measurements were carried out with variation of concentrations of each of the components in the reaction mixture at 25.0 °C and at pH 11.00 in order to determine the dependence of the reaction rate on the individual component.

The reaction kinetics were also studied by the variation in the concentration of dissolved oxygen in the reaction mixture. A 5.0-mL solution containing 1.25 mmol of SPG, 0.125 mmol of DPL, and 0.125 mmol of $CuCl_2$ was prepared and purged with argon gas. A 1.00-mL portion of this reaction mixture was diluted with 100.0 mL of carbonate-free water saturated with oxygen (by bubbling pure oxygen through it for about 48 h) in a sealed reaction vessel described earlier in this section. The volume of the reaction mixture was selected so as to leave no space for any gas above the reaction mixture in the cell. The ionic strength of the mixture was adjusted to 0.10 M by the addition of KCl crystals. The reaction was triggered by increasing the reaction pH to 11.00. The kinetics were studied by keeping the system closed from the external atmosphere throughout the reaction. In a second experiment, 1.00 mL of reaction mixture was added to 50.0 mL of water saturated with oxygen and 50.0 mL of water saturated with argon whereby the concentration of dissolved oxygen was reduced by half.

Oxygen Absorption Measurements. Reaction mixtures containing 1.0×10^{-2} M amino acid, 1.0×10^{-3} M pyridoxal derivative, and 1.0×10^{-3} M metal ion were placed in a thermostated cell. The ionic strength was adjusted to 0.100 M with KCl. The cell was closed with a lid fitted with glass and reference electrodes, a microburet containing 1.00 M KOH, and an inlet and outlet for oxygen. The acidic reaction mixture was first saturated with the purified oxygen. The gas outlet was closed, and the inlet was then switched to a thermostated reservoir containing a measured volume of oxygen at 1.0 atm of pressure. The reaction was triggered by raising the pH of the solution to the desired alkalinity. The amount of O_2 consumed was measured at regular intervals of time by raising or lowering the leveling mercury bulb to adjust the pressure of O_2 to 1.0 atm. Appropriate corrections were made to the volume of O_2 for changes in the atmospheric pressure.

Product Analyses. Ammonia. The Nessler's colorization test[24] indicated the evolution of ammonia from the reaction mixture. The amount of ammonia formed during the reaction was quantitatively analyzed by absorbing the effluent gases in a known quantity of standard acid solution and then determining the unreacted acid by titrimetry.

Nonvolatile Products. The solution containing the reaction products was acidified, and the Cu(II) was precipitated as insoluble sulfide with H_2S gas. The precipitate was filtered off, and the filtrate was concentrated.

The concentrated reaction mixture was tested for the presence of hydroxylamine by treating it with an aldehyde (*p*-anisaldehyde) to form the oxime adduct.[25] The mixture was also tested for the presence of hydrogen peroxide by iodimetric[26] titration.

The concentrate was loaded on a column containing strongly basic (BioRad AG1 X8, 100–200 mesh) anion exchange resin in the hydroxide form. The components were eluted with HCl solutions of varying concentrations. The components were isolated by evaporation of eluate fractions and were identified as follows.

Vitamin B_6 Coenzymes. The component eluted by 0.05–0.10 M HCl solution was recrystallized from absolute methanol. Electronic, mass, and NMR spectral properties of this compound were found to be the same as those found for authentic oximes of PLP and DPL. Authentic samples of oximes were prepared[25] by heating PLP or DPL with an alkaline solution of hydroxylamine for about 45 min. The oxime derivatives were extracted from the residue with absolute ethanol. The empirical formula of the compound obtained from the reaction mixture was further confirmed by elemental analysis.

Keto Acid. The keto acid was eluted with 6 M HCl from the column. The compound was recrystallized from hot water and analyzed by NMR, mass, and UV-visible spectra. An authentic synthetic (2,4-dinitrophenyl)hydrazone of the keto acid was prepared[27] to confirm its identity.

^{18}O-Labeling Experiment. A 200-mL aqueous solution of 5.0×10^{-4} M SPG, 5.0×10^{-4} M DPL, and 5.0×10^{-4} M $CuCl_2$ was placed in a flask with three openings: A sealed, stoppered reservoir containing $^{18}O_2$ was connected through the first, a three-way switchable connector for argon supply and evacuation was attached to the second, and the third was closed with a rubber septum. Initially, the system was completely evacuated and then filled with purified argon gas. The process of evacuating and filling the system with argon was repeated about 10 times to ensure that the system is devoid of $^{16}O_2$. When the system was under atmospheric pressure of argon, a predetermined amount of 2.0 M solution of KOH was injected in the solution so as to raise the pH of the solution to 11.0. The system was then partially evacuated, and $^{18}O_2$ was exposed to the reaction mixture by breaking the seal on the reservoir and opening the stopcock. The reaction flask was placed in a water bath at 35 °C to speed up the reaction. The completion of the reaction was inferred by the change in color of the reaction mixture from dark green to light blue. The reaction products were separated by the procedure described above, and the keto acid and oxime were characterized by mass spectrometry.

Computation of Reaction Rates. Successive changes in the absorption maximum for the Schiff base chelate complexes at 390 nm were measured, and pseudo-first-order rates for the conversion of Schiff base to the corresponding intermediate were computed as a function of pH and temperature by linear regression analysis with a FORTRAN program, LSQFIT.[28] Since the spectral changes ceased after initial conversion of the Schiff base complex to the oxime complex, the rates of the subsequent reactions could not be evaluated quantitatively by this method.

(22) Schwarzenbach, G.; Flaschka, M. *Complexometric Titrations*; Methuen: London, 1957.

(23) Motekaitis, R. J.; Martell, A. E. *Can. J. Chem.* **1982**, *60*, 2403.

(24) Struelli, C. A.; Averill, P. R. *The Analytical Chemistry of Nitrogen and Its Compounds, Part I*; Wiley: New York, 1970.

(25) Vogel, A. I. *Textbook of Practical Organic Chemistry*, 3rd ed.; Wiley: New York, 1962; p 270.

(26) Kolthoff, I. M.; Belcher, R. *Volumetric Analysis Vol. 3*; Interscience: New York, 1957; p 214.

(27) Roberts, R. M.; Gilbert, J. C.; Rodewald, L. B.; Wingrove, A. S. *An Introduction to Modern Experimental Chemistry*; Holt, Reinhart and Winstin Inc.: New York, 1974; p 265.

(28) Motekaitis, R. J. Personal communication, 1988.

Results and Discussion

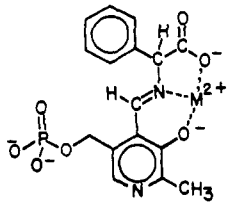
Oxidative deamination (eq 1) of (*p*-sulfophenyl)glycine (SPG), **1**, by pyridoxal 5′-phosphate (PLP), **2**, and 5′-deoxypyridoxal (DPL), **3**, in the presence of Cu^{2+} and molecular oxygen in basic aqueous solutions at 0.10 M ionic strength was studied as a function of pH, temperature, and stoichiometry of reactions.

$$RCH(NH_2)COOH + \tfrac{1}{2}O_2 \rightarrow RCOCOOH + NH_3 \quad (1)$$

Studies of oxidative deamination of phenylglycine (PG) and (*p*-methoxyphenyl)glycine (MPG) were complicated by the appearance of insoluble complex species in the reaction mixture. Upon analysis, these precipitates were found to be 2:1 amino acid to metal complexes. The formation of insoluble complexes was not observed in the case of SPG.

Oxygen uptake as well as spectrophotometric experiments indicated that Ni^{2+} and Zn^{2+} are not catalysts for oxidative deamination of SPG. The reaction mixtures containing Mn^{2+} and Co^{2+}, which do take up oxygen, resulted in precipitation, presumably due to oxidation of the metal ion to insoluble hydroxides. Kinetic studies were thus pursued with Cu(II) as the metal ion catalyst. No interference due to the formation of a solid phase was encountered with this metal ion in the initial states of the reaction.

Vitamin B_6 coenzymes catalyze[9] the formation of α-keto acids from amino acids by the transamination process. Schiff bases of coenzymes with amino acids and their metal complexes (e.g., see **4**) are understood to be intermediates for this reaction. This



4, metal complex of PLP–PG Schiff base

reaction proceeds[9] at an optimum rate between pH 4 and 5, and its rate is negligible[29] above pH 9. Therefore, pH values above 10 were considered best for the study of oxidative deamination to avoid the contribution of transamination toward the formation of keto acid. The amino acid substrate was used in 10-fold excess concentration over that of the coenzyme and Cu(II). Equilibrium calculations indicate the formation of a much higher concentration of Schiff base species under these conditions compared to the use of equivalent amounts of the reacting species.

Reaction Products. For the purpose of the isolation and analyses of the products of oxidative deamination, the reaction was carried out with 10:1:1 molar ratio of SPG to DPL to Cu(II), in aqueous solution at pH 11.0 by bubbling O_2 gas through the solution. The analysis of the final reaction mixture showed the formation of keto acid and ammonia under the reaction conditions employed in this investigation. Tatsumoto et al.[18] reported the formation of hydroxylamine when alanine and phenylalanine were deaminated in methanol solutions. In the present study, in aqueous media, no hydroxylamine was detected. Therefore, the formation of hydroxylamine is probably dependent on the medium. Also the formation of hydrogen peroxide, suggested (but not demonstrated) by Hamilton and Revesz[17] as a product of oxidative deamination, was not observed in this work.

Formation of Ammonia. The formation of ammonia as a product was qualitatively identified by the Nessler's test[24] and was confirmed by gas chromatographic analysis. The gas above the reaction mixture was injected into a chromatographic column (Alltech Porapa N, 80 mesh) capable of detecting ammonia. Although ammonia was detected, the strong peak for oxygen adjacent to that of ammonia in the chromatogram interfered with the quantitative analysis of ammonia by this method.

Ammonia formed in the reaction was analyzed quantitatively by titrimetry. The reactants SPG, DPL, and Cu(II) at a molar ratio of 10:1:1, respectively, were placed in a thermostated closed reaction flask with an inlet and an outlet for the gases. After the pH of the reaction mixture was raised to 11.0, a stream of oxygen was bubbled through the solution. The gas outlet was connected with a gas dispersion tube immersed in a known volume of standard HNO_3 (0.100 M). Unreacted acid was back-titrated with carbonate free standard 0.100 M KOH solution to determine the amount of ammonia absorbed. It was necessary to continue the process of absorbing the effluent gases over a long period of time to absorb a substantial amount of ammonia.

Table I. Analysis of Ammonia[a]

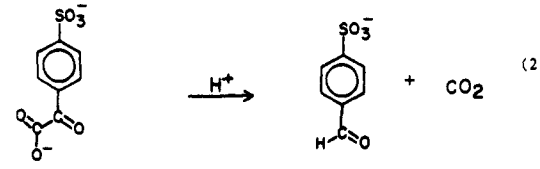
period (h)	NH_3 isolated (mol)	percent conversion
10	2.7×10^{-6}	0.1
72	4.1×10^{-5}	2.0
168	2.4×10^{-4}	12.0
260	5.8×10^{-4}	29.0
360	1.24×10^{-3}	61.2

[a] Initial moles of SPG = 2.0×10^{-3}; $[DPL]_{in}$ = 2.0×10^{-4}; $[Cu]_{in}$ = 2.0×10^{-4}; t = 25 °C and pH = 11.0; percent conversion = moles of NH_3/initial moles of SPG.

Table I shows the quantities of ammonia absorbed in the acid solution as a function of time. The percent conversion indicates the ratio of moles of ammonia isolated to moles of amino acid (SPG) employed. According to eq 1, the amount of ammonia formed at the completion of reaction should be equivalent to the initial concentration of SPG. The reaction was confirmed to be complete after a period of 15 days by product analysis, which indicated essentially complete reaction of SPG. The difference between the amount of ammonia isolated and the amount expected may be accounted for by the high solubility of ammonia in aqueous solution even at pH 11, and by the formation of Cu(II)–ammonia complexes under the reaction conditions employed. Also, as it turned out (see below), part of the ammonia that was expected to form was tied up in the reaction intermediate, in an amount equivalent to that of the coenzyme.

The solution containing the reaction products, after the completion of the reaction (about 15 days), was acidified and saturated with H_2S gas, and the precipitate was filtered off. The filtrate was concentrated and placed on a strongly basic anion exchange resin (Biorad AG-1 X8 in OH^- form). First, all the inorganic components were eluted with distilled water. The coenzyme was then eluted from the column with 0.5–1.0 M HCl, and the keto acid was subsequently eluted with 4.0–6.0 M HCl. The eluate fractions were concentrated, and products were isolated by crystallization.

Keto Acid Formation. The keto acid isolated from the product mixture was characterized by ^1H (see Figure 1) and ^{13}C NMR. The presence of a small amount of *p*-sulfobenzaldehyde was also noted. This is a product of partial decarboxylation of SPGA, (*p*-sulfophenyl)glyoxylic acid, during the acidification of the product mixture as evidenced by gas evolution. The formation of α-keto acid as the product of oxidative deamination was further confirmed by hydrazone formation[27] with (2,4-dinitrophenyl)-hydrazine.



(2)

5, (*p*-sulfophenyl)glyoxylic acid **6**, *p*-sulfobenzaldehyde

The NMR resonance of the proton on the α-carbon of SPG at 5.06 ppm in ^1H NMR and the resonance of the α-carbon at 58 ppm in ^{13}C NMR were not observed in the NMR spectra of separated product as well as the unseparated product mixture. Complete reaction of SPG is thus confirmed. Also new peaks were observed at 171 and 173 ppm in the ^{13}C NMR spectra and were assigned to the carboxylate carbon and the α-carbon of (*p*-sulfophenyl)glyoxylic acid, respectively. The relatively weaker

(29) Szpoganicz, B. Ph.D. *Dissertation, Texas A&M University*, 1984.

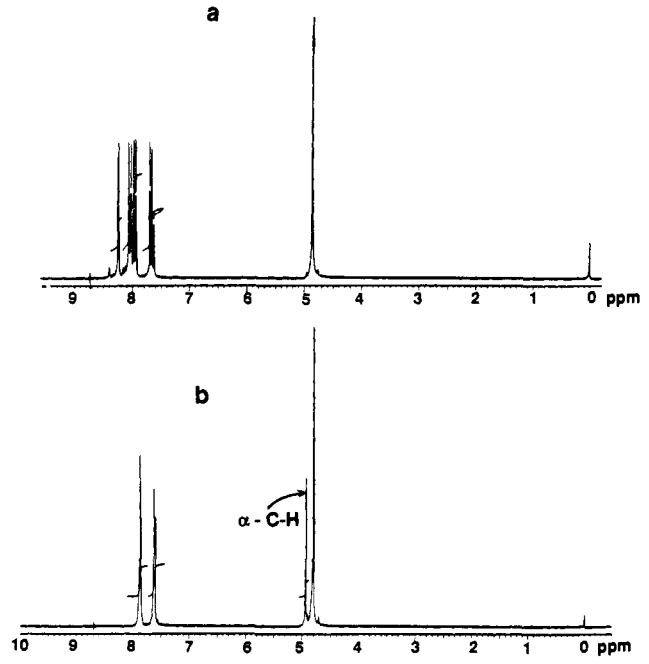


Figure 1. Proton magnetic resonance spectra (200 MHz) of (a) (*p*-sulfophenyl)glyoxylic acid and (b) (*p*-sulfophenyl)glycine in D_2O solutions with DSS as internal standard.

peak at 194 ppm was due to the aldehydic carbon of *p*-sulfobenzaldehyde.

Fate of Vitamin B_6 Coenzymes. The coenzyme separated from the product mixture was characterized by NMR, IR, UV-visible, and mass spectra as well as elemental analysis. The processes of characterization of the coenzyme after the reaction was carried out with DPL systems and PLP systems were analogous. The ^{13}C NMR of this compound showed a resonance at 164 ppm, characteristic of the imino carbon of an oxime (-C=NOH). Derivatization of the coenzyme was evident also by the absence of the resonance peak above 190 ppm expected for the aldehydic carbon. A pure sample of the oxime of DPL was prepared for the comparison. The NMR spectra of DPL-oxime and the compound isolated from the reaction mixture were identical.

The electron impact mass spectra of the compound isolated from the reaction mixture (DPL catalyzed) and the authentic DPL-oxime indicated a *m/e* of 166 for the molecular species. This agrees with the molecular weight of the oxime of DPL ($C_8H_{10}N_2O_3$, MW 166 au). The fragmentation patterns of the two were also identical (see Figure 6a,b).

UV-visible spectrophotometry also confirms that the compound isolated from the product mixture is the oxime of DPL. Figure 2 shows the absorbance of the compound isolated from the reaction mixture and the oxime of DPL. The absorption band at 345 nm in alkaline solutions and 330 nm with a shoulder at 358 nm in acidic solutions is characteristic of the oxime of DPL (also PLP). The IR spectra of the product compared very well with that of DPL-oxime and showed an absorption peak at 1650 cm^{-1} characteristic of the oxime (-C=NOH) stretching vibration. The elemental analysis further confirmed the formation of oxime in the reaction mixture: Anal. Calcd for $C_8H_{10}N_2O_2 \cdot 0.8KCl$: C, 42.56; H, 4.47; N, 12.41. Found: C, 42.50; H, 4.61; N, 12.85.

Kinetic Studies. Above pH 9.0, the reaction mixtures containing 10:1:1 ratio of SPG to DPL (or PLP) to Cu(II) absorb dioxygen with characteristic changes in the UV-visible absorption spectra. The absorption band of the Cu-Schiff base complex at 390 nm disappears with a concurrent appearance of a band at 345 nm (see Figure 3). The distribution curves of the species in aqueous solutions containing (sulfophenyl)glycine (SPG), PLP or DPL, and Cu(II) at a 10:1:1 molar ratio (see Figure 4) show the formation of CuSB and Cu(SB)OH as the only Schiff base species above pH 9. Also, the presence of a small amount of $Cu(SPG)_2$ and a large excess of uncomplexed SPG is indicated. SPG and

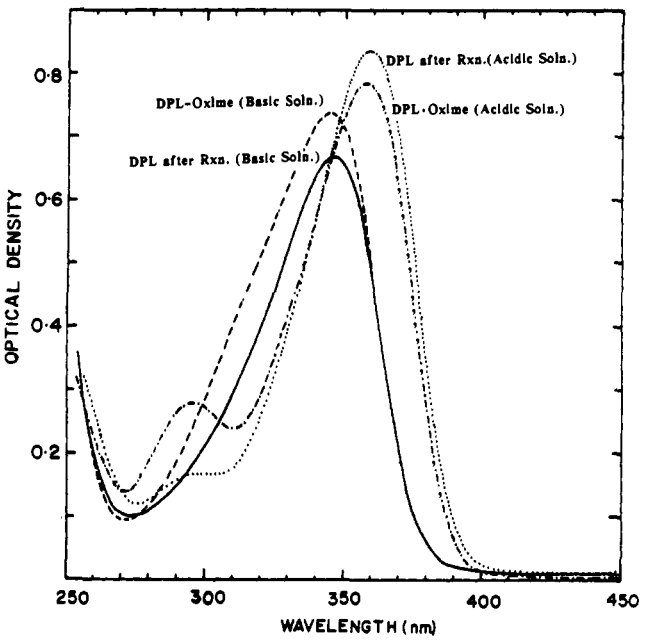


Figure 2. UV-visible absorption spectra of the DPL derivative isolated from the reaction mixture and the oxime of DPL: DPL derivative from reaction mixture (···, at pH 2.0; —, at pH 11.2); oxime of DPL (- - -, at pH 2.0; - · -, at pH 11.1) in aqueous solutions (1.0×10^{-4} M) in the wavelength range 250–450 nm at 25 °C.

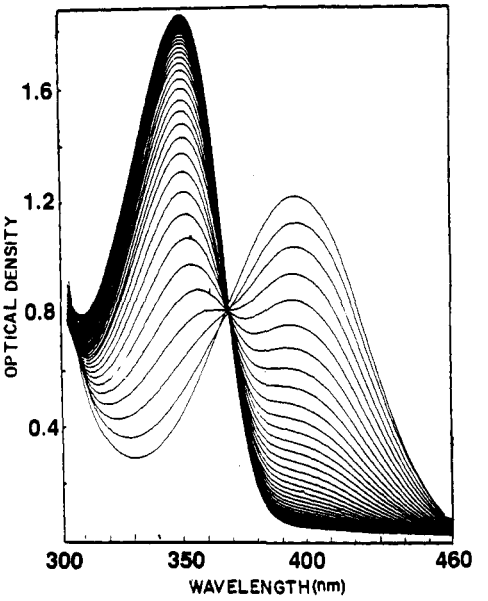


Figure 3. UV-visible spectra of the aqueous solution with SPG (2.50×10^{-3} M), PLP (2.50×10^{-4} M), and $CuCl_2$ (2.50×10^{-4} M): pH 11.20, m = 0.10 M (KCl), t = 25.0 °C. Oxygen was continuously bubbled through the solution. Successive scans are at 5-min intervals.

$Cu(SPG)_2$ absorb only below 280 nm, and CuSB and Cu(SB)OH both have absorption maxima around 390 nm. Thus, the disappearance of the 390-nm peak and the appearance of the new 345-nm band correspond to the reactions of CuSB and Cu(SB)OH only.

The disappearance of the 390-nm peak of Cu-Schiff base complexes and the oxygen uptake are confirmed to be characteristic only of oxidative deamination. Transamination of amino acids by pyridoxal analogues also results in the formation of keto acids. However, such reactions do not require oxygen and metal ions as catalysts. Also, they occur at optimal rates between pH 4 and 5. The intensity of absorption at 390 nm of Cu(II)-Schiff base chelate does not decrease in this pH range in the presence of oxygen. Also, no measurable oxygen uptake occurs in acidic solutions. Kinetics of oxidative deamination were thus studied

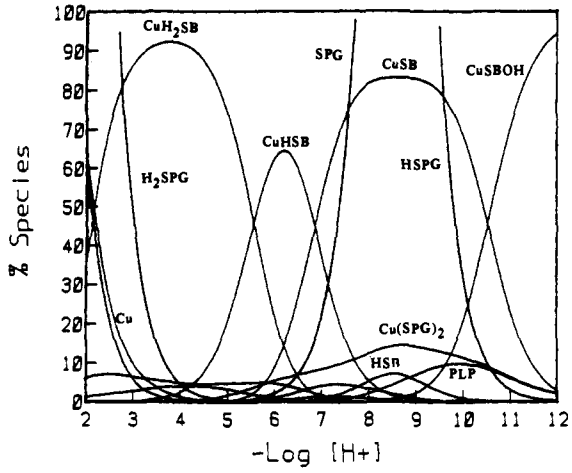


Figure 4. Species distribution curves calculated for the aqueous solution containing SPG, PLP, and Cu(II) at a molar ratio of 10:1:1. Percent species represents concentrations relative to PLP at an initial value of 2.00×10^{-3} M. CuSB, CuH(SB), CuH_2SB, and Cu(SB)OH represent unprotonated, monoprotonated, diprotonated, and hydroxo forms of the Schiff base chelate. H_2SPG and H(SPG) are di- and monoprotonated forms of SPG, (*p*-sulfophenyl)glycine; PLP is pyridoxal phosphate. Equilibrium data are from ref 21.

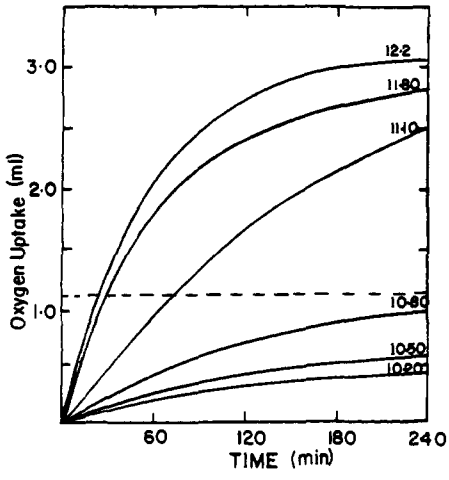


Figure 5. Initial rates of oxygen uptake in the aqueous solution containing 1.00×10^{-2} M SPG, 1.00×10^{-3} M PLP, and 1.00×10^{-3} M $CuCl_2$ (10:1:1 ratio) at pH values indicated: $P_{O_2} = 1.0$ atm, $t = 25.0$ °C, $V_{tot} = 50.0$ mL. The broken line indicates the volume of oxygen needed for one turnover (assuming all the oxygen corresponding to the change in the volume is utilized in deamination reaction).

by oxygen uptake and by measurements of absorption spectra between 300 and 460 nm at pH values above 10, with assurances of no interference by anaerobic transamination reactions.

Oxygen Uptake. In order to maintain a constant concentration of oxygen, the reaction mixtures were initially saturated with oxygen at 1.0 atm of pressure, which was maintained during the reactions. The reaction mixture was stirred at a uniform rate during measurements. The reaction was initiated by the addition of standard KOH solution, and additional amounts were added from time to time to maintain constant pH. In Figure 5, the amount of oxygen absorbed is plotted as a function of time for various pH values. Initially, oxygen uptake by the reaction mixture was rapid, and after some time, the absorption continued at a lower rate. Rates listed in Table II are calculated from the uptake of oxygen during the initial period.

$$-dV_{O_2}/dt = k_{O_2} \tag{3}$$

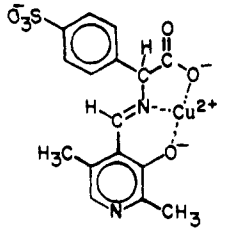
It is noted that, below pH 10.5, rates of oxygen uptake by the reaction mixture were very low. The data listed in Table II indicate the numbers of catalytic cycles with the assumption that all the oxygen absorbed by the reaction mixture is utilized in the deamination process. The data indicate that the rate of oxygen absorption increases considerably with pH.

Table II. Oxygen Uptake[a]

pH	$k_{O_2} \times 10^9$ (mol s^{-1})	O_2 adsorbed (mol) (time (min))	catalytic cycles
10.50	2.37	4.32×10^{-5} (180)	0.89
10.80	2.70	5.89×10^{-5} (205)	1.18
11.10	9.46	1.52×10^{-4} (502)	3.04
11.80	23.88	1.54×10^{-4} (450)	3.16
12.2	33.93	1.52×10^{-4} (395)	3.04

[a] $k_{O_2} = -dV_{O_2}/dt$ (initial); catalytic cycles = moles of O_2/moles of PLP; $[PLP]_{ini} = 1.0 \times 10^{-3}$ M in 50.0 mL.

Spectrophotometric Measurements. The Cu(II) complexes MSB and (MSB)OH of SPG–PLP Schiff base have absorption maxima at 390 nm, while those of SPG–DPL Schiff base have absorbance maxima at 393 nm. These bands disappeared with the development of new absorbance bands, respectively, at 343 and 345 nm upon introduction of oxygen to the solution (see Figure 3).

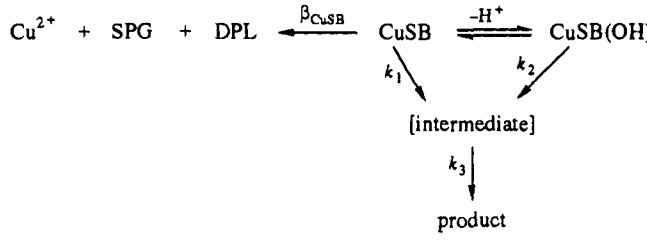


7, Cu(II) complex of DPL–SPG Schiff base

Oxidative deamination of SPG occurs only when pyridoxal 5′-phosphate or 5′-deoxypyridoxal, copper (II) ions, and molecular oxygen are present simultaneously in solution under the experimental conditions described above. In control experiments with the deliberate omission of any one of these constituents, the reaction did not proceed. This observation indicates that Cu(II)-free Schiff base species (H_nSB) do not undergo the oxidative deamination process.

The absorbance maximum around 390 nm disappears completely with the concurrent development of a maximum around 345 nm in 3–4 h when the reaction is carried out at and above pH 11. The analysis of the reaction mixture at this stage indicated the deamination of only a fraction of the SPG. Thus, the band at 345 nm was attributed to an intermediate. The rate of the disappearance of 390 nm is considered to be due to the disappearance of both CuSB and Cu(SB)OH, and the appearance of 345 nm is considered to be due to the formation of the intermediate (I).

The analysis of rate equations is based on the following reaction scheme:



where

$$k_{obs}[MSB] = k_1[CuSB] + k_2[Cu(SB)OH]$$
$$[MSB] = [CuSB] + [Cu(SB)OH] \tag{4}$$

With this reaction scheme, rate expressions for the disappearance of the Schiff base–Cu complex MSB and for the formation of the intermediate, I, are

$$-d[MSB]/dt = k_{obs}[MSB] \tag{5}$$

$$d[I]/dt = k'_{obs}[I] \tag{6}$$

Table III. Observed Rate Constants for Cu-SPG-DPL System at 25.0 °C, pH 11.0[a]

[SPG]:[DPL]:[Cu] ratio[b]	[dissolved oxygen] (M)	$k_{obs} \times 10^4$ (s^{-1})
10:1:1	[c]	2.40 ± 0.03
10:1:0.5	[c]	1.32 ± 0.01
10:2:1	[c]	2.43 ± 0.01
10:0.5:1	[c]	3.02 ± 0.01
5:1:1	[c]	3.66 ± 0.05
1:1:1	[c]	1.50 ± 0.01
10:1:1	1.41×10^{-3}	4.01 ± 0.02
10:1:1	0.70×10^{-3}	2.07 ± 0.02

[a] Same as Tables IV and V. [b] Concentrations are multiples of 2.5×10^{-4} M. [c] Constant concentrations approximately 0.80×10^{-3} M.

Table IV. Observed Pseudo-First-Order Rate Constants for Cu-SPG-DPL System[a,b]

pH	$k_{obs} \times 10^4$ (s^{-1}) at 25 °C	at 30 °C	at 35 °C
10.60	1.40 ± 0.01	2.57 ± 0.01	5.32 ± 0.01
10.80	1.62 ± 0.01	4.05 ± 0.01	7.70 ± 0.01
11.00	2.40 ± 0.01	6.21 ± 0.01	10.4 ± 0.01
11.20	5.23 ± 0.02	7.70 ± 0.01	12.4 ± 0.03
11.40	6.62 ± 0.03	9.99 ± 0.01	16.7 ± 0.03
11.60	7.56 ± 0.04	12.9 ± 0.02	22.2 ± 0.03
11.80	7.84 ± 0.03	15.2 ± 0.02	26.4 ± 0.04

[a] Deviations in k_{obs} are the errors in the slope of the best fit line obtained by the least-squares analysis of log $(A_t - A_\infty)$ vs time. [b] Reactant concentrations: [SPG] = 2.5×10^{-3} M; [DPL] = 2.5×10^{-4} M; $[Cu^{2+}] = 2.5 \times 10^{-4}$ M.

Table V. Observed Pseudo-First-Order Rate Constants for Cu-SPG-PLP System[a,b]

pH	$k_{obs} \times 10^4$ (s^{-1}) at 25 °C	at 30 °C	at 35 °C
10.60	0.37 ± 0.01	0.49 ± 0.01	0.97 ± 0.01
10.80	0.42 ± 0.01	0.75 ± 0.01	1.32 ± 0.01
11.00	0.44 ± 0.01	1.18 ± 0.01	1.70 ± 0.01
11.20	0.58 ± 0.02	1.68 ± 0.01	2.42 ± 0.01
11.40	0.99 ± 0.03	1.94 ± 0.01	3.06 ± 0.01
11.60	1.40 ± 0.04	2.11 ± 0.01	3.72 ± 0.01
11.80	1.70 ± 0.03	2.65 ± 0.01	5.80 ± 0.01

[a] Same as Table IV. [b] Reactant concentrations: [SPG] = 2.5×10^{-3} M; [PLP] = 2.5×10^{-4} M; $[Cu^{2+}] = 2.5 \times 10^{-4}$ M.

where k_{obs} and k'_{obs} are the rate constants for the disappearance of the Schiff base–Cu(II) complex and the formation of the intermediate, respectively.

The analysis of the spectrophotometric data suggested that the disappearance of the Schiff base chelate as well as the formation of the intermediate follow first-order kinetics and that k_{obs} and k'_{obs} were identical. This result is consistent with the presence of the isosbestic point at 367 nm (see Figure 3). The changes in absorbance of the 390-nm band were more reliable for following kinetics because there was no residual absorbance (A_∞) at the completion of the reaction, and the 345-nm band initially develops as a shoulder. Uniformity in the data treatment was maintained by selecting the data points for a period of 2 half-lives for the evaluation of rate constants. The rate constants determined by this method are listed in Tables III, IV, and V.

Data in Table III reflect the dependence of rate constants on the initial concentrations of reactants. The rates are higher in the systems where greater concentrations of Cu(II)–Schiff base complexes are formed. It is noted that the Cu(II) acts as a limiting reagent in determining the total concentrations of the metal Schiff base complexes. The data in Tables IV and V are in accord with the linear dependence of k_{obs} on the concentrations of the Schiff base chelate complexes.

Analysis of Rate Constants. The pseudo-first-order rate constants, k_{obs}, were calculated on the basis of the depletion of concentration of Cu–Schiff base complexes represented by the change in the absorption at 390 nm. The following expressions are used for the evaluation of the reactions of individual species:

$$k_{obs}([CuSB] + [Cu(SB)OH]) = k_1[CuSB] + k_2[Cu(SB)OH] \quad (7)$$

$$K^H_{CuSB} = \frac{[Cu(SB)OH][H^+]}{[CuSB]} \quad (8)$$

Thus

$$k_{obs} = \frac{k_1 + k_2K^H_{CuSB}[H^+]^{-1}}{1 + K^H_{CuSB}[H^+]^{-1}} \quad (9)$$

The species distribution curves indicate that the concentration of CuSB species reaches its maximum values below pH 9, but the reaction proceeds at insignificant rates at and below this pH. Thus, k_1 seems to be negligible compared to k_2, and eq 9 may be simplified as follows:

$$(k_{obs})^{-1} = [H^+](k_2K^H_{CuSB})^{-1} + k_2^{-1} \quad (10)$$

A plot of $(k_{obs})^{-1}$ as a function of $[H^+]$ is therefore expected to be a straight line with the slope equivalent to $(k_2K^H_{CuSB})^{-1}$. The specific rate constants calculated from the slope of eq 10 for both coenzyme systems at 25.0 °C are presented in Table VI.

The pseudo-first-order rate constants determined in this investigation depend on the concentration of Cu(II)–Schiff base complexes and oxygen, which is held at constant concentration corresponding to that of the saturated solutions of pure dioxygen in 0.10 M solution at 1.00 atm and at the temperature specified. The second-order rate constants may be obtained from the first-order constants reported by dividing them by molar concentrations of dioxygen under the reaction conditions specified, assuming saturated solutions.

The activation parameters were calculated with the use of an Eyring plot[30] and the kinetic data at 25.0, 30, and 35 °C and are listed in Table VI. A marginal difference in $\Delta G^\ddagger$ values can be noticed between the reaction systems containing PLP and DPL. This reinforces the idea that the mechanism of deamination is the same in both reaction systems. A sizeable difference can however be noticed in the cases of $\Delta H^\ddagger$ and $\Delta S^\ddagger$. The presence of the 5′-phosphate group seems to somehow affect the reaction rates adversely by increasing activation enthalpy.

Table VI. Specific Rate Constants[a] and Activation Parameters at 25 °C

Schiff base system	$k_2 \times 10^4$ (s^{-1})	$\Delta G^\ddagger$ (kcal/mol)	$\Delta H^\ddagger$ (kcal/mol)	$\Delta S^\ddagger$ (eu)
SPG–DPL–Cu	14.6	23	119	−13
SPG–PLP–Cu	1.79	23	20	−10

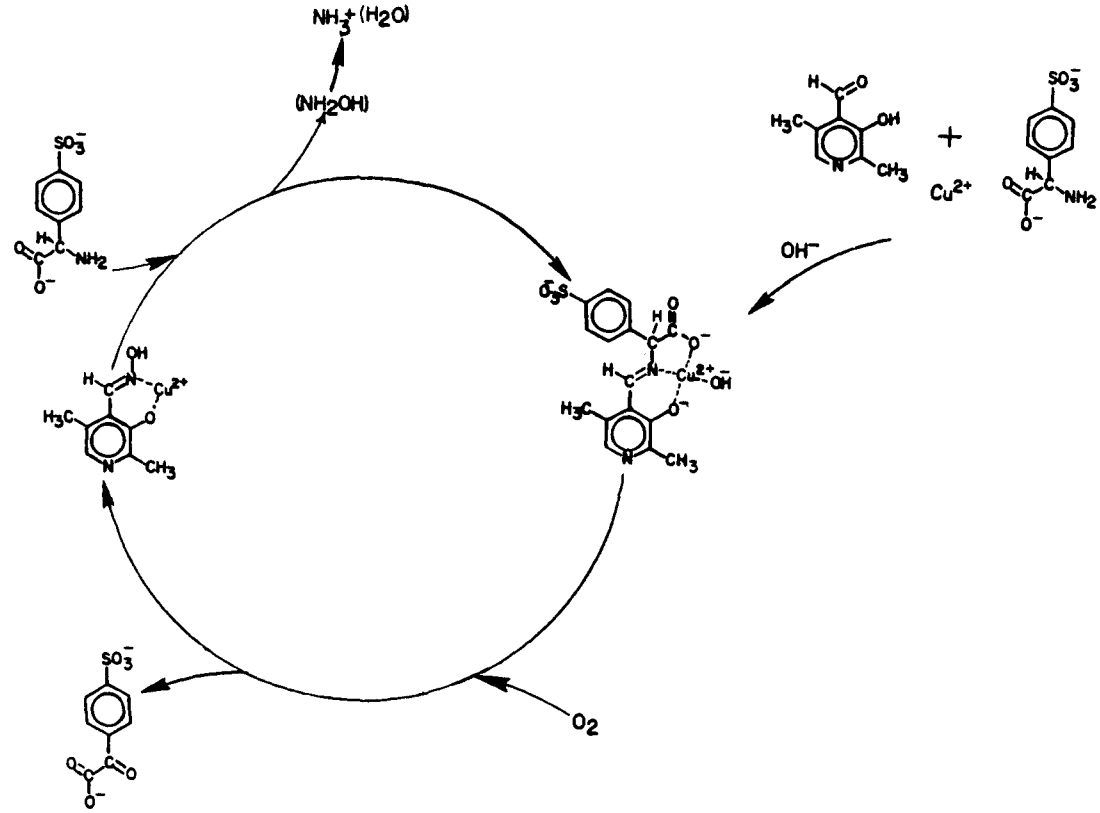
[a] Pseudo-first-order rate constants are given; they may be converted to second-order constants by dividing by the (constant) concentration of dioxygen.

Catalytic Systems. The reaction was demonstrated to be catalytic by the conversion of virtually all of a 10-fold excess of amino acid to keto acid. If the reaction was only stoichiometric, the uptake of oxygen and release of ammonia would have been equivalent to the amount of coenzyme (or Cu(II)) employed. Product analysis and characterization also confirmed complete reaction of SPG. The isolation of the oxime of the coenzyme from the reaction mixture demonstrates it to be an intermediate in the reaction. The catalytic cycle is illustrated in Scheme I.

The spectral changes are indicative of rapid depletion in the concentration of Cu–Schiff base complexes, while only a fraction of SPG undergoes deamination. This suggests that the Schiff base complexes are rapidly converted to the oxime derivative with the release of an equivalent amount of α-keto acid. After the first cycle, the intensity of the 345-nm band did not change and the absorption band of Cu(II)–Schiff base chelate complexes did not reappear, but the oxygen absorption and ammonia evolution

(30) Piszkiewicz, D. *Kinetics of Chemical and Enzyme Catalyzed Reactions*; Oxford University Press: New York, 1977; p 34.

Scheme I. Catalytic Oxidative Deamination Scheme



continued. Since the concentration of the oxime remains fairly constant after the initial conversion, it seems that only a small fraction of the coenzyme is converted back to Schiff base in the presence of a relatively large pool of amino acid substrate by the so-called transimination process.[31] The fact that the oxime concentration remains relatively constant indicates that the oxidative deamination reaction is much more rapid than the rate of displacement of hydroxylamine from the coenzyme by the amino acid, and that the Schiff base metal chelate is present at low steady-state concentration in accordance with Scheme I.

Thus, after the first cycle, the oxime is slowly converted to the SPG–Schiff base complex, which is rapidly oxidized to form the keto acid and regenerate the oxime, while the presumably released hydroxylamine decomposes to ammonia. The catalytic conversion of hydroxylamine to ammonia in alkaline solutions has been documented.[32,33] The release of ammonia by hydroxylamine in basic solutions was verified qualitatively by (a) placing hydroxylamine in aqueous solution at pH 11 and (b) addition of hydroxylamine to the reaction mixture after the completion of the reaction. In both cases, release of ammonia was observed. Also, Tatsumoto et al.[18] reported that a small amount of hydroxylamine was isolated in their deamination experiments. The evidence seems to point to a catalase-type reaction for the conversion of hydroxylamine to ammonia and dioxygen ($2NH_2OH \rightarrow 2NH_3 + O_2$). During the deamination process, there is no change in the oxidation state of the coenzyme, in contrast to transamination, in which the coenzyme undergoes two-electron reduction (e.g., PLP is converted to PMP).

The reaction pathway indicated in Scheme I for the oxidative deamination process was further confirmed by diagnostic experiments. A mixture of SPG, Cu(II), and the DPL–oxime (synthetic) in a molar ratio of 10:1:1, respectively, was placed in the reaction cell used for the kinetic measurements. The pH, concentration, and temperature conditions were maintained as in kinetic experiments with the exception that oxygen was completely excluded from the system. Absorption spectra were recorded as a function of time. A shoulder on the higher wavelength side of the 345-nm band of the oxime appeared with time, indicating the formation of a small amount of Cu(II)–Schiff base species. When oxygen was introduced into this solution, the shoulder disappeared. The analysis of the reaction mixture showed the presence of keto acid (SPGA) and ammonia, indicating that deamination of SPG had occurred in this system. Also, a first-order dependence of the reaction rate on the concentration of dissolved oxygen was inferred by experiment whereby the total concentration of dissolved oxygen was varied. The data in Table III indicate that the rate constant for the reaction was reduced by a factor of $^1/_2$ when the total concentration of dissolved oxygen was reduced by the same factor. The rate constants in Table III show first-order dependence on concentration of oxygen. This may become zero order in the later stages of the reaction when the oxime reaches steady-state concentration and the rate of loss of the Schiff base slows down. This should be tested with an oxygen monitor.

Data in Table III indicate that the variation in the concentration of Cu(II) has a greater effect on the rate constants than the variation in the concentrations of SPG and DPL. This suggests that the metal ions act as limiting reagents in determining the concentration of the Schiff base chelate complex. It must be noted that although the concentration of oxygen was maintained constant during most of the kinetic measurements, the reaction mixture was not completely saturated initially with dioxygen. When the solution was saturated initially over an extended period of time ([dissolved oxygen] $\simeq 1.4 \times 10^{-3}$ M), an enhancement in the rate constant was observed.

Proposed Reaction Mechanisms. The mechanism proposed by Hamilton and Revesz[17] for oxidative deamination of α-amino acids by pyridoxal and transition-metal ions (Scheme II) involves the coordination of oxygen to the metal ion of the Schiff base complex (**9** → **10**) and the transfer of a pair of electrons from amino acid through the coenzyme to the oxygen molecule via the metal ion (**10** → **11**). In the present study, a hydroxo complex of Cu-

(31) Weng, S. H.; Leussing, D. L. *J. Am. Chem. Soc.* **1983**, *105*, 4082.
(32) Holzapfel, H. *Wiss. Z. Karl-Marx-Univ. Leipzig, Math.-Naturwiss. Reihe* **1960**, *9*, 17.
(33) Schmidt, W. L.; Swinehart, J. H.; Taube, H. *Inorg. Chem.* **1968**, *7*, 1984.

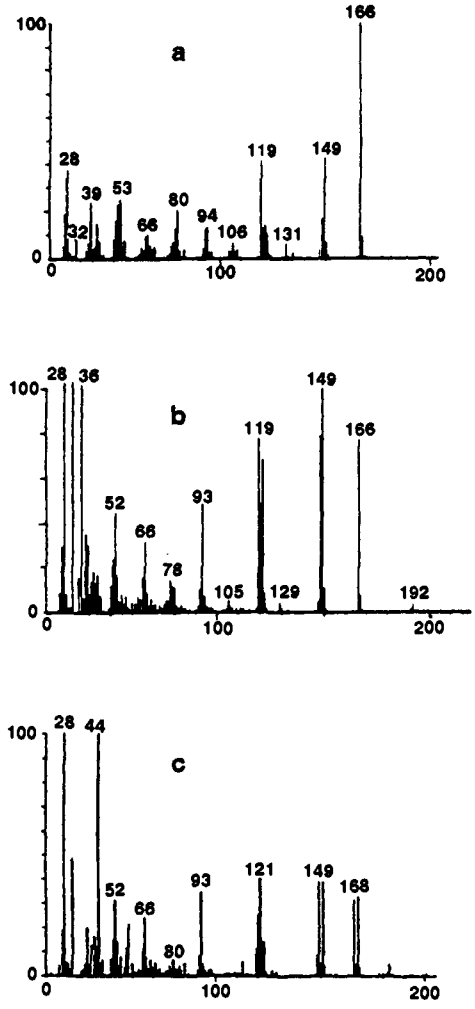
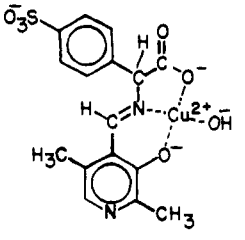


Figure 6. Electron impact mass spectra of (a) the compound (derivative of DPL) isolated from the reaction mixture when the reaction was run with $^{16}O_2$; (b) the oxime of DPL; and (c) the compound (derivative of DPL) isolated from the reaction mixture when the reaction was run with $^{18}O_2$.

(II)–Schiff base chelate (**8**) was found to be the reactive intermediate.

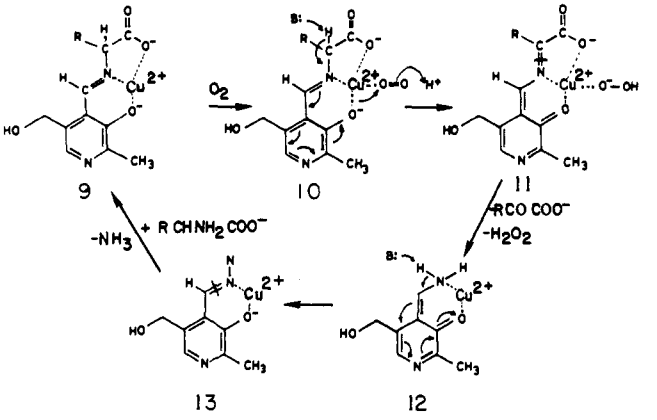


8, hydroxo complex of **7**

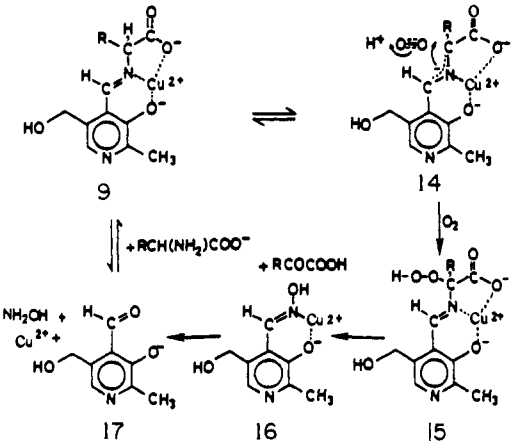
In **8**, the coordination of dioxygen with Cu(II) seems to be less probable because all four coordination sites of Cu(II) are occupied by strongly coordinating donor groups. Moreover, the formation of oxime may not require the mediation of metal ion in the electron-transfer process. Faleev et al.[34] reported the deamination of alanine in a 2:1 complex of alanine–salicylaldehyde Schiff base with Co(III) whereby dioxygen directly attacks the α-carbon of the amino acid. Tatsumoto et al.[18] also suggested a possibility of direct oxidation of the amino acid moiety (see Scheme III). However, one requires an activated form of oxygen for a direct attack on the substrate molecule. The coordination of the oxygen molecule with Cu(II) seems more reasonable because it assigns

(34) Faleev, N. G.; Belokon', Y. N.; Belikov, V. M.; Mel'nikova, L. M. *J. Chem. Soc., Chem. Comm.* **1975**, 85.102.

Scheme II. Proposed Mechanism for Vitamin B_6 Catalyzed Oxidative Deamination[17]



Scheme III. Proposed Mechanism for Vitamin B_6 Catalyzed Oxidative Deamination[18]

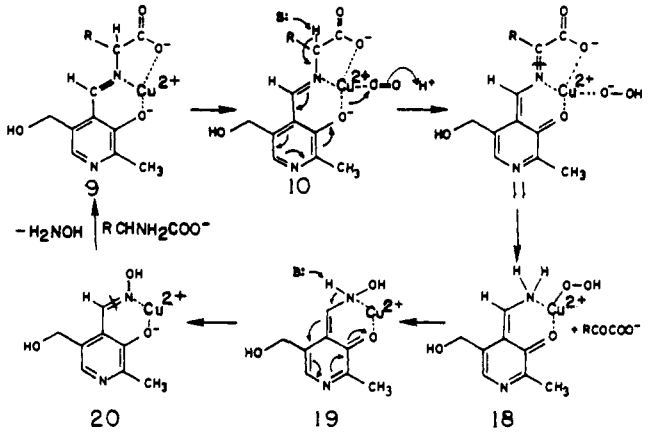


the metal ion a greater role in catalysis than otherwise. Tatsumoto et al.[18] also proposed an alternative mechanism (see Scheme IV) in which the formation of the oxime of the coenzyme was indicated as occurring through the interaction between the hydroperoxide formed in a previous step and the coordinated coenzyme. The sequence of O atom transfer, deprotonation, and electron shift shown in **18** → **20** is not considered critical, and the transformations could very well be concerted. This scheme seems to be the most plausible of the three reaction pathways suggested for the oxidative deamination reaction.

In order to obtain more information on the mechanistic aspects of the oxime formation, an $^{18}O_2$-labeling experiment was carried out. If the process occurs according to Scheme II, labeled oxygen will not be found in either the keto acid or the oxime. If Scheme III were correct, the keto acid will initially carry a labeled oxygen but not the oxime, unless in a concerted mechanism, oxygen interacts simultaneously with the α-carbon and azomethine nitrogen of the Schiff base chelate complex. However, the keto acid label would not be found. Cohn and Urey have reported[36] that a variety of carbonyl compounds exchange their oxygen rapidly in acidic as well as basic media, with both protons and hydroxyl ions catalyzing the process. However, if the hydroperoxide converts the imine to oxime as indicated in Scheme IV, only the oxime would carry the labeled oxygen. Mass spectra of the oxime samples obtained from experiments run with the use of $^{16}O_2$ and $^{18}O_2$ showed the molecular ion peaks at 166 and 168, respectively, indicating that the hydroxy group of the oxime (–N–OH) originates from dioxygen (see Figure 6 a,c).

The formation of keto acid, ammonia, and the original coenzyme or some derivative of the coenzyme (other than pyridoxamine) and lack of formation of hydrogen peroxide have been reported in all the previous oxidative deamination studies.[14-18] Schemes III and IV suggested here conform to these observations.

Scheme IV. Proposed Mechanism for Vitamin B_6 Catalyzed Oxidative Deamination[18]



The isolation of hydroxylamine from the methanol solution of the reaction mixture by Tatsumoto et al.[18] may be due to slower decomposition of hydroxylamine in methanol solution; thus in their systems, formation of ammonia was not observed as the only deamination product.

The formation of hydroxylamine seems to be characteristic of oxidative deamination only in model systems. In contrast, hydroxylamine has been found to inhibit oxidative deamination in enzyme systems.[35] Also, the formation of hydrogen peroxide, which is commonly observed in enzymic deaminase reactions, has not been observed in model studies. There is a parallel here, in that hydrogen peroxide and hydroxylamine are isoelectronic and represent half of the four-electron oxidative capability of dioxygen (the keto acid constitutes the other half). The lack of identification of hydrogen peroxide in model systems may very well be due to the catalase activity of the transition-metal complexes present, just as the loss of hydroxylamine in the present catalytic system may be due to similar catalase-like activity of Cu(II) complexes. The role of Cu(II) ion in oxidative deamination in biological systems is yet to be explored.

(35) Green, A. L. *Biochem. Pharmacol.* **1964**, *13*, 249. 3.
(36) Cohn, M.; Urey, H. C. *J. Am. Chem. Soc.* **1938**, *60*, 679.

Concluding Remarks

The oxidative deamination reaction occurs at optimum rates in alkaline solutions. The reaction rates are negligible below pH 9.0. Of the metal ions studied [Co(II), Mn(II), Ni(II), Cu(II), and Zn(II)], Cu(II) was found to be most effective in catalyzing the deamination of (sulfophenyl)glycine mediated by pyridoxal 5'-phosphate or 5'-deoxypyridoxal. The deamination reaction proceeded about 5 times faster in the presence of 5'-deoxypyridoxal than in the presence of pyridoxal 5'-phosphate. The principle Schiff base complex species involved in the rate-determining step of deamination of (sulfophenyl)glycine was found[21] to be the monohydroxo complex of the Cu-Schiff base chelate. The most novel and fundamental finding in this study is the rapid stoichiometric conversion of the Cu(II) complex of the Schiff base species to the oxime of the coenzyme. This oxime participates as an intermediate in the oxidative deamination cycle until the deamination of substrate amino acid reaches completion. In this process, the amino acid seems to displace a hydroxylamine molecule, which rapidly decomposes to ammonia. Reasonable mechanisms illustrated in Schemes III and IV (we prefer Scheme IV) are proposed that seem to account for formation of both keto acid and oxime of the coenzyme.

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Registry No. 1, 134653-57-9; **2**, 54-47-7; **3**, 1849-49-6; **3** (oxime), 826-71-1; **5**, 134653-58-0; **6**, 5363-54-2; MPG, 24593-48-4; D-PG, 875-74-1; NH_3, 7664-41-7; $Cu(NO_3)_2$, 3251-23-8; $Mn(NO_3)_2$, 10377-66-9; $Co(NO_3)_2$, 10141-05-6; $Ni(NO_3)_2$, 13138-45-9; $Zn(NO_3)_2$, 7779-88-6.

Stereochemical Studies on Reversible Metal–Nitrogen Transfer of Alkyl and Aryl Groups in Chiral Cobalt(III) Porphyrins. Relevance to the Mechanism of a Metabolic Heme Inactivation Process

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Abstract: Resolution of the antipodes of chiral σ-alkyl- and σ-aryl-cobalt(III) complexes of etioporphyrin I was first achieved, where the latter was shown to be much more stable than the former toward thermal racemization. Use of these antipodes for stereochemical studies on the mechanism of reversible cobalt–nitrogen transfer of alkyl and aryl groups in cobalt porphyrins revealed that the transfers from cobalt to nitrogen and from nitrogen to cobalt both take place in intramolecular fashions.

Introduction

During the xenobiotic metabolism of phenylhydrazine, the prosthetic group of the hemoprotein is denatured into an abnormal green pigment, which has been identified as *N*-phenylprotoporphyrin IX.[1] Ortiz de Montellano et al. have demonstrated that this reaction occurs via the transient formation of a protein-stabilized intermediate bearing a σ-phenyl-iron bond. Model studies have demonstrated that σ-aryl groups bonded to iron(III) porphyrins undergo an oxidative transfer reaction to the pyrrole nitrogen upon aerobic acid workup, giving the corresponding N-substituted porphyrins.[2] Furthermore, iron(II) N-substituted

(1) Lavallee, D. K. *The Chemistry and Biochemistry of N-Substituted Porphyrins*; VCH: New York, 1987; pp 261-298 and references cited therein.

(2) (a) Ortiz de Montellano, P. R.; Kunze, K. L. *J. Am. Chem. Soc.* **1981**, *103*, 6534. (b) Kunze, K. L.; Ortiz de Montellano, P. R. *J. Am. Chem. Soc.* **1981**, *103*, 4225. (c) Augusto, O.; Kunze, K. L.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1982**, *257*, 6231. (d) Kunze, K. L.; Ortiz de Montellano, P. R. *J. Am. Chem. Soc.* **1983**, *105*, 1380. (e) Ortiz de Montellano, P. R.; Kunze, K. L.; Beilan, H. S. *J. Biol. Chem.* **1983**, *258*, 45.

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