Oxidative Deamination and Oxygenation of a Cu^{II} Schiff Base Complex

## Arthur E. Martell* and Venkatesh M. Shanbhag

Department of Chemistry, Texas A&M University, College Station, TX 77843–3255, U.S.A.

The copper(II) complexes of Schiff bases formed from sulphophenylglycine with vitamin B_6 coenzymes, pyridoxal-5'-phosphate and 5'-deoxypyridoxal, react with dioxygen to produce the corresponding keto acid and new dioxygen insertion products, the oximes of the coenzymes.

It has been reported that vitamin B_6 catalyses the oxidative conversion of amino acids to keto acids by dioxygen through the formation of transition metal Schiff base complexes as intermediates.[1—5] Although this reaction had been advanced as a model for Cu^{II}-containing amine oxidase, it has been shown recently that the vitamin B_6 coenzyme, pyridoxal-

phosphate is not present in copper(II)-activated oxidase enzymes.[6—8] However, the vitamin B_6-catalysed oxidation of amino acids to keto acids, which was established by preliminary studies,[1—5] is considered worthy of further investigation, partly because it is a unique chemical reaction, and also because all of the reacting components are present in biological systems, thus still holding out the promise of possible biological significance. This paper describes conversion of the Cu^{II} complex of the 1:1 Schiff base (SB) of pyridoxal-5'-phosphate (PLP) and 5'-deoxypyridoxal (DPL) with p-sulphophenylglycine (SPG) to form the corresponding keto acid and the oximes of PLP and DPL, thus providing evidence for a Cu^{II}-catalysed oxygenase type reaction (oxygen insertion) rather than the expected oxidase (electron transfer) reaction.

Potentiometric equilibrium studies have demonstrated that 1:1 copper(II) chelates of the Schiff bases formed from PLP and DPL with SPG are the dominant metal complexes formed in solution over a wide pH range.[9] Figure 1 shows the distribution of species present in a typical solution containing an excess of amino acid. The pyridine N-protonated copper(II) Schiff base predominates in acidic solution, and the hydroxo complex is the major species in highly alkaline solution, while from pH 7—10 a small fraction of the predominant intermediate aquo Schiff base complex (1) is converted to the 2:1 amino acid chelate. This sytem is characterized by a strong absorption band of the Cu^{II} Schiff base chelate; the initial $t = 0$ curve for the Cu^{II} DPL–SPG Schiff base at pH 11 (Figure 2) is typical, with about 70% CuSB(OH) and 30% CuSB (both species have absorbance maxima at the same wave length). At constant concentration of dioxygen (i.e., at constant pressure of gaseous dioxygen), a psuedo first order reaction was observed in which a new compound with $\lambda_{max.}$ ~340 nm is formed. This compound was identified as the oxime of the

R = —⟨benzene⟩—SO_3^-;

R' = $CH_2OPO(OH)_2$ for PLP;

R' = Me for DPL
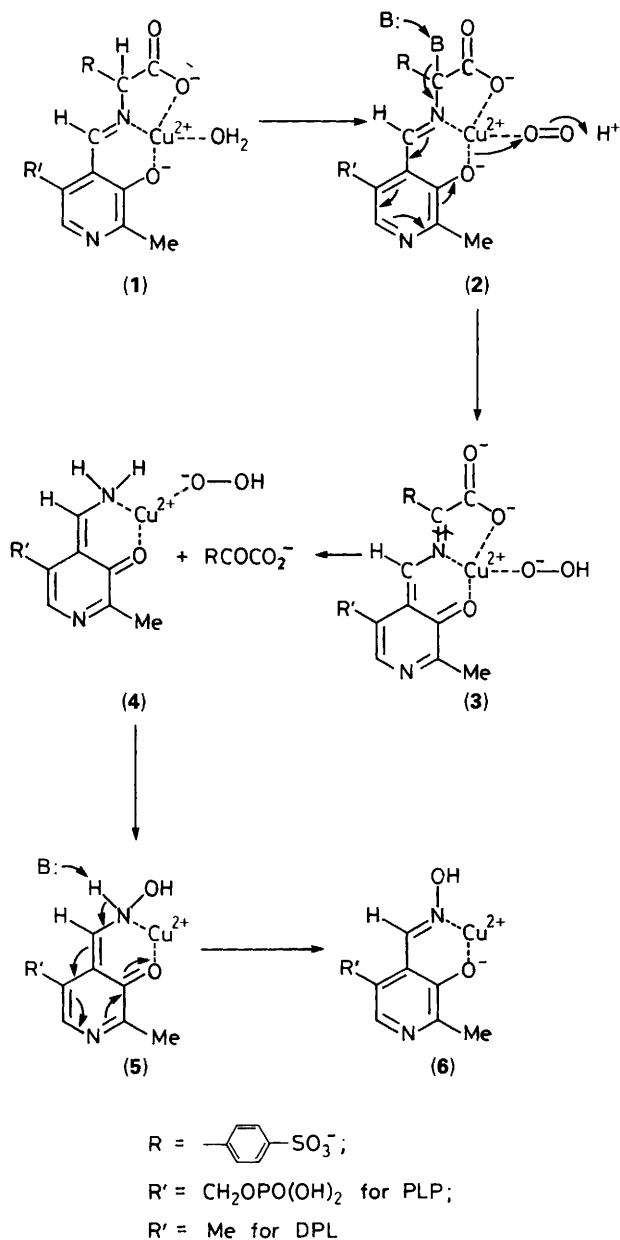
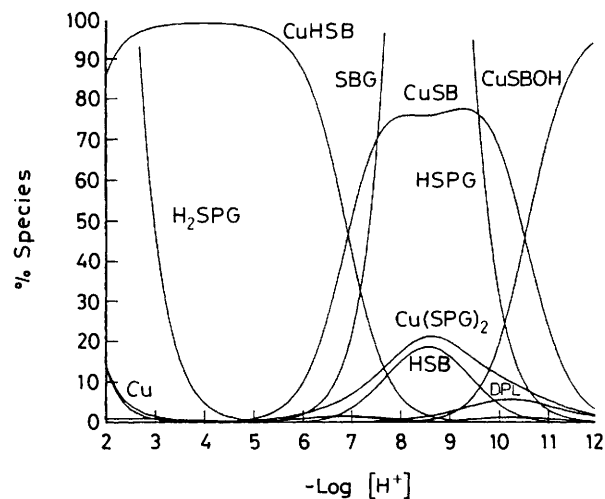**Scheme 1.** Proposed mechanism for vitamin B_6 catalysed deamination.

Figure 1. Species distribution curves for an aqueous solution containing SPG, DPL, and Cu^{II} at a molar ratio of 10:1:1. % Species represents concentrations relative to DPL.
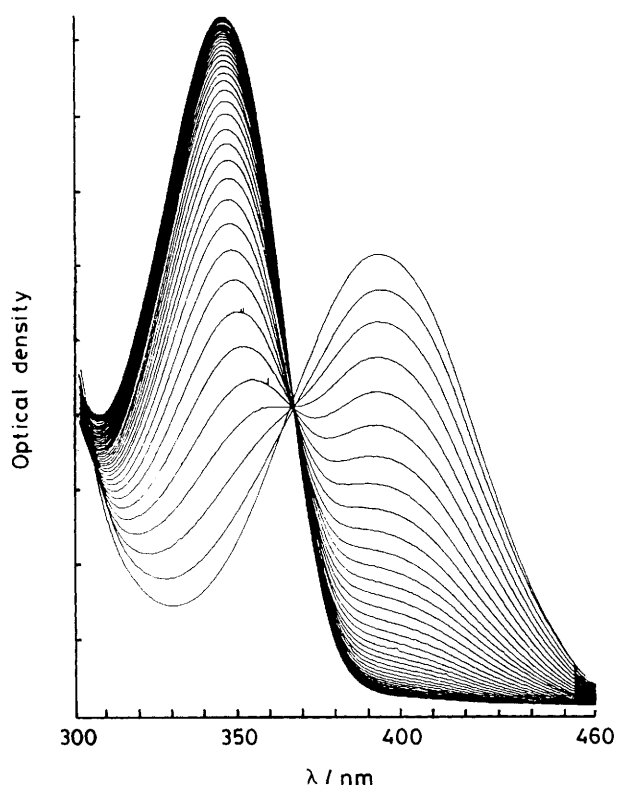
Figure 2. UV-VIS spectra of the aqueous solution with SPG (2.50 × 10^{-3} M), DPL (2.50 × 10^{-4} M), and $CuCl_2$ (2.50 × 10^{-4} M). pH = 11.00, μ = 0.10 M (KCl), T = 25.0 °C. Oxygen was continuously bubbled through the solution. Successive scans are at 3 min intervals.

coenzyme by product isolation and analysis, as well as by comparison with an authentic synthetic sample prepared from the coenzyme and hydroxylamine. The other reaction product, p-sulphophenylglyoxylic acid, was also isolated nearly quantitatively from the same reaction mixture. Parallel results were obtained from the analogous reaction system with PLP in place of DPL. The first order rate constants calculated from the disappearance of the Schiff base absorption band and from the appearance of the oxime absorbance were found to be identical. Typical pseudo first order rate constants measured at 25.0 °C and pH 11.0 are 2.40 ± 0.03 × 10^{-4} s^{-1} for the 10:1:1 SPG–DPL–Cu^{II} system and 4.4 ± 0.1 × 10^{-5} s^{-1} for 10:1:1 SPG–PLP–Cu^{II} system. For both systems, kinetic analysis of reaction rates as a function of pH showed that the specific rate constant involving CuSB was negligible compared to that of CuSB(OH). The second order nature of these reactions was demonstrated by measuring the pseudo first order rates at lower dioxygen concentration. Cutting the concentration of dioxygen to one half lowered the rate of oxime formation by 50%, thus confirming that the reaction

rate is first order in the concentration of Schiff base complex and first order in the concentration of dioxygen.

Finally, the reaction with DPL was carried out with only $^{18}O_2$ as the source of dioxygen. Isolation of the oxime and the keto acid from the reaction mixture and mass spectral analysis of the products (electron impact for the oxime and FAB MS for the keto acid) showed that all of the oxygen of the oxime comes from molecular oxygen, while the carbonyl oxygen generated in the formation of the keto acid comes from the solvent. With these results it is suggested that the insertion of the oxygen at the imino nitrogen of the Schiff base occurs through formation of a short-lived peroxo intermediate of the type first suggested by Tatsumoto et al.[5] According to this mechanism the dioxygen co-ordinates to the copper(II) of the Schiff base complex (1) to form an unstable intermediate (2) in which the dioxygen is partially reduced by two-electron oxidation of the co-ordinated coenzyme (as originally suggested by Hamilton[4]). After dissociation of the keto acid by hydrolysis (3, 4) the co-ordinated hydroperoxide or peroxide group converts the potential imino group in (5) to the oxime, as in (6) (Scheme 1).

The possibility of the involvement of Cu^I in dioxygen complex formation has been suggested by the referee of this communication. It is possible to postulate reduction of Cu^{II} to Cu^I in place of the two-electron reduction (of dioxygen) in (2), with the other electron remaining on the heterocyclic ring, stabilized by resonance. Combination of the Cu^I species with dioxygen would then lead to a Cu^{II}-superoxide species, which in effect would be a resonance form of (3). Consideration of the mechanistic details involving resonance forms of Cu^{2+} and Cu^{1+} co-ordinated to oxidizing and reducing species is beyond the scope of this communication.

Regardless of mechanistic considerations, however, the present reaction is the first clear-cut example of the formation of an oxime by metal ion-catalysed insertion of dioxygen on a co-ordinated azomethine Schiff base nitrogen.

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