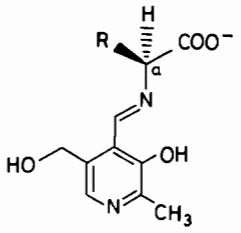
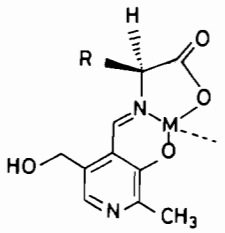
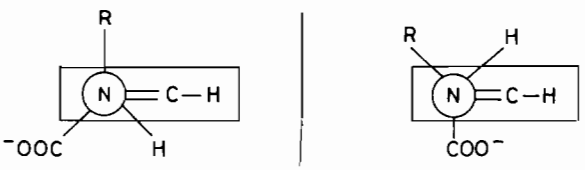


I



II

complex I in such a way as to allow a stereochemical differentiation between the groups at the amino acid C_α. The understanding of the stereochemical course of pyridoxal-dependent reactions usually relies upon the determination of the stereochemistry of the products in the hypothesis, put forward by Dunathan [2], that in the substrate–cofactor complex the bond to be broken is oriented perpendicular to the plane of the extended conjugated π system [3]. Direct evidence of the structure of the substrate–cofactor complex might however be obtained if we could relate the features of *e.g.* the CD spectra of I to the conformation of the C_α–N bond. We have shown that this is easily achieved in model metal complexes such as II, since their CD spectra correlate with the mode of binding of the amino acid residues [4, 5]. In particular, it is invariably found that the predominant conformation of the amino acid chelate ring of II contains the side chain R in the axial disposition. This conformation involves a ring chirality of sign λ for L-amino acids and is identified by Cotton effects of negative sign within the azomethine CD band. In free pyridoxal–amino acid Schiff bases like I a much wider range of conformations about the C_α–N bond is theoretically possible and correlations between CD spectra and conformations are more difficult to assess. The CD spectra of the Schiff bases I actually vary with the nature (polar, nonpolar, aromatic) of the L-amino acid side chain. However, a careful analysis of the chirality of the dominant interacting chromophores shows that the predominant conformers of I are restricted within the narrow range depicted by III and IV [6]. These CD results agree with previous NMR conformational studies [7].



III IV

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U5

The Properties and Structures of Glutathione–Cu(II) Complexes and SOD Activity

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The properties of Cu(II) complexes of reduced and oxidized glutathione ligands, respectively, were examined by potentiometric titration, electron spin resonance and visible absorption spectroscopy. Three Cu(II) complexes for reduced glutathione, [blue (I), green (II), and violet (III)], and three Cu(II) complexes for oxidized glutathione were obtained, respectively. The physiological concentration of reduced glutathione in human erythrocytes is 2 m*M* and that of the oxidized form is 4 μM. Thus, the complexation of reduced glutathione and Cu(II) is of biological interest.

Reduced glutathione and the Cu(II) 1:1 system below pH 6 forms a polymerized Cu(I) complex which does not dissolve even at high pH. Above pH 6, however, soluble Cu(II) complexes are obtained. The pH-dependent frozen solution ESR spectra reveal the presence of four Cu(II) species, namely, I, II, III and $[Cu(OH)_4]^{2-}$ (IV). The complexes I, II and III are interconvertible with protonation and deprotonation of peptide in the following manner.

$$2Cu(II) + 2GH_4SH \underset{pH \sim 6}{\rightleftharpoons} I \underset{pH \sim 9}{\rightleftharpoons} II \underset{pH \sim 10.5}{\rightleftharpoons} III \underset{pH \sim 12}{\rightleftharpoons} IV$$

The structure of III has been reported before [1]. The results show that the complexes, I, II, and III are binuclear, and the ligand coordinates with Cu(II) as its oxidized form. The coordination of Cu(II) to I at physiological pH involves the glutamic amine

TABLE I. Visible and ESR Spectral Data for 1:1 Reduced Glutathione–Cu(II) Complexes at Various pH.

Species	(pH)	λ_{max} (nm)	ϵ/Cu^{2+}	$g_\parallel$	$g_\perp$	$A_\parallel$ (G)	$A_\perp$ (G)
I, blue	(8.0)	620	60	2.256	2.055	163	–
II, green	(9.5)	615	70	2.258	2.059	165	–
III, violet	(11.3)	593	95	2.251	$g_x = 2.047$ $g_y = 2.038$	177	$A_x = 43.8$ $A_y = 36.0$
IV, $[Cu(OH)_4]^{2-}$	(12.5)	593	105	2.239	2.045	186	30.0

nitrogen, the glycinyl terminal carboxylate oxygen and the two protonated amide in an approximate planar coordination while the cystinyl sulfur is bonded apically to form a square-pyramidal. ESR parameters and absorption maximum are shown in Table I.

On the other hand, for the 1:1 and 1:2 oxidized glutathione and Cu(II) systems, our results are in agreement with those reported by White *et al.* [2] and Kroneck [3], respectively.

Since the discovery of superoxide dismutase (SOD) in 1969, there has been a search for low molecular weight complexes with high SOD activity. We examined the ability of I to act as a superoxide dismutating agent. The blue complex I at pH 7.8 inhibits the reduction of cytochrome c by the xanthine–xanthine oxidase system. The second rate constant of the reduction with O_2^- was estimated to be 2×10^7 M^{-1} sec $^{-1}$ which is close to the value (5×10^7 M^{-1} sec^{-1}) of the antiarthric drug, salicylate–Cu(II) complex. The Cu(II) chelate of glutathione is able to act as a stronger superoxide dismutating agent than the ligand (6.7×10^5 M^{-1} sec^{-1}). A role of complex I as a scavenger of O_2^- is suggested in biological systems.

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U6

Solid and Ethanolic Solution State Behavior of N-Tosylglycinate–Copper(II) System

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In the copper(II) ion–N-tosylglycinate system, in both the solid state and in aqueous solution, it was suggested that the ligand presents the properties of both L-α-amino acids [1] and their N-acetyl or N-benzoyl-derivatives [2] depending on the pH of the media [3]. In this paper we report an investigation on the same system in ethanolic solution in order to compare the coordination behavior of this amino acid with those of N-acetyl and N-benzoyl-amino acids also in this media.

From ethanolic solution two compounds of formula $[Cu(TsglyH)_2]_n$ (green) and $Na_2[Cu(Tsgly)_2]$ (blue) (TsglyH and Tsgly = N-tosylglycinate monoanion and dianion, respectively) were isolated. In the latter complex, which is similar to a compound previously separated from aqueous solution, the ligand acts as bidentate through the carboxylate oxygen atom and the deprotonated sulphonic nitrogen atom. For the green compound the crystal structure was also determined. Crystals are monoclinic, space group $P2_1/n$, with $Z = 4$ in a unit cell of dimensions: $a = 24.655(3)$, $b = 7.697(2)$, $c = 12.378(3)$ Å, and $\beta = 87.34(8)°$, and $R = 0.052$. The structure (Fig. 1) is built up of one dimensional polymeric chains of binuclear units, showing the cupric acetate