# AROMATIC COTTON EFFECTS INDUCED BY Cu(II) COORDINATION

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Abstract: Formation of Cu(II) complexes of peptides containing aromatic side chains induces major enhancement of the optical activity of the aromatic transitions. [(Gly-L-Tyr)Cu(II)] shows a peak at 233 mμ, [M] = +62,000°, and a trough at 198 mμ, [M] = -450,000°. Similar Cotton effects are shown by [(Gly-L-Phe)Cu(II)] and [(lysine vasopressin)Cu(II)].

Cotton effects associated with the d-d transitions of Cu(II) complexes of L-amino acids have been known since 1925 (Lifschitz, 1925; Pfeiffer and Christeleit, 1937). Much discussion has centered around the origin of these Cotton effects, since titration, spectral, and X-ray data indicate an essentially square planar structure for the complexes (see Martell and Calvin, 1952). The recent demonstration of multiple dichroic bands in the visible absorption spectrum of copper L-amino acid complexes supports the square planar structure (Yasui et al., 1965). A variety of unresolved Cu(II) dipeptide complexes show similar Cotton effects in the region of the d-d transitions (Bryce et al., 1965). In addition, Urry and Eyring (1964) have shown in the case of L-histidine that formation of complexes with first transition metal ions including Cu(II) results in marked alterations of the anomalous rotatory dispersion in the region of the ultraviolet chromophores of the ligand.

The present communication reports the finding that Cu(II) coordination of peptides containing aromatic side chains results in marked enhancement of the optical activity of the far ultraviolet aromatic transitions. Some of the Cotton effects reach molecular rotations of several hundred

thousand degrees and thus could significantly influence the optical rotatory dispersion (ORD) of copper proteins.

Visible and ultraviolet ORD and absorption spectra of [(Gly-L-Tyr)Cu(II)], [(L-Tyr-Gly)Cu(II)], and the free peptides are shown in Fig. 1. Titration data obtained in connection with the present study

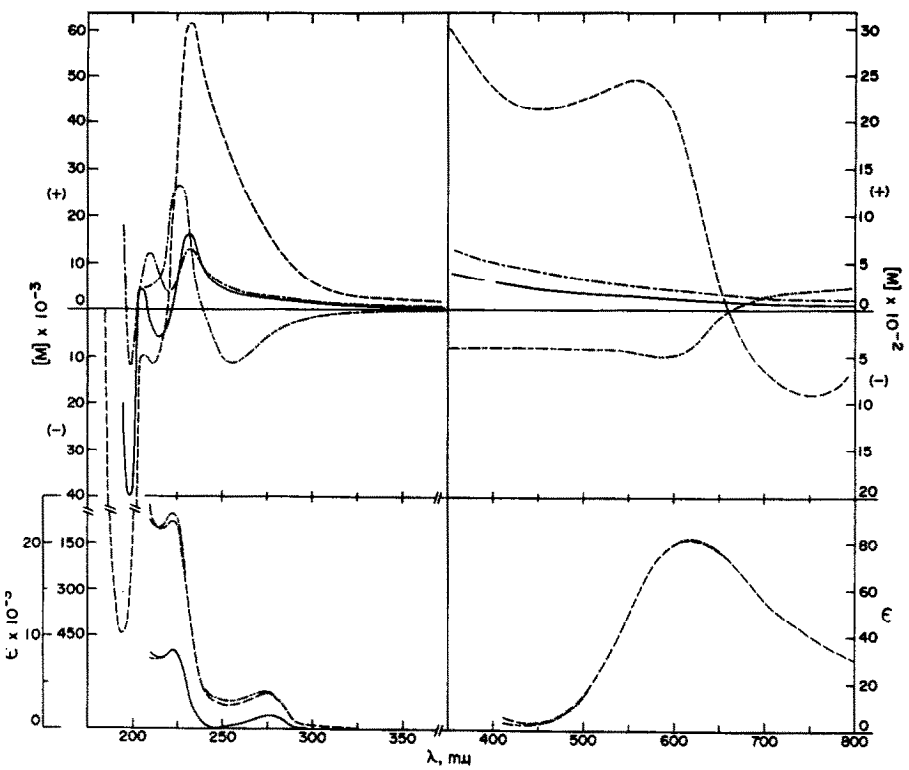
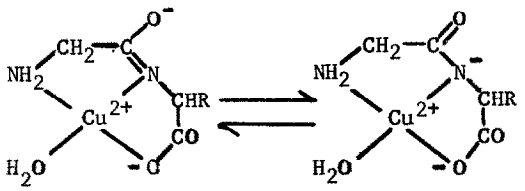



Figure 1. ORD and absorption spectra of Gly-L-Tyr and L-Tyr-Gly Cu(II) complexes: 2:1 mixtures, pH 7.2, 25°. ——, Gly-L-Tyr; – – –, [(Gly-L-Tyr)Cu(II)]; — · —, L-Tyr-Gly; — ·· —, [(L-Tyr-Gly)Cu(II)]. Solutions of 2:1 and 1:1 peptide:Cu(II) mixtures gave the same ORD profiles, supporting the previously held view that the 1:1 complex is the predominant form at neutral pH even in the 2:1 mixture. Peptides were obtained chromatographically pure from Mann Research Laboratories. ORD measurements were made with a Cary Model 60 recording spectropolarimeter. Rotation is expressed as molecular rotation, [M] = [α] x MW/100. Deviations of a series of curves for [(Gly-L-Tyr)Cu(II)] were as follows: 195 mμ, ± 5000°; 250 mμ, ± 2000°; 350 mμ, ± 1000°; 500 mμ, ± 50°; 750 mμ, ± 200°. Molar absorptivity for the complexes is plotted per Cu(II) ion.

show formation of these complexes at pH 7.2 to be accompanied by release of two protons in addition to the carboxyl proton, implying coordination

similar to that of the aliphatic dipeptide Cu(II) complexes. Titration and spectral data indicate that structure I is the predominant form of the latter complexes at neutral pH (Dobbie and Kermack, 1955; Datta and

I 

Rabin, 1956; Koltun et al., 1960; Kim and Martell, 1964). The visible absorption bands, maxima at 630 mμ (ε 81), are similar to those reported for the aliphatic dipeptide Cu(II) complexes. If the aromatic residue is C-terminal, the visible absorption band of the Cu(II) complex shows large negative asymmetric anomalous rotatory dispersion centered ~ 660 mμ, amplitude 3350°. If the aromatic residue is N-terminal, the absorption band undergoes practically no change, but the anomalous dispersion is much smaller and the overall sign appears reversed. Since the visible anomalous rotatory dispersion is a composite of several Cotton effects (Coleman, 1966), sign and position of the dichroic bands involved are difficult to predict.

In the ultraviolet, the tyrosine peptides have complex ORD spectra consisting of several Cotton effects beginning at 233 mμ and extending to 190 mμ, undoubtedly reflecting in part optical activity of aromatic transitions. As with the visible ORD, the most striking changes induced by Cu(II) coordination occur if the aromatic residue is C-terminal. The Gly-L-Tyr complex shows a very large peak at 233 mμ, [M] = +62,000°, followed by a crossover at 222 mμ. This Cotton effect seems to be associated with the 222 mμ band of the side chain. There follows an even more striking Cotton effect with a trough at 194 mμ, [M] = -450,000°, which appears to represent optical activity of the tyrosine absorption band near 192 mμ ( ε ~ 47,000) (Wetlaufer, 1962).

Aliphatic dipeptide Cu(II) complexes have large negative composite Cotton effects in the ultraviolet associated with an intense absorption

band at 235 mμ ($\epsilon \sim 4000$) (Bryce et al., 1965; Coleman, 1966). This absorption band is also present in the aromatic dipeptide complexes (Fig. 1), but optical activity associated with this band in the Gly-L-Tyr complex is obscured by the aromatic Cotton effects. In marked contrast, the L-Tyr-Gly complex does not show these striking aromatic Cotton effects, but has an ORD profile similar to the L-aliphatic amino acid-glycine Cu(II) complexes.

[(Gly-L-Phe)Cu(II)] shows analogous Cotton effects, but with the first peak at 222 mμ, [M] = +78,000°, in agreement with the location of the intense aromatic transitions of phenylalanine. L-Tyrosine and L-phenylalanine Cu(II) complexes also show enhancement of the optical activity of the aromatic transitions, except the signs of the Cotton effects are reversed; large troughs appear at 233 mμ and 222 mμ.

The Cu(II) complex of lysine vasopressin has been shown to involve coordination to the peptide nitrogens of the tyrosyl, phenylalanyl, and glutamyl residues, plus the α-amino group of the cystinyl residue (Campbell et al., 1963). Thus coordination about the tyrosyl and phenylalanyl residues approaches that of the dipeptide copper complexes and some of the analogous Cotton effects may be predicted (Fig. 2). The complex has an absorption maximum at 520 mμ ($\epsilon$ 155). This band shows asymmetric anomalous rotatory dispersion centered at 550 mμ, amplitude $\sim$ 8000°. The free peptide has an ultraviolet ORD similar to that expected for a random coil. On complexation with Cu(II), a peak appears at 233 mμ, [M] = +134,000°. Position and general profile fit that observed for [(Gly-L-Tyr)Cu(II)], but the coordinated phenylalanyl residue may also contribute. Like Gly-L-Tyr and Gly-L-Phe complexes, the vasopressin complex has a deep trough or combination of two troughs near 200 mμ, $[M]_{200} \simeq -300{,}000°$.

Reasons for enhancement of the optical activity of the aromatic side chain transitions on formation of the Cu(II) complexes are not evident from gross coordination geometry. Coupling between side chain transitions

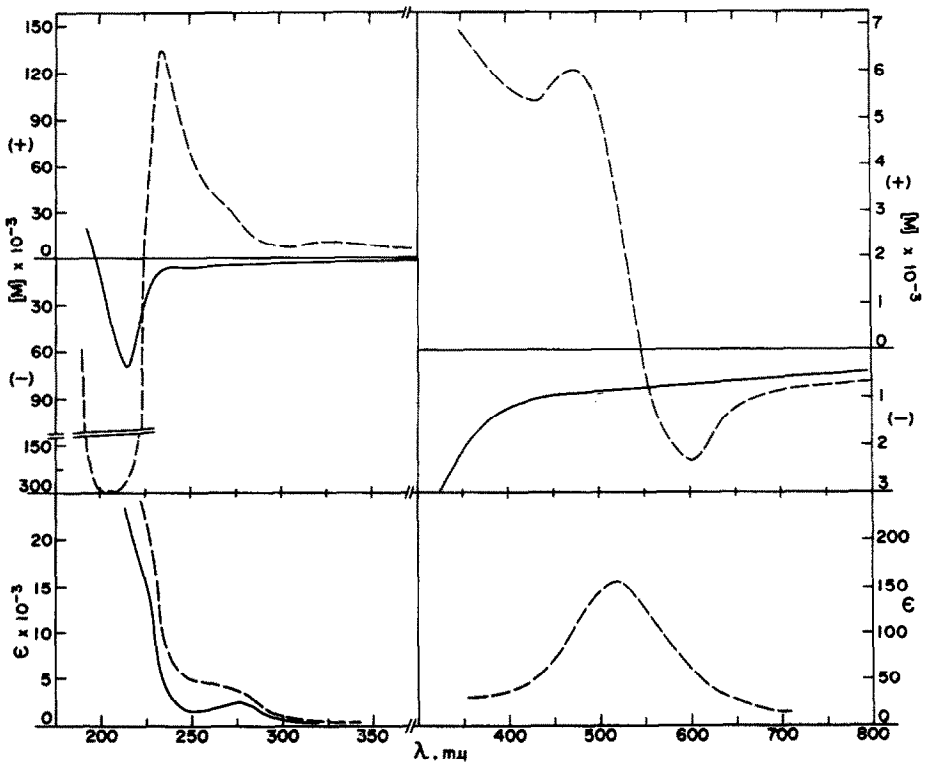



Figure 2. ORD and absorption spectra of lysine vasopressin and the 1:1 Cu(II) complex, pH 7.2, 25°. ——, lysine vasopressin; – – –, [(lysine vasopressin)Cu(II)]. Lysine vasopressin was the generous gift of Dr. Saul Lande and was purified by chromatography on carboxymethyl-cellulose and by gel filtration. The sample gave the expected amino acid analysis.

and transitions of the complex or a conformational preference for the ring could be invoked as possible explanations. A relationship between conformational mobility and the magnitude of Cotton effects associated with the near ultraviolet aromatic transitions (250 - 300 mμ) has been shown for a series of asymmetric 1-substituted indans (Brewster and Buta, 1966). Geometrical relationships between the ring, the coordinated groups, and the metal ion as examined in space-filling models indicate no obvious metal-ring interaction or conformational preference, although non-bonded atomic interactions may be the controlling factor. The X-ray structure of $C_6H_6CuAlCl_4$ shows a $\pi$ bond between Cu(I) and the benzene ring (Turner and Amma, 1966), but there is no evidence for such interaction between

Cu(II) and an aromatic ring in aqueous solution. Enhancement is closely related to the relative positions of the coordinated peptide nitrogen and the ring; it occurs only if the ring is adjacent, i.e., C-terminal. The spectra remain the same whether the ring is C- or N-terminal, indicating the same coordination. While the magnitudes of the Cotton effects for the "random state" of lysine vasopressin are only 1-5% of those expected for an average protein, the Cotton effects of the complex would make significant contributions to the ORD of even a large protein.

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