OPTICAL ROTATORY DISPERSION OF COPPER PROTEINS

Pseudomonas Blue Protein

Shiang-Pin W. Tang and Joseph E. Coleman

Department of Biochemistry, Yale University, New Haven, Conn.

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Anomalous rotatory dispersion associated with the d-d transitions of Cu(II) chelates has been known since 1925 (Lifschitz, 1925; Pfeiffer and Christeleit, 1937). In addition to the visible Cotton effects, recent work has demonstrated the induction of ultraviolet Cotton effects accompanying the formation of Cu(II) peptide and amino acid complexes (Urry and Eyring, 1964; Gurd and Bryce, 1966; Coleman, 1966). Apparently these arise from new optically active transitions of the complex as well as from alterations in optical activity of ligand transitions induced by conformational restrictions placed on the coordinated ligands.

We have recently examined the optical rotatory dispersion (ORD) of several copper proteins to determine the possible contribution of such Cotton effects to the rotation of copper proteins and to examine the role of the copper ion in maintaining structural features of these proteins. The present communication reports the ORD from 190-800 mμ of the small (M.W. = 16,300) blue copper protein which has been isolated from several species of Pseudomonas (Coval et al., 1961; Suzuki and Iwasaki, 1962; Ambler, 1963). The protein apparently functions in the electron transport chain of this organism adjacent to or near cytochrome c (Yamanaka et al., 1963). ESR data indicate most of the copper in the blue form of the protein to be present as Cu(II) (Mason, 1963). This protein proved to be an interesting example because its ORD has three distinct areas of anomalous rotatory dispersion:

multiple Cotton effects in the 500-800 mμ region associated with the copper absorption bands; multiple Cotton effects in the 270-300 mμ region associated with the aromatic side chains of the protein; and several far ultraviolet Cotton effects usually associated with the peptide bond chromophores (Fig. 1).

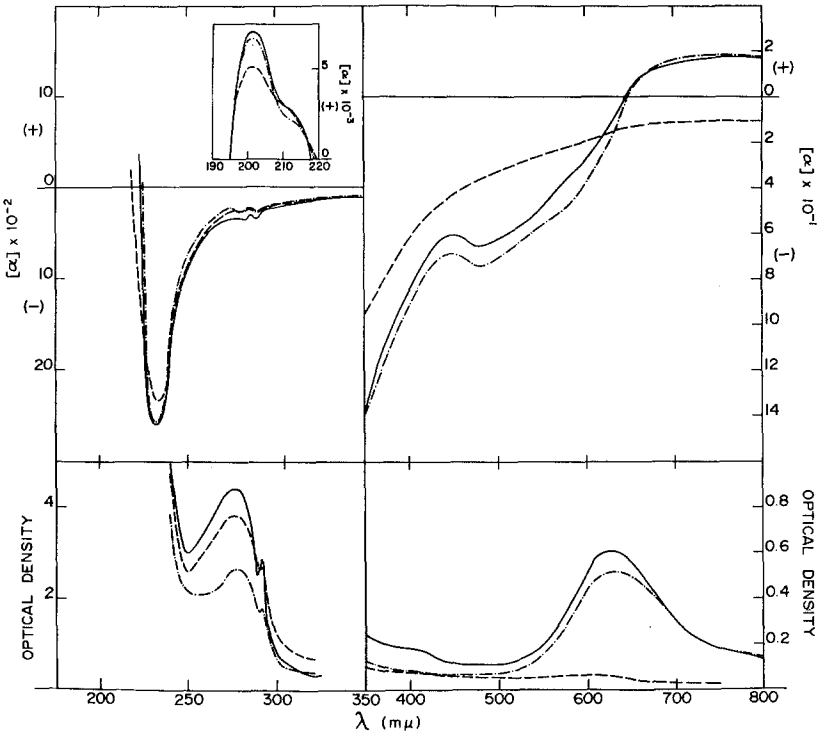

Figure 1. ORD and absorption spectra of native (——), apo- (— — —) and restored (—•—) Pseudomonas blue protein at pH 8.0, 0.025 M Tris, 25°. Apoprotein was prepared by dialysis against 0.5 M KCN at pH 7.0 (Yamanaka et al., 1963). The reconstituted protein was prepared by adding 5-fold molar excess of Cu(II) followed by dialysis of the free copper. Spectra are of the samples used for the ORD. Concentrations of native protein were determined by dry weight and were 6.5 mg/ml for the visible ORD, 1.3 mg/ml for the near ultraviolet ORD, and 0.28 mg/ml for the far ultraviolet ORD. Deviations for a series of curves were as follows: 200 mμ, ± 300°; 230 mμ, ± 150°; 400-700 mμ, ± 2°; 800 mμ, ± 5°. ORD measurements were made with a Cary Model 60 recording spectropolarimeter.

Thus the anomalous rotatory dispersion can be used to monitor changes in these three distinct parts of the protein structure accompanying removal or reduction of the metal ion and acid or alkaline denaturation.

Materials: Pseudomonas blue protein was isolated from P. aeruginosa by a

modification of the procedure of Ambler (1963). Additional samples were the generous gift of Dr. Henry A. Harbury and Dr. Serge N. Vinogradov. Copper analyses (atomic absorption spectroscopy) varied between 0.68 and 0.93 gm at per mole, generally less than one atom per molecule in agreement with previous data (Mason, 1963).

Results and Discussion: The visible ORD of the oxidized native protein is a composite of at least two Cotton effects (Fig. 1). A large positive peak occurs at ca. 700 mμ, crossover at ca. 630 mμ, followed by a trough at 490 mμ and a second peak at 445 mμ. The Cotton effect between 400 and 600 mμ and its pH-dependence have been reported by Maria (1966). There are several Cotton effects in the region 270-300 mμ with peaks at 279 mμ, 284 mμ, and 287 mμ reflecting the asymmetric environment of the aromatic side chains. These are followed by a trough at 233 mμ, $\alpha = -2600^{\circ}$, a crossover at 222 mμ, a peak at 202 mμ, $\alpha = +7000^{\circ}$, and a prominent shoulder from 205-215 mμ. Thus significant α-helical structure appears to be present in the native protein. Removal of the copper ion with KCN abolishes the visible Cotton effects, leaves the aromatic structure relatively unchanged, but causes a decrease in the depth of the 233 mμ trough and the height of the 202 mμ peak (Fig. 1). Hence, there appear to be significant changes in peptide bond conformation accompanying metal ion removal. While there are small differences in the contours of the ultraviolet anomalous dispersion and absorption spectra of the native protein, the apoprotein, and the reconstituted protein (Fig. 1), there do not seem to be major ultraviolet chromophores associated with formation of this Cu(II)-protein complex.

From the difference rotation between the metalloprotein and the apoprotein, the amplitude in molar rotation of the large Cotton effect associated with the copper absorption bands is ca. 1500°. This is comparable to the amplitudes of Cotton effects associated with a number of Cu(II)-peptide complexes. The blue color can be restored to the apoprotein by the readdition of Cu(II) (Yamanaka et al., 1963) (Fig. 1). The apoprotein is unstable and

some is lost by precipitation. Both the visible and ultraviolet ORD of the protein reconstituted from that remaining in solution after centrifugation are almost identical to that shown by the native protein. Thus the conformation surrounding the metal ion must be nearly the same in the native and reconstituted proteins. There is some deviation from the native protein in the structures affecting rotation in the aromatic region.

Although the presence of the visible Cotton effects indicates an asymmetric environment for the Cu(II) ion, they are not diagnostic of any particular configuration. The ORD of model Cu(II) chelates indicates that a complex array of conformational factors may contribute to the optical activity of the transitions centered on the metal ion, perhaps through minor distortions of the coordination geometry (Gurd and Bryce, 1966; Blumberg, 1966; Coleman, 1966). With the proper distortion, even a small deviation from a square planar configuration may give rise to significant optical activity.

The protein structure is stable over a wide pH range (Fig. 2). The trough at 233 mμ, the peak at 202 mμ, and the aromatic Cotton effects show only minor differences between pH 3.5 and 12. On the other hand, there are significant changes in amplitude of the Cotton effects associated with the transitions of the Cu(II) site, apparently reflecting local changes in the protein. Reduction of the metal ion appears to occur at pH 12, since both color and Cotton effects disappear while copper analyses show the protein to contain copper not removable by Chelex resin or dialysis. Below pH 2.0, the protein undergoes a marked conformation change as reflected in the ORD of the protein at pH 1.5 (Fig. 2) determined after 24 hr of equilibration at this pH. Copper begins to dissociate under these conditions. This change is characterized by a loss of the aromatic Cotton effects, a deepening and broadening of the trough near 230 mμ resulting in much more negative rotation in the visible, and a loss of the large peak near 202 mμ. The latter is replaced by a relatively small peak near 195 mμ. The ultraviolet ORD pattern of the acid-denatured protein does not bear a close resemblance to any of

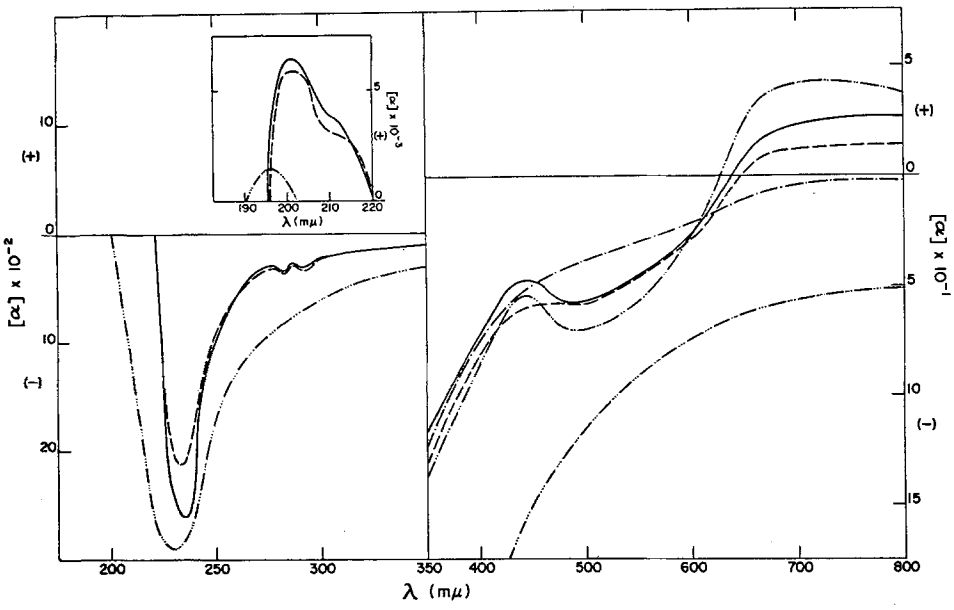

Figure 2. ORD of Pseudomonas blue protein in acid and alkaline solution. (———), pH 6.65; (—•—), pH 12; (—••—), pH 3.45; (—•••—), pH 1.54; (— — —), pH 8.0, readjusted from pH 1.54. Ultraviolet ORD at pH 12.0 and 3.45 is the same as at pH 6.65.

the patterns reported for known polypeptide structures or to the variety of ORD profiles reported for native proteins. This pattern may be the result of several configurations assumed by different parts of the peptide chain. Return of the acid-denatured protein to neutral pH results in slow but relatively complete restoration of the original conformation (Fig. 2). Most of the features of the original ORD profile including the detailed aromatic Cotton effects (Fig. 3) are reestablished over a period of 72 hr at 5°. Hence, the interactions inherent in the polypeptide sequence seem sufficient to reestablish the native conformation from that of the acid-denatured form.

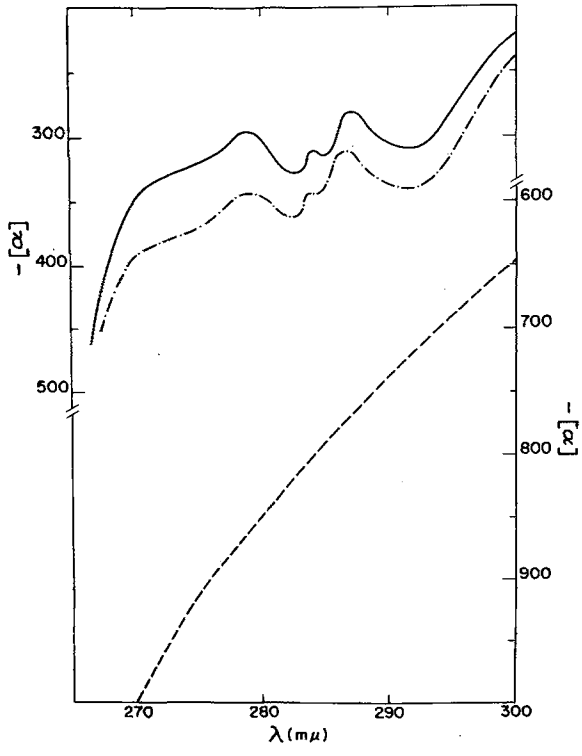

Figure 3. Detail of the aromatic Cotton effects as a function of pH. (———), pH 6.65; (— — —), pH 1.54; (—•—) pH 8.0, readjusted from pH 1.54. Protein concentration was 3.5 mg/ml.

Amino acid analyses show the protein to contain two moles of tyrosine, six moles of phenylalanine, and <0.2 moles of tryptophan per mole (Ambler, 1963). Preliminary analyses of the present preparation support these findings. The apparent absence of tryptophan would indicate that the aromatic Cotton effects in the 270-300 mμ region arise from the asymmetry of the tyrosine residues. The prominent absorption peak at 292 mμ is largely abolished in urea, hence this peak must also reflect the specific environment of the aromatic side chains. The amplitudes of the small aromatic Cotton effects are ca. 8-15,000°, expressed as molar rotation. This is an order of magnitude greater than rotation observed in this region for free tyrosine. Conformational restrictions can, however, increase the rotation of aromatic transitions by at least an order of magnitude through such mechanisms as chelation (Coleman, 1966) or steric limitations on the conformational mobility of the ring (Brewster and Buta, 1966). Such restrictions may well be imposed on the aromatic rings by the three-dimensional structure assumed by the protein at neutral pH. This work was supported by grant AM-09070-03 from the National Institutes of Health, U. S. Public Health Service.

References

Ambler, R. P., Biochem. J. 89, 346, 1963.
Blumberg, W. E., in Peisach, Aisen, and Blumberg, editors, "The Biochemistry of Copper", Academic Press, New York, 1966, p. 49.
Brewster, J. H., and Buta, J. G., J. Am. Chem. Soc. 88, 2233, 1966.
Gurd, F. R. N., and Bryce, G. F., in Peisach, Aisen, and Blumberg, editors, "The Biochemistry of Copper", Academic Press, New York, 1966, p. 115.
Coleman, J. E., Biochem. Biophys. Res. Commun. 24, 208, 1966.
Coval, M. L., Horio, T., and Kamen, M. D., Biochim. Biophys. Acta 51, 246, 1961.
Lifschitz, J., Z. physik. Chem. 114, 491, 1925.
Maria, H. J., Nature 209, 1023, 1966.
Mason, H. S., Biochem. Biophys. Res. Commun. 10, 11, 1963.
Pfeiffer, P., and Christeleit, W., Z. physiol. Chem. 245, 197, 1937.
Suzuki, H., and Iwasaki, H., J. of Biochem. (Japan) 52, 193, 1962.
Urry, D. W., and Eyring, H., J. Am. Chem. Soc. 86, 4574, 1964.
Yamanaka, T., Kijimoto, S., and Okunuki, K., J. of Biochem. (Japan) 53, 256, 1963.