OPTICALLY ACTIVE METALLOPROTEIN CHROMOPHORES I. METALLOTHIONEIN[+]

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Received June 15, 1962

Spectropolarimetric studies have thus far delineated three distinct sources of asymmetry in proteins which contribute to their optical rotatory properties: (1) the constituent amino acids; (2) the backbone rotation or conformation; and (3) chromophores, such as prosthetic groups, bound to proteins asymmetrically (Blout, 1960; Urnes and Doty, 1961; Ulmer et al., 1961). In certain metalloproteins we have recently found that the metal binding site can constitute a fourth source of rotatory power.

Metallothionein, a protein isolated from horse kidney cortex (Margoshes and Vallee, 1957), contains about 6% cadmium, 2% zinc, and more than 9% sulfur in the form of cysteine (Kägi and Vallee, 1961). Both metal atoms bind to the protein at the same site through mercaptide linkages: the ratio of metal/sulfur is 1:3. The cadmium-sulfur bond exhibits an intense absorption maximum near 250 mμ with a molar extinction coefficient of 1.45×10^4 liter $mole^{-1}$ cm^{-1}. The absorption is abolished when cadmium is removed as by dialysis against EDTA at pH 4.5 (Kägi and Vallee, 1961).

This cadmium-mercaptide chromophore is optically active. Its visualization is greatly facilitated by lack of the usual protein absorption near

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[+] This work was supported by a grant from the National Science Foundation and by the Howard Hughes Medical Institute.

[*] Research Career Development Awardee of the National Institutes of Health, Department of Health, Education, and Welfare.

280 mμ, because of the virtual absence of aromatic amino acids. Optical rotation was measured as described previously (Ulmer et al., 1961). In the region of the chromophore, the rotatory dispersion of metallothionein is anomalous presumably due to the ascending limb of a positive Cotton effect (Fig. 1). The high extinction coefficient of the chromophore precludes accurate measurements below 270 mμ. The rotatory dispersion of the protein is plain at wavelengths longer than 300 mμ. In contrast, the rotatory dispersion of thionein, the metal-free protein, is plain to wavelengths as short as 244 mμ. Readdition of cadmium to thionein reconstitutes cadmium-thionein (Kägi and Vallee, 1961) and restores the anomalous dispersion originally present. The rotatory power of the zinc chromophore, at 215 mμ, has not been accessible to examination with our present instrumentation as yet.

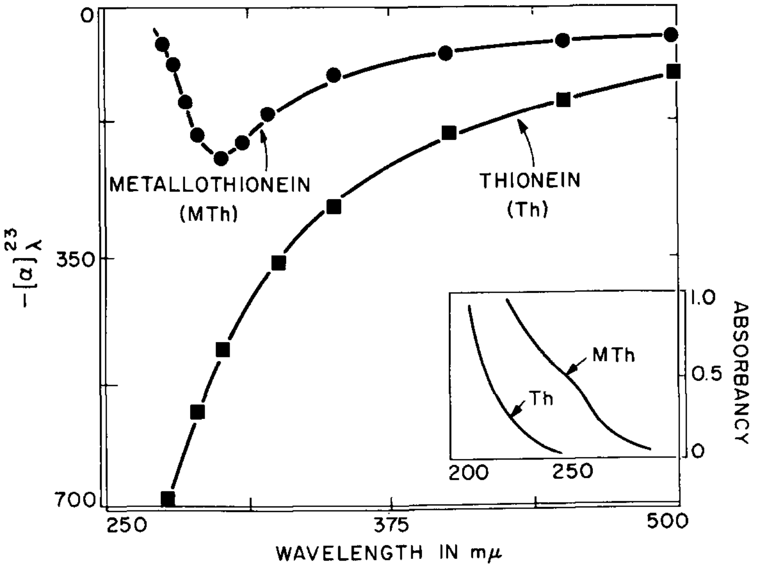

Figure 1. Optical rotatory dispersion of metallothionein and thionein. Specific rotation at 23°, $-[\alpha]^{23}_{\lambda}$, and absorbancy are plotted as a function of wavelength. The cadmium-mercaptide chromophore at 250 mμ is optically active and generates a positive Cotton effect (ascending limb shown). Removal of cadmium abolishes the 250 mμ absorption and results in the plain negative rotatory dispersion of thionein. MTh, metallothionein, the cadmium-containing protein. Th, thionein, the metal-free protein. Conditions: The proteins were dissolved in metal free distilled water, pH 5.5, and placed into polarimeter cells of 1 cm. and 5 cm. path lengths. Optical rotatory dispersion was determined in the Rudolph Spectropolarimeter (200S-80Q) employing a symmetrical angle setting of 5° and a monochromator slit width of ≤ 0.15 mm. Variation in protein concentration from 0.5 to 5 mg. per ml. failed to alter the characteristic anomalous dispersion of metallothionein eliminating the possibility of a spurious Cotton effect (Urnes and Doty, 1961).

The Cotton effect of metallothionein is detectable only by measuring rotation below 300 mμ. However, the rotatory power of the optically active cadmium-mercaptide absorption band renders the specific rotation more positive at longer wavelengths. This is reflected at wavelengths as far removed from the chromophore as the sodium D line where measurements reveal a shift from -59 to -29° upon addition of cadmium to thionein. In the absence of recognition of a rotational contribution of the metal chromophore, the change in specific rotation consequent to cadmium removal or restoration might be attributed, erroneously, to an alteration in protein conformation. This is analogous to previous observations in other systems (Ulmer et al., 1961).

The optically active stereoisomers of many metal coordination complexes have been resolved (Woldbye, 1959; Kling and Woldbye, 1961), and their pronounced Cotton effects have been characterized. Certain metalloproteins, in analogy with such simple metal-ligand systems, similarly exhibit optical activity due to conformational orientation of their multidentate metal-ligand sites. Indeed, a unique orientation of these groups is implied for specificity of metal-protein binding; presumably, the asymmetry and optical activity of the metal chromophore reflects this high degree of chemical organization of the protein binding site and its singular reactivity toward particular metal ions. In the present instance the large number of cysteine residues in the primary structure of thionein condition protein conformation to allow the constitution of metallothionein; three -SH groups must be arranged sterically such that the coordination of one metal atom can take place (Kägi and Vallee, 1961).

It appears likely that a critical steric organization about the metal atom will be found frequently in proteins which exhibit a marked specificity in metal binding. In this regard, we have recently identified optically active metal-ligand absorption bands in proteins involving iron (transferrin and conalbumin) and copper (ceruloplasmin) (Ulmer et al., 1962; Ulmer and Vallee, 1962). Cotton effects in such proteins are altered upon complexation of the metal with chelating agents. As with metallothionein, the Cotton effects are abolished by removal of the metal from the protein.

The rotatory dispersion curves of isomers of many simple metal coordination complexes are now being recognized (Woldbye, 1959; Kling and Woldbye, 1961). It seems probable that the comparison of their optical rotatory properties to those of metalloproteins will provide a novel and highly informative approach to the elucidation of the chemical details of the metal binding sites of proteins.

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