OPTICALLY ACTIVE METALLOPROTEIN CHROMOPHORES II. TRANSFERRIN AND CONALBUMIN *

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In a previous communication (Ulmer et al, 1962a) we reported the identification of an optically active cadmium-mercaptide chromophore in metallothionein, a protein isolated from horse kidney cortex. This observation suggested that the metal-binding site of metalloproteins may constitute a source of rotatory power in addition to that of protein conformation, the constituent amino acids, and -- when present -- bound prosthetic groups. In this regard, we have now found that the visible absorption bands of the iron complexes of transferrin and conalbumin are optically active. It has been suggested that the properties of the metal-binding sites of these two proteins are remarkably similar (Warner and Weber, 1953; Inman, 1956). Notably, the Cotton effects which appear upon formation of their iron complexes are virtually identical.

Experimental

Conalbumin was a 4 x crystallized, salt-free preparation (Sigma Chemical Co.). Human serum transferrin[1] was rendered metal-free by dialysis at pH 5.5 against

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α, α' -bipyridyl (G. F. Smith Chemical Co.) or EDTA after reduction with sodium hydrosulfite. The proteins were dissolved in either 0.1 M Tris (hydroxymethyl) aminomethane or 0.02 M Veronal buffer, pH 8.5, in the presence of 2.5×10^{-4} M bicarbonate, and iron was added from a freshly prepared solution of ferrous ammonium sulfate. Spectrophotometric and spectropolarimetric measurements were performed after the samples were stored overnight at 4°. Rotatory Dispersion Titration was carried out as described previously (Li et al, 1962).

Results

Metal-free conalbumin exhibits a plain negative rotatory dispersion between 300 and 675 mμ with a specific rotation, $[\alpha]_D^{10}$ of -30°. In the presence of iron, the dispersion becomes anomalous due to the appearance of a negative Cotton effect (Fig. 1). The magnitude of the Cotton effect is a function of the amount of iron bound to the protein: maximally, 2 moles of iron bind to each mole of conalbumin by Rotatory Dispersion Titration (Fig. 1), in accord with previous spectrophotometric titrations (Warner and Weber 1953). At saturation, the Cotton effect has a trough at 505 mμ, a peak at 420 mμ, a breadth of 85 mμ, and an amplitude of 16°. The inflection point, λ_o, near 470 mμ, corresponds to the absorption maximum of the iron-conalbumin complex.

The Cotton effect appearing upon addition of iron to transferrin is nearly identical to that observed for conalbumin (Fig. 2), and the Rotatory Dispersion Titration of transferrin with iron similarly reveals the binding of a maximum of 2 moles of metal per mole of protein. At saturation the amplitude of the Cotton effect of iron-transferrin is 13°, slightly less than that of conalbumin. The specific rotation, $[\alpha]_D^{10}$, of metal-free transferrin is -45°, and its rotatory dispersion, like that of metal-free conalbumin, is plain.

Both conalbumin and transferrin form colored complexes with metals other than iron but which are thought to interact at the same protein binding site (Warner and

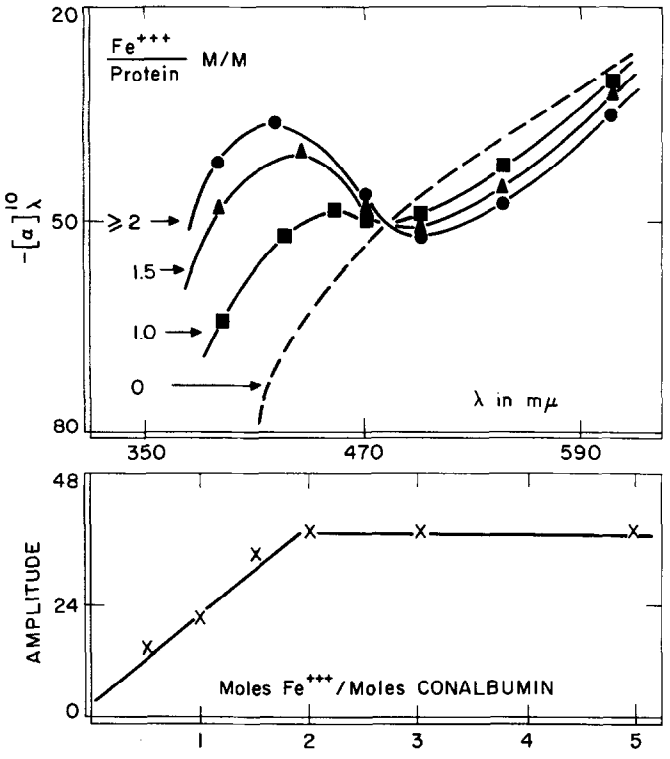

Figure 1. Rotatory Dispersion Titration of Conalbumin with Fe^{+++}. In the upper portion of the figure, specific rotation at 10°, $-[\alpha]^{10}_{\lambda}$, is plotted against wavelength. In the presence of Fe^{+++}, the rotatory dispersion of conalbumin becomes anomalous due to a negative Cotton effect centered about the absorption maximum of the metal-protein complex at 470 mμ. At a fixed conalbumin concentration of 6.6×10^{-5} M, the magnitude of the Cotton effect increases with increasing Fe^{+++} concentration to become maximal at 2 moles of iron per mole of conalbumin. In the lower portion of the figure, the data are plotted according to the method of molar proportions (Li et al, 1962). In this instance, the amplitude was calculated from the difference rotation between the metal-free and the metal-containing protein at the peaks and the troughs of the Cotton effects. At saturation, 2 moles of Fe^{+++} bind to each mole of protein. Conditions: 5 mg per ml conalbumin in 0.1 M Tris, pH 8.5. Rotatory Dispersion Titration determined in the Rudolph Spectropolarimeter (200 S-80Q) employing a symmetrical angle setting of 5°, monochromator slit width of ≤ 0.15 mm, and an A-H 6 mercury lamp (G. E.) as a light source. The characteristics of the anomalous dispersion remained constant at variable protein concentrations, eliminating the possibility of a spurious Cotton effect (Urnes and Doty, 1961).

Weber, 1953; Inman, 1956). We have examined the optical rotatory dispersion of the Mn^{+++} and Cu^{++} complexes of these proteins. The absorption bands at 429 mμ of the manganic complexes of conalbumin and transferrin are optically active and generate a Cotton effect which, however, differs both in sign and shape from that

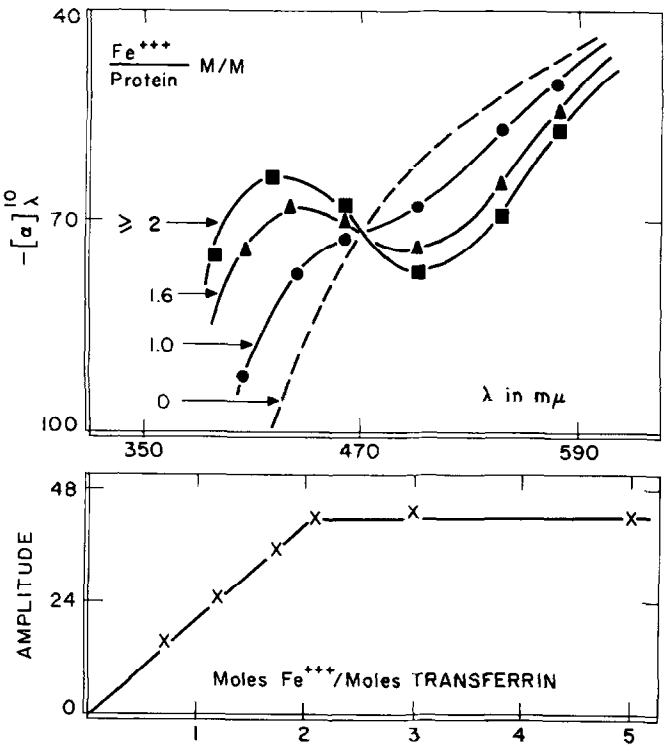

Figure 2. Rotatory Dispersion Titration of Transferrin with Fe^{+++}. In the upper portion of the figure, specific rotation at 10 , $-[\alpha]^{10}_{\lambda}$, is plotted against wavelength. In the presence of Fe^{+++}, the rotatory dispersion of transferrin becomes anomalous due to a negative Cotton effect centered about the absorption maximum of the metal-protein complex at 470 mμ. As with conalbumin (Fig. 1), the magnitude of the Cotton effect is a function of the amount of Fe^{+++} bound to transferrin and becomes maximal at 2 moles of Fe^{+++} per mole of protein (lower portion of figure). Conditions: identical to those for conalbumin (Fig. 1).

of the iron complexes. Significantly, the absorption bands, near 440 mμ, of the cupric complexes, although of intensity comparable to those of Fe^{+++} and Mn^{+++}, are not optically active and the binding of Cu^{++} does not alter the rotatory dispersion of either protein.

Discussion

It has been shown previously that the asymmetric interaction of certain organic chromophoric molecules with proteins may induce anomalous optical rotatory dispersion (Ulmer et al, 1961). Upon binding to the protein, the absorption bands of the chromophore become optically active, generating a characteristic Cotton effect.

Considerations pertinent to the interpretation of these rotational changes in relation to those resulting from alterations in protein conformation have been discussed (Ulmer and Vallee, 1961; Ulmer et al, 1961). The present data indicate that the interaction of metal atoms with specific sites of proteins may, in a similar manner, induce anomalous rotatory dispersion.

Earlier observations suggested that metal atoms may constitute asymmetric loci on the surface of metalloenzymes and, thereby, serve as centers of orientation for the binding of chromophoric coenzymes, substrates, or inhibitors (Ulmer and Vallee, 1961; Ulmer et al, 1961). Recently, we have studied the protein thionein, which exhibits plain negative rotatory dispersion but which develops a Cotton effect upon binding cadmium to form metallothionein (Ulmer et al, 1962a). In this instance, anomalous dispersion appears to be attributable to the highly specific cadmium-mercaptide bond. The physical-chemical characteristics of this system, including its unique spectral properties attributable directly to the cadmium-SH bond, have strongly suggested that the optical asymmetry which generates the Cotton effect is controlled by the limited configuration of the metal-protein ligand binding site. This observation would indicate that the formation of optically active complexes might bear importantly on the three-dimensional configuration of the metal-binding sites of proteins as expressed by the chemical specificity of metal-protein interactions.

The present studies of conalbumin and transferrin constitute further support for this hypothesis. The Cotton effects of the iron complexes of these proteins are nearly identical although the metal-free proteins exhibit dissimilar optical rotatory properties: transferrin displays significantly greater levorotation than does conalbumin (Fig. 1 and 2). This is in keeping with the postulate that rotatory power, manifested as a Cotton effect, arises from discrete asymmetric loci on proteins, the metal-binding site in this instance. Strong similarities in metal-binding properties by the two proteins

have been noted and it has been suggested that phenolic hydroxyl groups constitute the metal-ligand sites (Warner and Weber, 1953; Inman, 1956).

The specificity of the Cotton effect is indicated by the variations in optical rotatory power which result from the substitution of other metals for iron. Fe^{+++} Mn^{+++}, and Cu^{++} are all thought to bind to the same site of the protein and all three of these ions induce strong absorption bands. The extinction coefficients, k, of a 1 per cent solution of these complexes vary from 0.4 to 1.2 cm^{-1}. While manganese induces a distinctive Cotton effect at the maximum of absorption of its complexes with both proteins, copper does not alter the rotatory dispersion of either. Thus, copper fails to enter into the configuration at the protein binding site which could render its absorption band optically active. This distinction between these complexes of Fe^{+++} and Mn^{+++} on the one hand, and those of Cu^{++} on the other, may be related to the coordinating properties of these metal ions. Fe^{+++} and Mn^{+++} presumably form octahedral complexes with the protein and thus might function both as chromophores and as centers of asymmetry. Since Cu^{++}, although chromophoric, prefers a square planar configuration this characteristic might account for its failure to establish a center of asymmetry.

The apparent specificity of these Cotton effects supports the suggestion that spectropolarimetric studies will provide useful means to investigate the chemical details of the interaction of metals with proteins. In this regard, we have now observed highly distinctive Cotton effects, associated with the metal-binding sites of hemerythrin, photosynthetic pyridine nucleotide reductase, and hemocyanin, and with metal-prosthetic groups of many proteins, e.g., heme (Ulmer et al, 1962b). Comparison of the rotatory dispersion curves of these and other metalloproteins under study may be expected to yield new information concerning both the nature of protein ligands to which the metal binds as well as the coordination state of the metal in such systems.

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