OPTICAL ROTATORY DISPERSION OF EGG PROTEINS. II. ENVIRONMENT SENSITIVE SIDE CHAIN CHROMOPHORES IN CONALBUMIN[1]

William Gaffield, Lidia Vitello and Yoshio Tomimatsu

Western Regional Research Laboratory,[2] Albany, California 94710

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Recent optical rotatory dispersion (ORD) and circular dichroism (CD) measurements of proteins from 250-300 mμ have shown the presence of conformation dependent Cotton effects, presumably due to side-chain chromophores (Myers and Edsall, 1965; Beychok, 1965; Glazer and Simmons, 1965, 1966). We report preliminary results concerning the sensitivity to the environment of Cotton effects due to side-chain chromophores of hens egg-white conalbumin. Our results indicate that these Cotton effects are related to the degree of exposure to solvent of these chromophores and that these anomalies may help elucidate the nature of conalbumin's metal binding sites.

Conalbumin, an analog of serum transferrin and lactotransferrin, is known to specifically bind two atoms of certain metals (Fraenkel-Conrat and Feeney, 1950; Warner and Weber, 1953) and to undergo a reversible unfolding at pH's below 4 (Wishnia et al., 1961; Glazer and McKenzie, 1963). The iron complex of conalbumin is more stable to denaturation (Azari and Feeney, 1958, 1961; Glazer and McKenzie, 1963) than native conalbumin.

Earlier it was noted (Tomimatsu and Gaffield, 1965) that Schecter-Blout plots for conalbumin, as well as other egg proteins, deviated from linearity

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[1] For previous paper: Tomimatsu, Y. and Gaffield, W., Biopolymers, 3, 509 (1965).

[2] Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

at wavelengths below 300 mμ. Careful examination of the 250-300 mμ region of native conalbumin's ORD curve revealed reproducible anomalies (Fig.1) at 295, 289 and 285 mμ and smaller perturbations between 260-285 mμ. Moffitt plots (Moffitt and Yang, 1956) consistently deviated (Fig.2) from a straight line below 300 mμ. These plots have been used previously (Kronman et al., 1965; Timasheff et al., 1966) to amplify small rotatory perturbations. On the other hand, the ORD curve (Fig.1) became featureless and the Moffitt plot (Fig.2) linear when conalbumin was examined in 2-chloroethanol.

Examination of conalbumin's ORD in mixed chloroethanol-water revealed no difference in rotatory parameters between zero and 10% chloroethanol solutions (Table I). In 20% chloroethanol the rotatory perturbations at 285-295 mμ were absent but the anomalies at 260-285 mμ were still weakly evident in the Moffitt

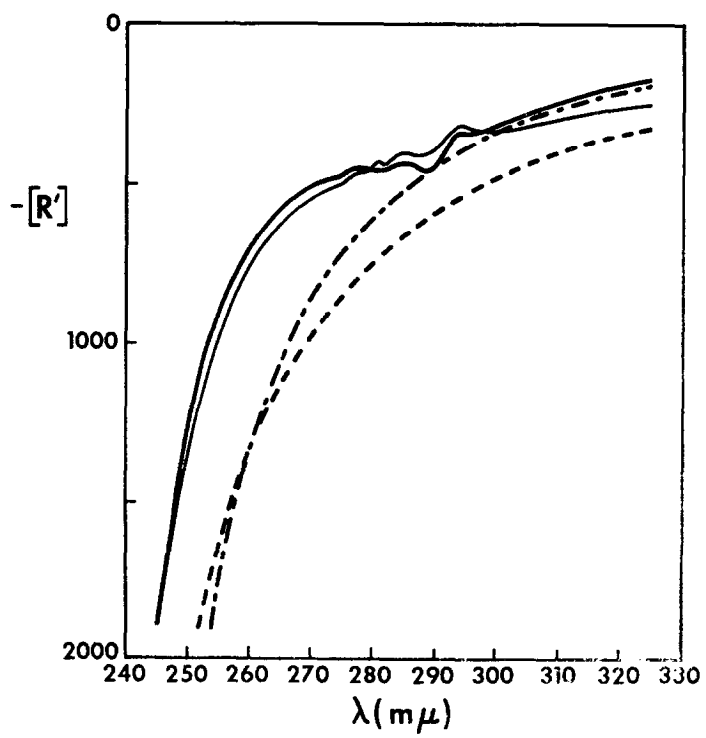

Fig. 1. ORD of conalbumin and iron conalbumin in the 250-325 mμ region: (——) conalbumin (.05 M NaCl); (▬) iron conalbumin (.05 M borate); (- —) conalbumin (100% chloroethanol); (— —) conalbumin (.05 M SDS).

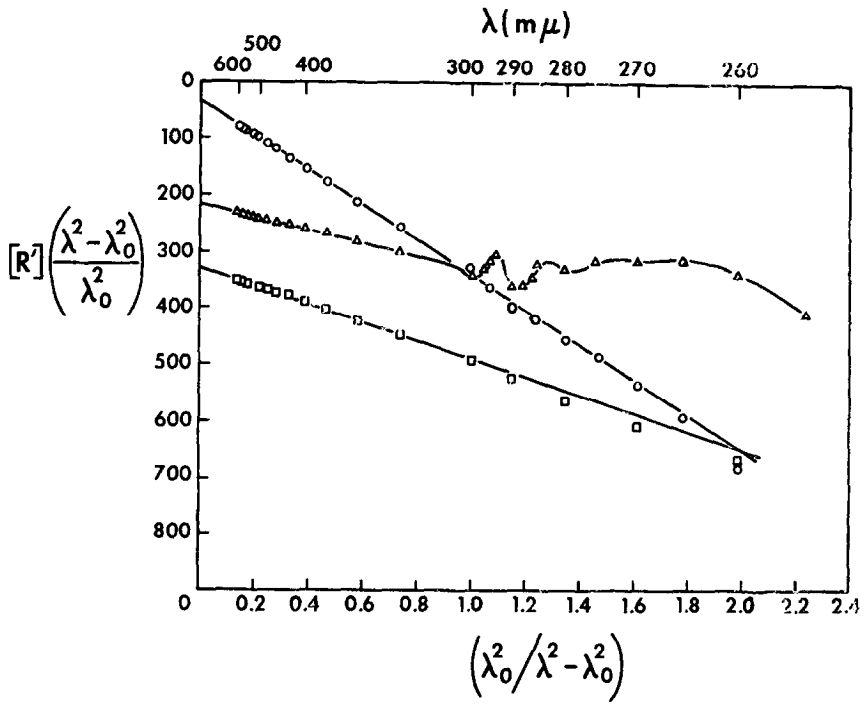

Fig. 2. Moffitt plots of conalbumin: (△) conalbumin (.05 M NaCl); (○) conalbumin (100% chloroethanol); (□) conalbumin (.05 M SDS).

plot. Increased chloroethanol concentration resulted in further diminution of the small side-chain Cotton effects and an increase in the amount of apparent α-helical character. Solutions of higher chloroethanol concentration are more acidic. Since conalbumin is known to undergo a transition in acidic environment (Wishnia et al., 1961; Glazer and McKenzie, 1963), the disappearance of anomalies with increasing amounts of chloroethanol may be related to this transition. Low concentrations of chloroethanol have been shown to disrupt the native state of some proteins with subsequent refolding at higher chloroethanol concentration (Martin and Bhatnagar, 1965; Hamaguchi and Kurono, 1963). A similar mechanism might occur in conalbumin. Two independent observations confirm an exposure of chromophoric groups with increasing chloroethanol concentration. The absorption maximum of conalbumin is shifted from 280 to 278 mμ and an increased number of tryptophyls is reactive towards

Table I. Optical rotatory parameters[a] of conalbumin

Sample	Solvent	$-b_o$	$-a_o$	$-[R']_{233}$	250-300 mμ perturbations[b]
A. Chloroethanol-water mixtures.					
Conalbumin	0%	120	220	2700	present
Conalbumin	10%	135	250	3300	present
Conalbumin	20%	95	350	4000	weak
Conalbumin	100%	300	40	6800	absent
B. Aqueous solutions (.05M NaCl) with variable pH.					
Conalbumin	pH 8.7	130	200	2900	present
Conalbumin	pH 4.1	110	280	3000	present
Conalbumin	pH 3.7	80	380	3300	weak
Conalbumin	pH 2.8	80	440	2700	absent
C. Metal chelates of conalbumin					
Fe conalbumin[c]	pH 8.5	--	--	2800	present
Cu conalbumin[c]	pH 8.5	125	320	2700	present
Mn conalbumin[c]	pH 8.5	--	--	2800	present
D. Other solvents					
Conalbumin	8M urea	10	530	2000	absent
Conalbumin	.05M SDS pH 6.2	170	320	4500	absent
Acetylated conalbumin[d]	H_2O	--	--	3200	weak
Acetylated iron conalbumin[d]	pH 6.4	--	--	2900	present
Iron conalbumin[e]	.05M SDS pH 7.3	115	360	3800	weak

[a]ORD was measured on a Cary Model 60 spectropolarimeter (Tomimatsu and Gaffield, 1965). [b]Criteria for perturbations in the 250-300 mμ region were Moffitt plots (see Fig. 2). [c]Prepared by the procedure of Ulmer and Vallee, 1963. [d]Acetylated by the procedure of Riordan et al., 1965. Difference spectra showed 18 tyrosyls acetylated in conalbumin and 12 tyrosyls acetylated in iron conalbumin. [e]See text.

a tryptophan specific reagent (Koshland et al., 1964). The Koshland reagent modified 1.8 of conalbumin's 13 tryptophyls in aqueous solution, 5.3 tryptophyls in 20% chloroethanol and 7.4 tryptophyls in 100% chloroethanol. ORD studies of conalbumin in aqueous solution as a function of pH (Table I) confirmed the sensitivity of the side-chain Cotton effects to acidic environment. The rotatory parameters were invariant from pH 9 to pH 4 and the 250-300 mμ perturbations

were undisturbed. Below pH 4 unfolding of the protein commenced and the side-chain region of the ORD became devoid of anomalous behavior. Therefore, the loss of rotatory perturbations at 250-300 mμ appears to be independent of helical content but closely related to the degree of exposure of chromophoric side-chains. The transfer of these groups from an interior region to the surface of the protein could result in lower amplitude Cotton effects due to greater conformational freedom (Kronman et al., 1966).

Iron conalbumin and manganese conalbumin exhibit Cotton effects in the Soret region while copper conalbumin does not (Ulmer and Vallee, 1963). All three metal chelates gave similar ORD curves below 300 mμ (Table I) and in this region were almost identical (Fig. 1) with native conalbumin. The b_o value for copper conalbumin was similar to that observed for the native protein although the a_o of the copper chelate was more negative. These results indicate that a gross conformational change does not occur upon binding of metal ions by conalbumin although a smaller conformational alteration cannot be ruled out.

A loss of helical structure and disappearance of side-chain optical activity occurs when conalbumin was dissolved in 8 M urea (Table I). The ORD curve (Fig. 1) of conalbumin in .05 M sodium dodecyl sulfate (SDS) showed loss of rotatory perturbations at 250-300 mμ as evidenced by a Moffitt plot (Fig. 2) in addition to a slight increase (Table I) in helical content. When iron conalbumin (.05 M borate, pH 7.5) was treated so that the final solution was .05 M in SDS (pH 7.3), the chelate dissociated as shown by disappearance of the absorption band at 470 mμ. The resultant ORD curve was devoid of anomalies from 600-300 mμ and the rotatory parameters (Table I) were roughly similar to iron-free conalbumin. Iron conalbumin is stable in aqueous media down to pH 5 (Warner and Weber, 1951). Apparently the detergent either displaces the metal atoms from their binding sites or effects a conformational change in the protein secondary structure at these specific sites so that metal binding is impossible. Further experiments

are necessary to decide between these alternatives.

Conalbumin, in which all 18 tyrosyls had been acetylated by N-acetylimidazole (Table I), gave an ORD curve which showed much less optical anomaly in the side-chain region than unmodified protein. Initial turbidity upon addition of the acetylating reagent followed by eventual clarification of the reaction mixture suggested conformational change in native conalbumin during acetylation. Acetylation of the 12 available tyrosyls (Table I) in iron conalbumin gave no turbidity upon reaction and the ORD curve was nearly identical to that of unmodified iron conalbumin.

In Fig. 3 are shown the circular dichroism spectra of conalbumin and iron conalbumin. The native protein (.05 M borate) gave a negative dichroism band at 296 mμ, a broad negative band centered at 274 mμ and a positive band at 252 mμ. Iron conalbumin (.05 M borate) showed bands relatively similar to the

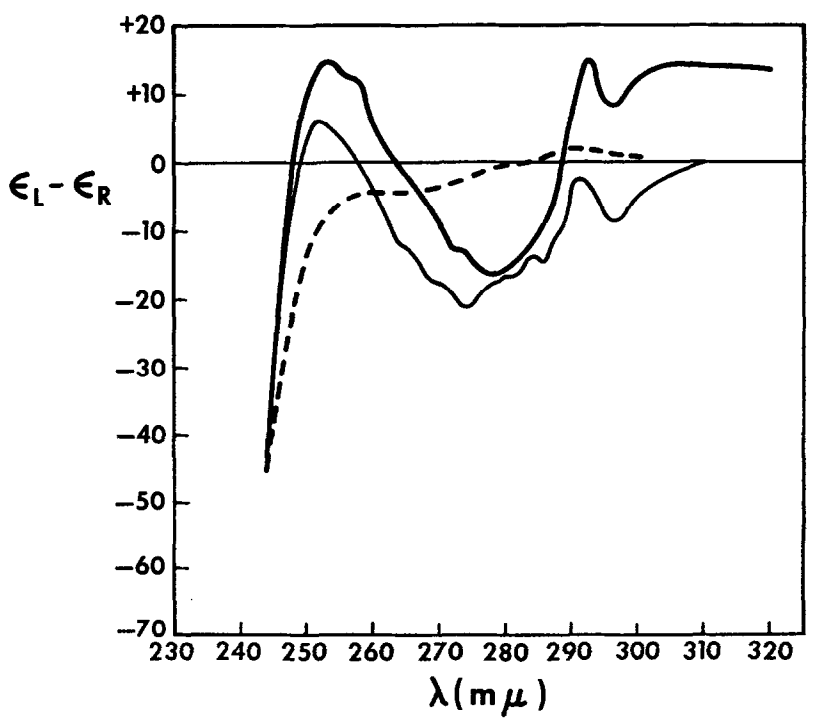

Fig. 3. CD of conalbumin and iron conalbumin: (——) conalbumin (.05 M borate); (▬) iron conalbumin (.05 M borate); (— —) conalbumin (.05 M SDS). CD spectra were measured on a Durrum-Jasco Recording Spectropolarimeter.

latter two but the long wavelength dichroism band (293 mμ) was positive.[3] The dichroism bands at 293-296 mμ may be associated with tryptophyls and the different signs exhibited by the two systems could be indicative of slight conformational alterations between certain residues of native conalbumin and the iron complex. The CD of conalbumin in .05 M SDS (Fig. 3) showed much weaker dichroism than native protein in accordance with ORD results (Fig. 1) and Moffitt plots (Fig. 2). The CD results emphasize the caution necessary in making definite assignments to individual Cotton effects from ORD data. The strong background contribution of the peptide chromophore in the 250-300 mμ region of the ORD curve renders the resolution of small Cotton effects in this region extremely difficult.

Titration studies on conalbumin (Wishnia et al., 1961) revealed that 13 of the 18 tyrosyls are buried in the molecule and that 6 of these 13, possibly those involved in metal binding (Windle, et al., 1963), are hydrogen bonded. Our results suggest that several side-chain chromophores (tyrosyls, tryptophyls and possibly cystines and phenylalanines), which exist in an interior portion of conalbumin, are spatially oriented in such a manner so as to enhance the optical activity of their long wavelength absorption bands. This ordered structure of conalbumin is highly susceptible to acidic environment and is also sensitive to detergent at neutral pH. However, any definite conclusion regarding contributions of specific side-chain chromophores to the ORD of conalbumin and its chelates is premature at this time. The CD spectra appear to offer great promise insofar as separation of various Cotton effects is concerned and in conjunction with other spectroscopic and chemical methods may yield valuable information concerning the specific binding sites of conalbumin.

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[3] As seen in Fig. 3, iron conalbumin showed positive CD above 300 mμ. This chelate gave a broad negative dichroism at 380-550 mμ and a broad positive signal at 300-375 mμ apparently due to the optically active Soret transitions. The CD spectra of conalbumin chelates are being studied further.

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