OPTICAL ROTATORY DISPERSION STUDIES OF THE HEAT DENATURATION OF AVIDIN AND THE AVIDIN-BIOTIN COMPLEX*

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Avidin, a protein isolated from egg white, combines firmly with biotin to yield a complex with a dissociation constant reported as 10^{-15} M (Green, 1963a). The avidin-biotin complex has been shown to be relatively stable toward proteolytic enzymes (György and Rose, 1943) and over a wide pH range (Eakin, Snell, and Williams, 1940; György, Rose, and Tomarelli, 1942). Steam sterilization was shown to release biotin from its complex with avidin (Eakin, Snell, and Williams, 1940), but the increased affinity of biotin for avidin in buffer solutions of high ionic strength affords considerable protection toward disrupting the complex with heat (Wei and Wright, 1964; Pai and Lichstein, 1964).

In agreement with a recent report (Green and Melamed, 1966), the present study shows that the optical rotatory dispersion (ORD) spectrum of avidin exhibits prominent Cotton effects which are negative at 245 mμ and positive at 285 mμ. The curve approximates zero rotation into the visible region. The ORD spectrum of avidin is not markedly changed upon complexing with biotin, and only moderate changes occur in 8 M urea.

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However, we have now found that heating of avidin solutions above 80° causes a precipitous increase in levorotation below 330 mμ reflecting irreversible denaturation which can be prevented by complexing with biotin.

Experimental

Crystalline D-biotin and crude avidin were purchased from Nutritional Biochemicals Corp. The avidin was purified by chromatography on carboxymethyl-cellulose (Melamed and Green, 1963) and biotin-cellulose (McCormick, 1965). The ORD measurements were obtained using a Cary 60 Spectropolarimeter with a thermostated cell compartment and 2.5 cm cells. Spectra were determined at room temperature or after 15 minutes at such temperatures as specified. Solutions contained a constant concentration of avidin (0.5 mg/ml) in NH_4OH-NH_4Cl buffer (pH 9, μ = 1) with and without urea or biotin as specified.

Results and Discussion

The ORD spectra of native avidin, avidin-biotin complex, avidin with 8 M urea, and heat-denatured avidin are shown in Fig. 1.

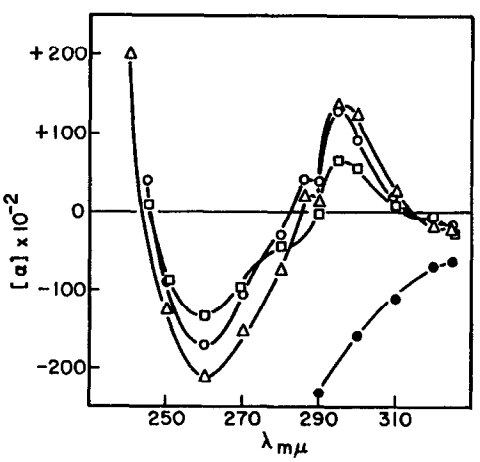

Fig. 1. ORD spectra of avidin in buffer (o), with 2.9 x 10^{-5} M biotin (Δ), in 8 M urea (□), and when heated at 85° (●).

Avidin in distilled water or buffer favorable for binding of biotin exhibits a maximal positive rotation at 295 mμ with a shoulder at 287 mμ and a maximal negative rotation at 260 mμ. The overall spectrum is somewhat different from those of many globular proteins, but the positions of Cotton effects in the ultraviolet expectedly reflect contributions from the aromatic amino acid composition, reportedly high in L-tryptophan (Melamed and Green, 1963). Inclusion of biotin in the solution elicits a slight intensification of specific rotations at both 295 and 260 mμ. It has been shown that complexing of biotin by avidin impedes oxidation of tryptophan residues by N-bromosuccinimide (Green, 1963b). Addition of 8 M urea to avidin somewhat diminishes the dispersion at both 295 and 260 mμ, but concentrations of this solute as high as 9 M have been reported to cause little denaturation of avidin (Green, 1963c). As with prolonged treatment with 6 M guanidine hydrochloride (Green and Melamed, 1966), heat denaturation causes complete loss of the positive rotation at 295 mμ and a large increase in levorotation at wavelengths below 330 mμ. These changes are irreversible, as the spectrum of native avidin does not reappear upon keeping a previously heated solution at room temperature for many hours. Also earlier work has shown that such heat-denatured avidin will not complex with biotin (Wei and Wright, 1964). The conditions used (15 minutes at 85°) may disrupt secondary bonding forces, probably including those involved in the sub-unit structure reported for avidin (Green, 1963c).

The heat denaturation of avidin can be followed readily by the changes in specific rotation at either 295 or 260 mμ as shown in Fig. 2. The structure of avidin remains stable at temperatures below 70° as evidenced by lack of significant change in the ORD spectrum. Above this temperature, rapid disruption of structure occurs with an apparent Tm of 80°. By 85°, extensive loss of the original structure and ability to bind biotin are found.

The effect of biotin concentration on preventing heat denaturation of avidin is shown in Fig. 3. All concentrations of biotin effect some protection of avidin when heated at a temperature (85°) sufficient to irreversibly denature this protein alone. Increasing the level to that required for a 1:1 molecular complex affords maximum protection, and higher concentrations of biotin do not alter this situation. The avidin-biotin complex remains stable until temperatures near that of boiling water are reached.

Fig. 2

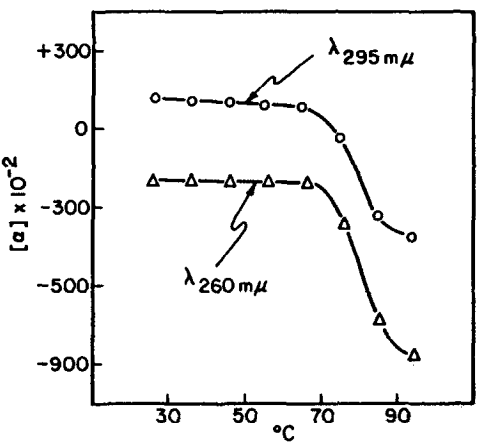

Fig. 3

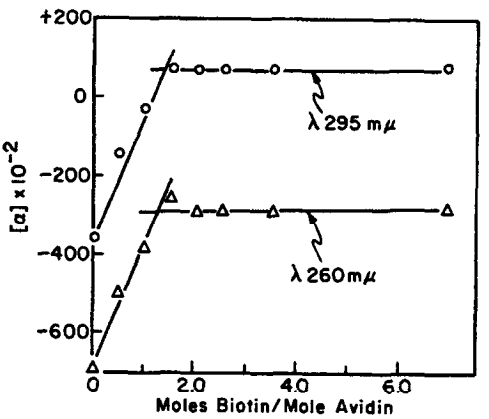

Fig. 2. Heat denaturation of avidin as measured by ORD changes at two wavelengths.

Fig. 3. Effect of biotin concentration on preventing the heat denaturation of avidin at 85° as measured by ORD changes at two wavelengths.

Acknowledgments

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