# Circular Dichroism Studies on the Binding of Ligands to Tryptophan Synthase

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Tryptophan Synthase (E.C. 4.2.1.20) from Escherichia coli which catalyzes the conversion of L-serine + indoleglycerol phosphate to L-tryptophan + D-glyceraldehyde 3-phosphate + $H_2O$ is a tetrameric bienzyme complex with $\alpha\beta\beta\alpha$ structure. The isolated $\beta_2$ subunit cooperatively binds two molecules of the coenzyme pyridoxal 5'-phosphate (PLP) yielding internal aldimines with microscopic dissociation constants $K_{d1,2}$ of $8.7 \times 10^{-6}$ M and $2.3 \times 10^{-7}$ M respectively. Cofactor binding to the native $\alpha_2\beta_2$ complex is non-cooperative with $K_d$ equal to $1.0 \times 10^{-6}$ M. [1] At high PLP concentrations additional, however unproductive internal aldimine groups can be traced. [2]

Although lacking the reactive 4'-aldehyde group the coenzyme analogues pyridoxine 5'-phosphate and N-phospho pyridoxyl-L-serine [3] form inactive complexes with the $\beta_2$ subunit. The respective dissociation constants are two orders of magnitude greater than for the active holoenzyme.

Since significant conformational alterations are expected to occur in both the protein and the ligands upon binding circular dichroism (CD) should provide a sensitive probe to follow the formation of the different complexes.

The far-uv CD spectrum of the $\beta_2$ subunit (190-240 nm) shows no characteristic changes in the helix content (~30%) when PLP and its analogues interact with the active center of the enzyme. In the near-uv CD spectrum (250-320nm) all three ligands induce marked positive Cotton effects in the absorption of the aromatic side chains of the protein.

In the coenzyme itself a large positive Cotton effect at 415 nm [6] is induced upon formation of the productive internal aldimine whereas unproductive pyridoxylation as well as binding of the coenzyme analogues shows no effect. The amplitude of the induced CD is ~80% greater for the native bienzyme complex than for the isolated $\beta_2$ subunit.

These findings are of particular advantage for titrations of the enzyme with cofactor. The dissociation constant $K_d$ so determined for the $\alpha_2\beta_2$ complex is equal to $0.9 \times 10^{-6}$ M and agrees well with the value obtained by equilibrium dialysis. Titration of the isolated $\beta_2$ subunit with PLP leads to the result that the T-state as defined in the proposed nonexclusive concerted binding mechanism [3,5] obviously is characterized by a three-times greater extrinsic Cotton effect than the corresponding R-state. Based on this assumption the obtained binding parameters sufficiently fit to the values determined by equilibrium dialysis.

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