CIRCULAR DICHROISM OF BIODEGRADATIVE THREONINE DEAMINASE*

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Biodegradative threonine deaminase of Escherichia coli (Umbarger and Brown, 1957) contains pyridoxal phosphate (PLP) as a cofactor and is activated by AMP (Wood and Gunsalus, 1949; Phillips and Wood, 1964; Hirata et al., 1965). In addition to the catalytic function through Schiff base formation with substrate, the enzyme-bound PLP was shown to contribute to the stabilization of the enzyme conformation (Tokushige, 1967). In this paper is described the circular dichroism (CD) of PLP in the biodegradative threonine deaminase in relation to the catalytic reaction; the results are pertinent to the mode of binding of PLP to the enzyme protein, and their interaction.

Materials and Methods

CD, absorption spectra, and optical rotatory dispersion (ORD) were measured with a Jasco ORD/UV-5 recording spectropolarimeter with a CD attachment. All measurements were performed in 1.0 cm

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silica cuvettes with enzyme concentrations of 5-10 mg/ml in 25 mM potassium phosphate, pH 7.4 containing 5 mM each of AMP and β-mercaptoethanol to stabilize the enzyme (Hirata et al., 1965). Samples were clarified by centrifugation prior to the measurements to eliminate turbidity. Threonine deaminase in this work was purified from cells of anaerobically grown E. coli W by the method described previously (specific activity, 210-250 units/mg of protein) (Tokushige, 1967). The final solution was kept in the potassium phosphate, AMP, β-mercaptoethanol mixture described above. Protein was determined by the method of Lowry et al. (1951).

Results and Discussion

The purified threonine deaminase exhibited a positive CD in the absorption band of the protein-bound PLP with a maximum at 415 mμ (Fig. 1). The CD spectrum appeared to be asymmetric with the

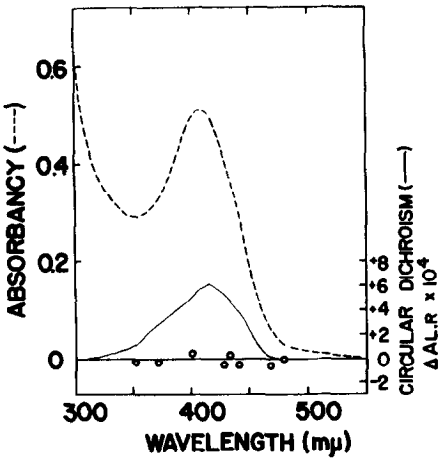

Fig. 1. CD (——) and absorption spectrum (----) of threonine deaminase. The enzyme (10.8 mg/ml) was dissolved in 0.1 M potassium phosphate buffer, pH 7.4 containing 5 mM AMP and 5 mM β-mercaptoethanol. Effect of L-threonine on the CD is also shown. Open circle (o); threonine deaminase in the same buffer as above, 1 minute after the addition of 0.05 M L-threonine.

right side of the curve steeper than the left side, indicating that

at least two optically active bands contribute to the CD spectrum. When the ORD of the enzyme was measured, a positive Cotton effect was also observed with an inflection point at 415 mμ. Upon addition of urea at 4 M, the CD as well as the positive Cotton effect in the ORD completely disappeared with a concomitant decrease in the absorption at about 408 mμ by 70% and an increase at 325 mμ. It is therefore plausible that the conformational integrity of the enzyme protein is required for the occurrence of the CD, especially since PLP itself has no asymmetricity.

Upon addition of L-threonine at 0.05 M, the yellow enzyme solution changed instantaneously to a more reddish color with a concomitant and complete disappearance of the positive CD (Fig. 1). As the catalytic reaction proceeded[1/], however, the CD reappeared returning to the original level in several minutes (Fig. 2). Changes in the CD by L-threonine were observed repeatedly. When D-threonine, a competitive inhibitor, was added at 0.05 M, the

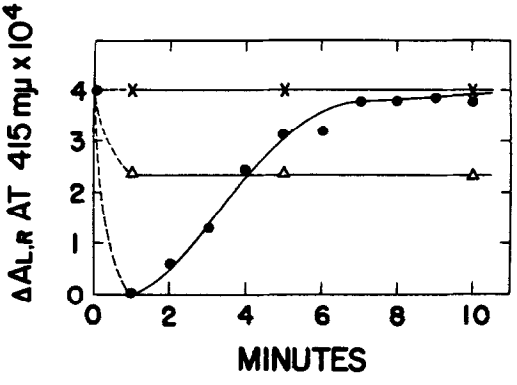

Fig. 2. Time course of the CD at 415 mμ after the addition of L-threonine (●——●), D-threonine (Δ——Δ) or L-valine (X——X) at 0.05 M each into the enzyme solution (8.0 mg/ml) in 25 mM potassium phosphate buffer, pH 7.4 containing 5 mM AMP and 5 mM β-mercaptoethanol.

1/ A separate experiment showed that the catalytic reaction proceeded during disappearance and reappearance of the CD band as judged from the increase of the absorption of α-ketobutyrate.

positive CD decreased by 40%, but no reversibility was observed, unlike the case of L-threonine (Fig. 2). Further addition of D-threonine up to 0.5 M led to the decrease in CD to as low as 20% of the original level, whereas the absorption of the enzyme bound-PLP increased by 20%. When L-valine, a non-substrate analogue, was added at 0.05 M, no change in CD was observed.

In order to examine the effect of alteration in the aldimine linkage between PLP and the enzyme protein, the following experiments were conducted. When hydroxylamine, a carbonyl reagent and an inhibitor of the enzyme, was added at 5 mM, the absorption at 408 mμ decreased by 40% and the CD band disappeared completely. The addition of $NaBH_4$ in 0.1 M potassium phosphate, AMP and β-mercaptoethanol as described in Methods followed by dialysis against the same buffer mixture caused the absorption at 408 mμ to decrease by 80% with the appearance of a new shoulder at about 320 mμ; the CD band disappeared completely. These results suggest that the aldimine linkage is required for the optical activity of PLP in the threonine deaminase.

In order to evaluate the effect of AMP on the optical activity of enzyme-bound PLP, the nucleotide was removed from the enzyme solutions by passage through a small column of charcoal. The enzyme preparation thus obtained, however, exhibited the same CD spectrum as the original, and did not change on addition of AMP to 5 mM. Similar results were reported with glycogen phosphorylase, in which the presence of substrate and activator (AMP) did not affect the optical activity associated with enzyme-bound PLP (Johnson and Graves, 1966).

In the native threonine deaminase, PLP is known to be bound by the enzyme protein to form a Schiff base between the ε-amino group of a lysine residue and the aldehyde group of PLP (Phillips and Wood, 1965). Upon addition of the substrate, no appreciable change

in the absorption maximum at 408 mμ was observed in spite of the change in the optical activity, indicating that a new optically inactive Schiff base is formed between PLP and threonine replacing the original one between PLP and the enzyme protein. The optical activity of PLP in this enzyme must, therefore, be ascribed to the specific manner in which the coenzyme is bound to the enzyme protein. Although the optical activity of the coenzyme has been reported with aspartate-glutamate transaminase (Breusov et al., 1963; Fasella and Hammes, 1964), aspartate β-decarboxylase (Wilson and Meister, 1966), phosphorylase (Torchinsky et al., 1965; Johnson and Graves, 1966), and glutamate decarboxylase (Huntley and Metzler, 1967), the present study shows in addition that the interaction between the substrate and enzyme-bound PLP in this case at least results in a reversible loss of this optical activity.

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