The reconstitution kinetics of tryptophanase from Escherichia coli.

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The reconstitution of apotryptophanase with pyridoxal-P (PLP) can be considered as the final step on the biosynthetic pathway to active holotryptophanase. Raibaud and Goldberg obtained knowledge about the reconstitution kinetics by merely measuring the increase of enzymatic activity and they proposed a two-step mechanism for the binding of PLP to tetrameric apotryptophanase (1). Since the reconstitution reaction is discontinously assayed for enzymatic activity this technique can give only information about the appearance of active enzyme but not about faster steps preceding the formation of holotryptophanase. To study the mechanism of reconstitution on conventional and stopped-flow time scales spectrophotometric measurements were performed at 440 nm where changes of the Schiff base absorbance were optimally under the reaction conditions used. At a 10 to 40-fold excess of PLP per monomer two reaction steps were observed. Half-times of 0.5-2 s and 2-3.5 min were found for the fast and the slower step, respectively.

To distinguish between several reaction mechanisms the trace and the determinant of the kinetic matrix of the total reaction system were determined at each PLP concentration (2). Within the PLP concentration range studied the trace is a linear function of the PLP concentration whereas the determinant exhibits a parabolic dependency, indicating two independent binding steps for PLP. Since each of the 4 subunits of apotryptophanase binds one PLP (3) but only 2 independent PLP binding steps have been observed 2 subunits behave kinetically indistinguishable with respect to their PLP binding. Our kinetic data obtained in the presence of excess PLP are consistent with the mechanism proposed by Raibaud and Goldberg (3): $E_4 + 2P = E_4P_2$; $E_4P_2 + 2P = H$ (P=PLP, E=apotryptophanase subunit, E_4P_2=Schiff-base intermediate, H=holoenzyme).

To confirm this mechanism stopped-flow experiments at an equimolar ratio of PLP to apotryptophanase subunit were performed at different wavelengths. In contrast to the data obtained with excess PLP already two reaction steps can be seen on the stopped-flow time scale. The interpretation of the second reaction step is difficult due to the fact that under those reaction conditions the kinetics of the aldehyde hydratation of PLP cannot be neglected. Further investigations using kinetical multiwavelength analysis are necessary to evaluate the experiments performed at second-order conditions.

1) Raibaud, O. and Goldberg, M.E., (1976) J. Biol.Chem. 251, 2814.
2) Lachmann, G., Lachmann, H. and Mauser, H., (1980) Z. Physik. Chem. N.F. 120, 19.
3) Snell, E.E., (1975) Adv. Enzymol. 42, 287.