STUDIES ON ENZYME-SUBSTRATE INTERACTIONS IN THE REGULATION OF TRYPTOPHAN OXYGENASE ACTIVITY*

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Tryptophan oxygenase is a heme enzyme which catalyzes the insertion of molecular oxygen into the pyrrole moiety of tryptophan forming formylkynurenine (Knox and Mehler, 1950). This enzyme is a likely site of regulation as it is the initial reaction in a metabolic sequence which in mammals leads to the formation of the nicotinic acid precursor to NAD and in certain microorganisms permits the degradative utilization of tryptophan as sole carbon source. The level of tryptophan oxygenase activity is indeed subject to several interesting types of regulation. The rate of enzyme synthesis is hormonally controlled in the mammalian liver (Knox, 1951; Feigelson et al., 1962) and by substrate induction in certain microorganisms (Palleroni and Stanier, 1964; Rosenfeld and Feigelson, 1967). The rate of enzyme degradation is modified during substrate induction in mammals (Schimke et al., 1964) which is in turn a consequence of a tryptophan mediated saturation of the enzyme with its heme cofactor (Feigelson and Greengard, 1962). In addition to the above mechanisms which determine the total amount of enzyme protein present, several types of regulation of the catalytic activity of pre-existing enzyme molecules has become evident. These include for

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mammalian tryptophan oxygenase the control by tryptophan of the ratio of catalytically inactive apoenzyme to catalytically active holoenzyme (Greengard and Feigelson, 1962) and for both microbial and mammalian tryptophan oxygenase the existence of latent forms of enzyme which are convertible to the catalytically active state following preincubation with tryptophan or tryptophan analogues (Feigelson and Dashman, 1959; Feigelson et al., 1964; Schimke et al., 1965; Knox et al., 1966).

The present investigation using highly purified preparations of microbial tryptophan oxygenase demonstrates the existence of a regulatory site within the enzyme molecule which also influences the functional capacity of pre-existing enzyme. This study indicates: (a) the catalytic activity is a sigmoidal function of the tryptophan concentration, (b) α-methyltryptophan, which is neither a substrate nor an inhibitor of the enzyme, eliminates the sigmoidicity and enhances enzyme activity at low tryptophan level, (c) the $K_M{}^{O_2}$ decreases as the enzyme becomes saturated with either tryptophan or α-methyltryptophan. These findings are compatible with the existence of a regulatory site on the enzyme which when saturated with tryptophan or α-methyltryptophan modifies the properties of the catalytic site.

RESULTS & DISCUSSION

Tryptophan oxygenase was induced in cultures of Pseudomonas acidovorans by growth on 0.1% L-tryptophan as sole carbon source. The enzyme was purified from sonnicates of the bacteria as previously described (Maeno and Feigelson, 1967) and further purified using DEAE-Sephadex gel and electrophoresis to a specific acitivity of 14.0 μmoles of formylkynurenine/min/mg protein. The rate of formylkynurenine formation was estimated by continuous spectrophotometric

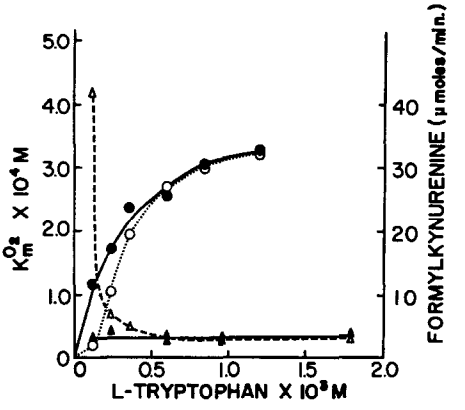

Fig. 1 The effect of L-tryptophan on the activity and $K_M^{O_2}$ of tryptophan oxygenase in the presence and absence of ∝ -methyl-DL-tryptophan. A series of cuvettes containing 390 μ moles sodium phosphate (pH 7.0), 0.92 mμ moles hematin, 0.25 μ moles ethylene diamine tetraacetic acid and the indicated amounts of tryptophan in a total volume of 2.5 ml were equilibrated with increasing ratios of O_2:N_2. The reaction was initiated by the addition of 0.01 ml of the purified enzyme (specific activity of 14.0 μ moles formylkynurenine/min/mg protein) and 4 μ moles of ascorbate. The rate of formylkynurenine formation was measured by continuous spectrophotometric recording at 321 mμ at 25°C. At each tryptophan level, $K_M^{O_2}$ in the presence (▲ ——— ▲) and absence (Δ ----- Δ) of 3 x 10^{-3} M ∝ -methyl-DL-tryptophan was determined. The enzyme activity depicted as a function of tryptophan concentration in the presence (● ——— ●) and absence (0-------0) of 3 x 10^{-3}M ∝ -methyl-DL-tryptophan was determined in equilibrium with air.

recording at 321 mμ (Feigelson et al., 1965) and the protein concentration was determined turbidometrically (Layne, 1957).

When tryptophan oxygenase activity was measured as a function of tryptophan concentration a sigmoidal substrate saturation curve was observed (Fig. 1) suggesting the existence of, and possible interaction between, more than one tryptophan binding site on the enzyme. At low tryptophan concentration the addition of 0.003 M ∝ -methyltryptophan to the reaction cuvettes more than tripled the rate of formylkynurenine formation, whereas it had no effect upon the catalytic rate when the enzyme was saturated with tryptophan. The net effect of ∝ -methyltryptophan was the elimination of the sigmoidicity and the

appearance of a classical hyperbolic tryptophan saturation curve. Control experiments indicated that α-methyltryptophan does not inhibit tryptophan oxygenase activity nor when incubated alone with large amounts of enzyme overnight could any catalytic modification of α-methyltryptophan be detected. Hence α-methyltryptophan is neither an inhibitor nor a substrate of this enzyme and presumably exerts its effect upon the tryptophan saturation curve by binding to a site on the enzyme other than the catalytic site.

It was of import to ascertain whether the binding of tryptophan or α-methyltryptophan to the enzyme modifies its interaction with its other substrate, oxygen. Therefore at each of a variety of tryptophan levels, and in both the absence and presence of α-methyltryptophan, enzyme activity was studied as a function of oxygen concentration. Tryptophan oxygenase activity was always a hyperbolic function of the oxygen concentration and thus the $K_M^{O_2}$ was estimated in the traditional manner (Lineweaver and Burk, 1934). As depicted in Fig. 1, the apparent $K_M^{O_2}$ is dependent upon the tryptophan concentration undergoing a marked decrease as the enzyme becomes saturated with tryptophan. α-methyltryptophan completely eliminates the dependency of $K_M^{O_2}$ on tryptophan. Thus binding of either tryptophan or α-methyltryptophan to what is apparently a non-catalytic site on the enzyme modifies it in a manner which facilitates its interaction with oxygen.

In a two substrate enzyme catalyzed reaction a dependence in the apparent K_M of one substrate upon the concentration of the other substrate is a possible consequence assuming certain rate constants (Reiner, 1959). That such a purely kinetic explanation underlies the lowered $K_M^{O_2}$ by tryptophan is rendered unlikely by the finding that a non-substrate, α-methyltryptophan, also has this effect. The most reasonable model which seems compatible with these facts is to assume the existence of a regulatory as well as a catalytic site on the enzyme

and that tryptophan or α-methyltryptophan may combine with the regulatory site but that only tryptophan and oxygen combines with the catalytic site. Combination of tryptophan or α-methyltryptophan with the regulatory site evokes an unknown modification within the enzyme, possibly conformational, which enhances its ability to combine with oxygen.

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