PRELIMINARY NOTES

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Liver tryptophan pyrrolase activity during thyroxine-induced metamorphosis of *Rana catesbeiana*

Studies on mammalian liver tryptophan pyrrolase (EC 1.11.1.4) are numerous. The enzyme activity can be greatly enhanced by the injection of substrate tryptophan, histidine, tyrosine, histamine, adrenalin and adrenal steroid hormones. GREENGARD AND FEIGELSON[1] demonstrated that the enzyme activity was increased by its prosthetic group, hematin, following substrate induction but not after cortisone induction. This result suggested two alternative mechanisms involved in the enzyme induction. The result that the administration of actinomycin D abolished the hydrocortisone-mediated increase in tryptophan pyrrolase activity but did not influence the tryptophan-mediated rise in the enzyme activity further suggested a basic difference between hormonal and substrate-induced stimulation of the enzyme biosynthesis[2]. Recent evidence has indicated that tryptophan prevents the conversion of the active reduced holotryptophan pyrrolase into the inactive oxidized form, as well as stabilizing the combination of the prosthetic group and the apoenzyme[3]. On the other hand, histidine and hydrocortisone do not seem to have any inducing action on the enzyme in the liver of *Rana catesbeiana*[4]. In the present paper we report that tryptophan pyrrolase in tadpole liver is somewhat different from the one in rat liver in respect to the stimulation of the enzyme activity with methemoglobin and ascorbic acid, and sensitivity to heat. Evidence for the existence of inhibitor on the enzyme is also presented.

Bull frog tadpole, *R. catesbeiana*, in premetamorphic stage was obtained from Connecticut Valley Biological Supply Co. (Valley Rd., Southampton, Mass.) and was treated with thyroxine at the concentration of $2.6 \cdot 10^{-8}$ M by the method described previously[5]. In order to induce tryptophan pyrrolase in tadpole or frog liver either 2.5% tryptophan solution was injected intraperitoneally (0.2 ml/1 kg body weight; in the case of tadpole, injected through tail muscle in order to minimize leakage), or tadpole was immersed in 0.005 M tryptophan solution for 16 h at 21–23° (room temperature). The livers were excised, pooled and homogenized in 0.14 M KCl solution containing 0.0025 M NaOH as 20% homogenate. The homogenate was passed through a double layer of cheese-cloth. Tryptophan pyrrolase activity was measured by the method described[6]. Protein concentration was determined by the method of LOWRY *et al.*[7]. Molar absorbance coefficient (ε_M) of 4870 at 365 mμ for kynurenine is used. In tadpole or frog liver, formylase (catalyzes the conversion of formylkynurenine to kynurenine, EC 3.5.1.9) is probably present in excess[4]. Therefore, the enzyme activity is expressed as mμmoles of kynurenine formed per h per mg protein.

Fig. 1 illustrates the effect of thyroxine treatment on the activity of tryptophan pyrrolase and its inducibility by tryptophan in tadpole liver. Firstly, it is seen in the figure that there is a large scattering in the results; this was not only due to variations in individuals, but also to variations in shipments of tadpoles. During the early phase of thyroxine treatment the enzyme activity was induced 2–3-fold by tryptophan. However, as the duration of the hormonal treatment progressed, the degree of inducibility gradually decreased. Furthermore, thyroxine treatment does not seem to

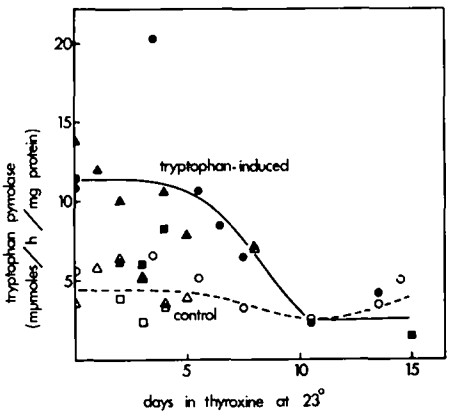

Fig. 1. Effect of thyroxine treatment on tryptophan pyrrolase activity and its inducibility in tadpole liver. Tadpoles were treated in $2.6 \cdot 10^{-8}$ M thyroxine solution at room temperature. In order to induce the enzyme, tadpoles were immersed in the thyroxine (or none in zero day thyroxine treatment) solution together with 0.005 M L-tryptophan solution for 16 h. Each point represents a value of the liver pooled from three tadpoles. Duplicate runs were carried out. Different symbols in the figure indicate different shipments of tadpoles. Closed symbols indicate the value of the tryptophan-induced and the open symbols for the control. The rest of the procedures are described in the text.

increase the enzyme activity. The result shown in the figure, therefore, is in close agreement with the observation made by SPIEGEL AND SPIEGEL[4].

It is of interest to note that even though level of constitutive enzyme is relatively high at the later stage of thyroxine treatment, inducibility by tryptophan of the enzyme becomes very small. This indicates that the level of constitutive enzyme does not necessarily correlate proportionately with the degree of inducibility. The decrease in the enzyme activity as well as the inducibility by tryptophan could be due to many factors: appearance of inhibitor, disappearance of activator, decreased biosynthesis

TABLE I

INHIBITORY EFFECT OF THYROXINE-TREATED TADPOLE LIVER ON THE TRYPTOPHAN PYRROLASE ACTIVITY OF THE CONTROL (NON-THYROXINE-TREATED)

Thyroxine (T_4) treatment and induction with tryptophan were carried out by immersion of tadpole in the solution. The rest of the procedures are described in the text.

Expt. No.	*Profile of homogenate*	*Enzyme activity**	*Expected enzyme activity**	*Found*
1	(a) Treated with T_4 for 9 days. No induction with tryptophan	2.01		
	(b) Control (no T_4, induced with tryptophan)	7.76		
	a + b		4.89	3.02
2	(a) Treated with T_4 for 15 days. No induction with tryptophan	1.44		
	(b) Control (no T_4, and no tryptophan induction)	6.76		
	(c) Control (no T_4, and induced with tryptophan)	10.06		
	a + b		4.10	0.14
	a + c		5.75	1.87

* mμmole kynurenine per h per mg protein.

of the enzyme, *etc.* As a preliminary study on these alternatives, liver homogenate from thyroxine-treated tadpole was mixed with an equal volume of the homogenate from the control, and was examined for the presence of an inhibitory factor in the thyroxine-treated tadpole liver. As shown in Table I, in both experiments a mixture of the homogenates definitely decreased the control enzyme activity, thereby showing the activity far below the calculated expected value of two homogenates. Similar observation that spontaneously metamorphosing tadpole liver contained an inhibitor for tryptophan pyrrolase has been reported previously[4]. Since reduced triphosphopyridine nucleotide inhibits the purified rat liver tryptophan pyrrolase allosterically[8], it is quite possible that increased amount of this coenzyme might be involved in the case mentioned above.

TABLE II

EFFECT OF METHEMOGLOBIN AND ASCORBIC ACID ON TRYPTOPHAN PYRROLASE OF RAT AND TADPOLE LIVER

Liver homogenate was prepared as 15%, and the supernatant at 105 000 × *g* for 60 min was used as the source of tryptophan pyrrolase. The incubation mixture contained the following: 1.0 ml of the supernatant, 0.3 ml 0.03 M L-tryptophan, 1.0 ml 0.2 M phosphate buffer at pH 7.0, and 0.3 ml methemoglobin (0.72 mg) or 0.3 ml 0.3 M fresh neutralized ascorbic acid in a total volume of 4.0 ml. Incubation was carried out at 37° for 1 h in Dubnoff shaker. The rest of the procedures are described in the text.

Addition	*mμmoles kynurenine per h per mg protein*	
	Rat liver	*Tadpole liver*
Control	2.73	10.64
Methemoglobin	14.81	11.64
Ascorbic acid	9.49	9.34
Methemoglobin + ascorbic acid	24.87	11.93

Table II compares the requirement of methemoglobin and ascorbic acid for the tryptophan pyrrolase of tadpole and rat liver. It is seen that rat enzyme absolutely requires these two factors for the enzyme activity. However, tadpole liver enzyme does not need both for full enzyme activity. Since the enzyme from both sources is the supernatant at 105 000 × *g* for 60 min, the result in Table II constitutes a rather interesting difference between the source of the enzyme and its requirements. Non-requirement of the tadpole enzyme for methemoglobin suggests that the enzyme is more strongly bound with the prosthetic group than rat enzyme which is easily detached from its hematin and is easily oxidized[3]. On the other hand, when the bull frog enzyme was induced with tryptophan, it required the addition of methemoglobin and ascorbic acid for full enzyme activity: the supernatant of tryptophan-induced bull frog liver had an enzyme activity of 0.35 mμmole/h per mg protein without the two factors and achieved 3.43 mμmoles/h per mg protein in the presence of the added cofactors.

The results presented in this paper offer very useful materials for future investigations concerning the inhibitory substance, comparative aspects of the enzyme from two sources as well as the problems of the biological significance of synthesis of the inhibitor at the certain stages of metamorphosis. Unlike the rat enzyme[8], tryptophan-

induced frog enzyme did not increase its specific activity at 55° in up to 10 min and decreased sharply to about 30% of the original activity in another 5 minutes. It should be noted here that the fact that L-histidine and hydrocortisone had no effect on the tadpole liver tryptophan pyrrolase activity[4] also suggests a difference in the system between rat and tadpole.

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Reinvestigation of the pH optimum in terms of the action and properties of rabbit muscle amylo-1,6-glucosidase–oligo-1,4 → 1,4-transferase

Amylo-1,6-glucosidase (dextrin 6-glucanohydrolase, EC 3.2.1.33) and oligo-1,4 → 1,4-transferase (α-1,4-glucan: α-1,4-glucan 4-oligoglucantransferase, EC 2.4.1.24) comprise the glycogen debranching system in rabbit muscle. Both glucosidase and transferase activities appear to be associated with each other throughout purification[1] and it has been suggested that they may represent a "two-headed" enzyme[2].

Although glucosidase–transferase has been extensively studied, there have been discrepancies between the properties of various preparations. The pH optimum for hydrolysis was found to be 7.2 in one case[3] and 5.8–6.5 in others[1,4–7]. Likewise, the pH optimum for glucosyl incorporation has been reported as 7.4 (ref. 8) and 6.4 (ref. 7). In the latter two cases neither optimum coincided with that of the hydrolytic reaction.

Several suggestions have been put forth to explain these differences. The incorporation reaction has been suggested to proceed by a mechanism different from simple reversal of the hydrolysis by the glucosidase[6]. In the case of hydrolysis, evidence has been obtained suggesting that glucosidase–transferase activities with differing pH optima may be present in partially purified preparations[9].