Occurrence of Sepiapterin Deaminase in the Silkworm, *Bombyx mori*

Two yellow pteridines, xanthopterin-B_1 and xanthopterin-B_2, were isolated from the mutant lemon of the silkworm, and identity of the former with sepiapterin (2-amino-4-hydroxy-6-lactyl-7,8-dihydropteridine)[1] has been established[2]. Recently GOTO et al.[3] proposed for xanthopterin-B_2 the structure of 2,4-dihydroxy-6-lactyl-7,8-dihydropteridine (lumazine of sepiapterin). During the studies on these pigments, the author found a silkworm enzyme which deaminates sepiapterin to xanthopterin-B_2. The present paper describes identification of the reaction product and some properties of the enzyme.

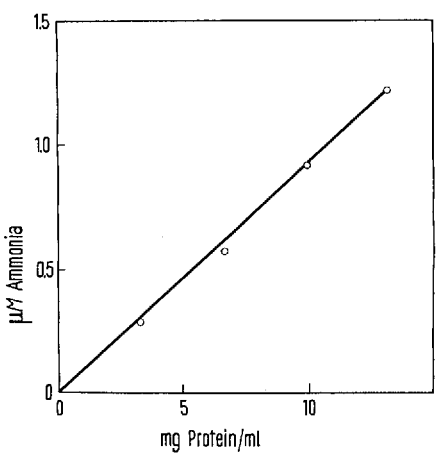
The yellow pteridines were obtained by the method previously described[2]. Since the fat body of normal strain of the silkworm contained sepiapterin reductase[4] as well as deaminase, the mutant lemon which is devoid of the reductase was preferable as a starting material.

Subsequent procedures were carried out at 0–3 °C. 10 g of the fat bodies of lemon larvae (5th instar, 4 or 5 days after last moulting) were homogenized with 10 volumes of 0.05 *M* potassium phosphate buffer, pH 6.5. After standing for 20 min, the homogenate was centrifuged at 8000 rpm for 20 min. Then the supernatant fluid was strained through a cheese-cloth and the filtrate was used as a crude enzyme. The crude enzyme solution was fractionated with ammonium sulphate between 55 and 74% saturation. The precipitate was dissolved in 3 ml of 0.05 *M* phosphate buffer, pH 6.5, and ammonium salt was removed by passing through a Sephadex G-25 column (2 · 19 cm) that was eluted with the buffer.

When sepiapterin was incubated with the crude enzyme at neutral pH, formation of another yellow fluorescent substance was detected by paper chromatography. Simultaneous liberation of ammonia was also demonstrated by the alkaline phenol method using Conway's apparatus[5]. As shown in the Figure, the amount of ammonia released is proportional to the concentration of the enzyme.

To obtain yellow fluorescent product, 3 ml of freshly prepared crude enzyme was mixed with 3 mg of sepiapterin dissolved in 3 ml of 0.02 *M* potassium phosphate buffer, pH 7.0. After incubating at 25 °C for 60 min, the reaction mixture was heated in a boiling water bath for 3 min. The resulting supernatant solution was applied to an Ecteola-cellulose column (1 · 15 cm) that was eluted with distilled water. After elution of sepiapterin, the reaction product remaining in the column as a yellow fluorescent band was eluted with 0.01 *M* acetic acid. The concentrated eluate was applied to a Sephadex G-50 column (1 · 15 cm) that was eluted with distilled water. The yellow eluate gave a single fluorescent spot on paper chromatogram and its behaviour was indistinguishable from that of xanthopterin-B_2 (Table I). The UV-absorption spectrum in 0.1 *N* HCl as well as in 0.1 *N* NaOH was also identical with that of xanthopterin-B_2.

Although the ammonium sulphate fraction had negligible adenosine-deaminase activity, it showed an appreciable activity of guanase. Therefore, there may be a possibility that sepiapterin is deaminated by guanase. However, as shown in Table II, sepiapterin deaminase activity is considerably inhibited by xanthopterin[6], whereas guanase



Liberation of ammonia as a function of enzyme concentration. The reaction mixture contained the following components: potassium phosphate buffer, pH 8.0, 100 μM; sepiapterin, 2 μM; enzyme as indicated, in a final volume of 1.4 ml. After incubation at 25 °C for 30 min the reaction was stopped by the addition of 0.1 ml of 1 *M* potassium dihydrogen phosphate, followed by heating in a boiling water bath for 3 min. Ammonia released in deproteinized solution was determined. Protein was determined by the biuret method using the bovine serum albumin as the standard.

Table I. Comparison of Rf values of the reaction product and xanthopterin-B_2

Solvent system	Rf value	
	Reaction product	Xanthopterin-B_2
1	0.64	0.64
2	0.49	0.49
3	0.47	0.47
4	0.27	0.27
5	0.35	0.35
6	0.41	0.43
7	0.55	0.55

Solvent system: (1) *n*-propanol/1% ammonia (1:1, v/v). (2) *n*-propanol/1% ammonia (2:1). (3) iso-propanol/water (7:3). (4) 95% ethanol/*n*-amylalcohol/water (7:5:3). (5) *n*-propanol/ethylacetate/water (7:1:2). (6) *n*-butanol/acetic acid/water (4:1:2). (7) 3% ammonium chloride. Ascending method. Toyo filter paper No. 51.

Table II. Inhibition of sepiapterin deaminase by xanthopterin

Addition	Guanase	Sepiapterin deaminase
	(μM NH_3/mg protein)	
None	0.037	0.242
Xanthopterin (1 μM)	0.037	0.079

The reaction mixture contained the following components: potassium phosphate buffer, pH 8.0, 100 μM, guanine or sepiapterin, 2 μM; xanthopterin as indicated; enzyme solution (ammonium sulphate fraction), 0.3 ml, in a final volume of 1.3 ml. Incubation at 25 °C for 60 min. Ammonia was determined as described in the Figure.

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activity affected little in contrast with mammalian guanase[7]. In addition, sepiapterin could not be attacked by guanase from rat liver[8].

Both sepiapterin and isosepiapterin were deaminated by the enzyme, whereas the following pteridines were inactive as a substrate: 2-amino-4-hydroxypteridine[9], 2-amino-4-hydroxy-6-methylpteridine[9], 2-amino-4-hydroxy-6,7-dimethylpteridine[10], xanthopterin, and 2-amino-4-hydroxypteridine-6-carboxylic acid[11]. It was reported that bacterial pterin deaminase[12] has rather low substrate specificity and attacks some pteridines, including 2-amino-4-hydroxypteridine, 2-amino-4-hydroxy-6-methylpteridine and 2-amino-4-hydroxypteridine-6-carboxylic acid. From the substrate specificity, it seems likely that sepiapterin deaminase is different from the bacterial enzyme.

The enzyme exhibits its activity in the range of pH 6 to 10. The enzyme deaminates sepiapterin both under aerobic and anaerobic conditions. From the structure of sepiapterin and xanthopterin-B_2 together with the above fact, the deamination reaction may be formulated as follows:

$$\text{sepiapterin} + H_2O \rightleftharpoons \text{xanthopterin-}B_2 + NH_3$$

Purification and further characterization of the enzyme are being undertaken.

Zusammenfassung. Im Seidenspinner (*Bombyx mori*) wird ein Enzym nachgewiesen und angereichert, welches das gelbe Pigment Sepiapterin zu Xanthopterin-B_2 desaminiert. Ferner wird die Substratspezifität des Enzympräparates abgeklärt.

M. Tsusue

Department of Biology, Faculty of Science, Metropolitan University, Tokyo (Japan), October 4, 1966.

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Primary Afferent Depolarization Produced by Vagal Visceral Afferents

Previous work[1] showed that stimulation of vagal and aortic afferents depressed laryngeal reflexes with a time course similar to that of primary afferent depolarization (PAD) in the spinal cord[2-4]. This suggested that the depression in reflex activity could be due, at least in part, to PAD produced by the visceral afferents which course through the vagus nerve. This seemed of interest because, in addition to its possible significance for the understanding of the mechanisms involved in the inhibitions produced by vagal and aortic stimulations[5-7], previous studies on PAD produced by afferent stimulation have been concerned only with the actions of somatic, i.e. muscle and cutaneous, afferents[2-4].

Methods. The observations were performed in 20 adult cats anaesthetized with sodium pentobarbital (35–40 mg/kg i.p.), paralysed with gallamine triethiodide and maintained by artificial respiration. The floor of the fourth ventricle was exposed by cerebellectomy, and a stimulating microelectrode was placed in the solitary tract nucleus (STN) region, 4–6 mm rostral to the obex and 1.5–2.5 mm lateral to the midline, at depths ranging from 0.7–1.2 mm from the bulb surface. Antidromic responses were monophasically recorded from the central end of the ipsilateral superior laryngeal nerve (SL), which consists of afferent fibres only[8]. Stimulating and recording electrode pairs were placed on the central end of the ipsilateral vagus and aortic nerves. Arterial blood pressure was recorded from the femoral artery.

Results. The antidromic SL responses produced by STN stimulation were usually increased when preceded by vagal and aortic nerve stimulation. As shown in Figure 1A and D, hyperexcitability of the SL nerve terminals resulted mainly from the activation of vagal afferents whose threshold ranged from 1.6–5.0 times that of the most excitable fibres in the nerve (T). In 5 experiments stimulus strengths of 5–10 T added a small excitability increase, and this was correlated with the appearance of an intermediate-threshold group of fibres in the vagal electrogram (Figure 1B). No changes were observed for stimulus strengths of 10 up to 50 T.

The vagal afferents producing excitability increases of the SL terminals also induced a blood-pressure fall when stimulated at 50 c/sec (Figure 1C). Both effects had about the same threshold and grew similarly with increasing stimulus strengths. It is therefore concluded that the afferent fibres producing the blood-pressure fall are the same ones producing hyperexcitability of the SL terminals.

The lowest-threshold afferent fibres in the vagus nerve seem to originate from pulmonary stretch receptors, while those with intermediate threshold are attributed to arterial pressoceptors and rapidly adapting tracheal receptors[9]. In order to test possible actions from pul-

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