Pteridines
Vol. 6, 1995. pp. 135-137

Short Communication

Evidence of the Phenylalanine Hydroxylase Involvement in Biosynthesis of Pteridines and Hydroxylation of Tryptophan in *Drosophila melanogaster*

S. Alcañiz, P.Ruiz-Vázquez and F.J. Silva§

Department of Genetics, University of Valencia, 46100 Burjassot, Spain

(Received June 15, 1995)

Introduction

Phenylalanine hydroxylase (PAH) is an enzyme which catalyses the tetrahydropteridine-dependent hydroxylation of L-phenylalanine to L-tyrosine. In mammals, this enzyme and its structural gene have been studied thoroughly (1, 2, 3). In the insect *Drosophila melanogaster*, the analysis of the phenylalanine hydroxylation system have been carried out more recently (4, 5, 6, 7). In this organism. only two genes coding for aromatic amino acid hydroxylases have been cloned and sequenced, one corresponding to phenylalanine hydroxylase (8) and the other to tyrosine hydroxylase (9). In contrast to the situation found in mammals, no specific tryptophan hydroxylase enzymes have been found in insects. Several results have led to the hypothesis that in *Drosophila* as well as in other insects, the same enzyme performs in vivo the hydroxylation of both tryptophan and phenylalanine (10). These results included the presence of PAH in serotonin neurons and the *in vitro* ability of the recombinant phenylalanine hydroxylase protein to hydroxylate tryptophan.

A third function for the PAH enzyme in *Drosophila* is the tetrahydropterin oxidase activity of this enzyme. As previously shown by several authors (11, 12) in an *in vitro* system the mammalian PAH enzyme is susceptible to oxidase several tetrahydropteridines in coupled or uncoupled reactions to the hydroxylation of the amino acid. The physiological meaning of these reactions has not yet been completely clarified. In *Drosophila melanogaster*, the pteridine pattern of the PAH-defective mutant *Henna recessive-3* have led to the hypothesis that PAH participates directly as tetrahydropterin oxidase in the synthesis of pteridines (6), including for example drosopterins which act as screening pigments in the eyes.

Our aim is to present several pieces of evidence to support the theory that Drosophila PAH enzyme functions as a tryptophan hydroxylase and a tetrahydropterin oxidase.

Material and Methods

Supplementation experiments. 0.5 g of either L-Phe, L-Tyr or L-Trp were added to a glass tube containing 10 ml of a standard culture medium. Around 80 eggs from the wild type strain *Oregon R* were transferred to these tubes. When the insects reached the whitepupa, they were collected, immersed in liquid nitrogen and stored frozen till use. To determine PAH concentration, proteins were separated by SDS-PAGE, transferred to a nitrocellulose filter (Schleicher and Schuell, BA85) and immunodetected with PH8 monoclonal antibody following the procedure described by (6). The amounts of cross-reacting-material detected in the filters were determined using a densitometer (model 2202 Ultrascan, LKB) and an integrator (model 3390A, Hewlett-Packard). The statistical analysis used was a

---

§ Author to whom correspondence should be addressed.
Dr. Francisco J. Silva
Departament de Genètica, Facultat de CC. Biològiques
Universitat de València
C/Dr. Moliner 50, 46100 Burjassot (València)
SPAIN

t-Student test for paired samples. Each value obtained for a supplemented medium was a percentage of the nonsupplemented medium value which was always obtained from the same nitrocellulose filter.

Drosopterin synthesis. Correlation between drosopterin/isodrosopterin content and PAH concentration was determined in head samples at different stages of development. Pteridines were separated by HPLC using a Nova-Pak C18 column (3,9 x 150 mm, Waters) with a mobile phase of 50 mM sodium phosphate buffer pH=4.6, including 6% methanol. Detection was carried out with a fluorescence detector F-1050 (Merck-Hitachi) using an excitation wavelength of 490 nm and an emission of 560 nm. The amount of drosopterin was determined in five independent samples. The PAH protein was immunodetected as described previously except that values were a percentage of the amount detected in newly-born adults (96 hours after pupariation).

Table 1. PAH concentration. Relative amounts (PAH in supplemented medium/PAH in control medium × 100). SE standard error of mean.

Supplemented amino acid	n	PAH mean ± SE	Statistical analysis
Phenylalanine	12	232 ± 39	$P<0.01$
Tyrosine	12	91 ± 10	NS
Tryptophan	12	156 ± 22	$P<0.025$

Table 2. Correlation between PAH concentration and drosopterin/isodrosopterin content. ND=nondetected. Values are percentages of the ones obtained at adult emergence (96 hours). Values between brackets are standard errors.

	Time (hours)[a]							
	48	54	66	72	78	90	96	100
Drospterin	ND	ND	1	6	30	62	100	126
			(1)	(4)	(12)	(1)	(1)	(15)
PAH	33	51	26	58	88	106	100	96
	(3)	(8)	(2)	(2)	(7)	(7)	(0)	(8)

[a] Hours after pupariation

Results

In order to determine the involvement of PAH in the metabolism of tryptophan the diet given to the insects, was supplemented with one aromatic amino acid, either phenylalanine, tyrosine or tryptophan. The amount of the PAH protein was significantly higher in white pupae from media supplemented with phenylalanine or tryptophan, while the aromatic amino acid tyrosine did not show effect (Table 1).

One of the physiological functions of pteridines is their role as screening pigments in the compound eyes of the flies. The most abundant of these pigments are the isomers droso- and isodrosopterin. Their synthesis starts after the adult head is formed inside the pupal case, and continues till some days after the adult emerges. In table 2, a correlation between the relative concentration of PAH and drosopterins is shown. Between the 66-hour-pupa and the emergence of the adult (96 hours) there is a four-fold increase in the PAH concentration. This increment is simultaneous with the deposition of pteridines in the eyes.

Discussion

In the present paper we have shown several pieces of evidence connecting the PAH protein with the tryptophan and pteridine metabolism.

Supplementing the diet of *Drosophila* with L-Phe has recently been reported to cause a two-fold increase in the PAH enzyme activity when tetrahydrobiopterin was used as cofactor (7). We have shown that this increased activity is due to an elevated concentration of this protein in the flies, and that a similar result is observed when the supplemented amino acid was tryptophan. In contrast, the supplementation with tyrosine did not alter the PAH concentration. These results reveal that PAH participates in the Trp as well as the Phe metabolism and show an *in vivo* connection between Trp and PAH, supporting the hypothesis of a single locus encoding both PAH and TPH activities in *Drosophila melanogaster* (10). Additional support for this idea was the low level of serotonin detected in the PAH-defective mutant strain *Henna recessive-3* (data not shown), indicating that the presence of PAH is the serotonin neurons was directly related with the synthesis of serotonin.

The second putative function for PAH was the ability to act as a tetrahydropterin oxidase. This activity has been stated in several *in vitro* assays using the enzyme from mammalian sources, and it may be coupled or uncoupled to the amino acid hydroxylation (13). However, the physiological relevance of this reaction need to be clarified. In our work, we present preliminary evidence in favour of a physiological function in the insect *D. melanogaster*. Our results show that the synthesis of the red pigments in the head is correlated with a four-fold increase in the amount of the protein PAH. An easy explanation for this fact would be that this enzyme is working as a tetrahydropterin oxidase (probably in an uncoupled reaction) using one or several tetrahydropteridines as substrates and producing that are required for the formation of drosopterin.

Finally in order to fulfil these three enzyme activities and the metabolic roles asscciated with all of them, the expression of the *pah* gene should be tissue and temporally regulated. The analysis of the sequence of a genomic clone including this gene have shown the existence of six putative TATA boxes in the 5' end of the gene (data not shown). Whether they function *in vivo* as promoter sequences, is a matter that will require a deeper molecular analysis.

Acknowlegment

This work has been supported by the commission of the European Communities (Human Capital and Mobility: Network contract number ERBCHRXCT 93-0243).

Reference

1. Kaufman, S. The phenylalanine hydroxylation system from mammalian liver. Adv. Enzymol. 1971; 35: 245-319.
2. Ledley, F.D., Dilella, A.G., Kwok, S.C.M., Woo, S.L.C. Homology between phenylalanine and tyrosine hydroxylases reveals common structural and functional domains. Biochemistry 1985; 24: 3389-3394.
3. DiLella, A.G., Kwok, S.C.M., Ledley, F.D., Marvit, J., Woo, S.L.C. Molecular structure and polymorphic map of the human phenylalanine hydroxylase gene. Biochemistry 1986; 25: 743-749.
4. Geltosky, J.E. ,Mitchell, H.K. Developmental regulation of phenylalanine hydroxylase activity in *Drosophila* melanogaster. Biochem. Genet. 1980; 18: 781-791.
5. Bel, Y., Ferré, J. Regulation of pteridine biosynthesis and aromatic amino acid hydroxylation in *Drosophila melanogaster*. Biochem. Genet. 1989; 27: 59-76.
6. Silva F.J., Bel, Y., Botella, L.M., Cotton, R.G.H., Ferré, J. Immunological detection of phenylalanine hydroxylase protein in *Drosophila meleanogaseter*. Biochem. J. 1992; 287: 85-89.
7. Bel, Y. Jacobson, K.B., Silva F.J., Ferré, J. Developmental and biochemical studies on the phenylalanine hydroxylation system in *Drosopila melanogaster*. Insect Biochem. Molec. Biol. 1992; 22: 633-638.
8. Morales, G., Requena, J.M., Jimenez-Ruiz, A., López, M.C., Ugarte, M., Alonso, C. Sequence and expression of the Drosophila phenylalanine hydroxylase mRNA. Gene 1990; 93: 213-219.
9. Neckameyer, W.S., Quinn, W.C. Isolation and characterization of the gene for Drosophila tyrosine hydroxylase. Neuron 1989; 2: 1167-1175.
10. Neckameyer W.S., White, K. A single locus encodes both phenylalanine hydroxylase and tryptophan hydroxylase activities in *Drosophila*. J. Biol. Chem. 1992; 267: 4199-4206.
11. Fisher D.B., Kaufman, S. Tetrahydropterin oxidation without hydroxylatiom catalyzed by rat liver phenylalanine. J. Biol. Chem. 1973; 248: 4300-4304.
12. Davis, M.D., Kaufman, S. Evidence for the formation of the 4a-carbinolamine during the tyrosine-dependent oxidation of tetrahydrobiopterin by rat liver phenylalanine hydroxyiase. J. Biol. Chem. 1989; 264: 8585-8596.
13. Davis, M.D., Kaufman, S. Studies on the partially uncoupled oxidation of tetrahydropterins by phenylalanine hydroxylase. Neurochem. Res. 1991; 16: 813-819.