Decarboxylases for Polyamine Biosynthesis in *Drosophila melanogaster* Larvae

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Ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) and S-adenosyl-methionine decarboxylase (S-adenosyl-L-methionine carboxy-lyase, EC 4.1.1.50) were assayed in *Drosophila melanogaster* larvae. The highest enzyme activities were detected in 24 and 48 h larvae, with diminishing activities in subsequent larval stages. Stimulation of S-adenosylmethionine decarboxylase by putrescine was demonstrable in late but not in early stages of larval development.

Polyamines are believed to play an important role in a variety of growth processes (Herbst & Bachrach, 1970), possibly in the control of RNA synthesis (Dion & Herbst, 1967; Cohen, 1971; Bachrach, 1973; Herbst *et al.*, 1973). Polyamines have been observed to increase, paralleling similar increases in RNA, during periods of rapid growth induced by liver regeneration (Dykstra & Herbst, 1965; Jänne & Raina, 1966) and hormones (Bachrach, 1973) and during development of chick embryos (Raina, 1962; Caldarera *et al.*, 1965), foetal rat (Russell & McVicker, 1972), *Xenopus laevis* (Russell, 1970) and *Drosophila melanogaster* (Herbst & Dion, 1970). Although polyamine concentrations have been studied in several insect tissues (Gilmour, 1961; Herbst & Dion, 1970; Heby, 1972; Wyatt *et al.*, 1973), a polyamine-biosynthetic enzyme, ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) has been studied only in the silk moth *Hyalophora cecropia* (Wyatt *et al.*, 1973).

In order to contribute to the further understanding of the metabolism of polyamines in *D. melanogaster*, a study was undertaken to determine whether ornithine decarboxylase and S-adenosylmethionine decarboxylase (S-adenosyl-L-methionine carboxy-lyase, EC 4.1.1.50) activities were present in *Drosophila* larvae, to develop reliable assay conditions for the enzymes in these tissues, and to investigate the variation in these enzyme activities during larval development.

Materials and Methods

The assay for ornithine decarboxylase used for *Drosophila* larvae was a modification of the procedure used by Wyatt *et al.* (1973). Larvae obtained as previously described (Herbst *et al.*, 1973) were thoroughly washed, weighed and homogenized in a sufficient volume to yield a 25% (w/v) homogenate. The homogenization medium consisted of 10 mM-Tris/HCl, pH 7.7, 10 mM-EDTA, 5 mM-dithiothreitol and 0.25 M-sucrose. The larvae were homogenized in a

Duall homogenizer (Kontes Glass Co., Vineland, N.J., U.S.A.) and centrifuged at 3000g for 15 min at 4°C; 0.1 ml of the supernatant was used in each assay. The ornithine decarboxylase assay was performed in 15 ml conical centrifuge tubes in a total volume of 0.25 ml consisting of 42 mM-Tris/HCl, pH 7.1, 0.04 mM-pyridoxal phosphate, 5 mM-dithiothreitol and 0.2 µCi of DL-[1-¹⁴C]ornithine monohydrochloride (29 mCi/mmol; ICN, Cleveland, Ohio, U.S.A.) plus sufficient L-ornithine to bring the concentration of the L-isomer to 1 mM. The tubes were capped with special rubber stoppers fitted with a plastic centre well (Kontes Glass Co.) which contained 0.1 ml of a 1 M solution of Hyamine hydroxide in methanol. The reaction was allowed to proceed for 60 min at 37°C and was terminated by the injection of 0.85 ml of 0.5 M-H₂SO₄. The tubes were kept at room temperature for 15 min, and the ¹⁴CO₂ trapped in the centre well was counted for radioactivity in liquid-scintillation vials containing toluene Omni-fluor (New England Nuclear Corp., Boston, Mass., U.S.A.). The assay as described was linear throughout the 60 min incubation period and yielded increased activity proportional to the amount of supernatant containing 100–600 mg of protein.

S-Adenosylmethionine decarboxylase was assayed in a manner similar to the ornithine decarboxylase assay. The homogenization medium consisted of 100 mM-sodium phosphate, pH 7.0, 10 mM-EDTA and 0.25 M-sucrose. The assay contained 100 mM-sodium phosphate, pH 7.0, 0.04 mM-pyridoxal phosphate and 0.1 µCi of S-adenosyl-L-[1-¹⁴C]methionine (51.2 mCi/mmol; ICN) with sufficient S-adenosyl-L-methionine added to yield a final substrate concentration of 0.2 mM.

Results and Discussion

Considerable ornithine decarboxylase activity was detected in larvae under the above assay conditions. The enzyme appeared to be stabilized by reducing conditions, since the addition of 5 mM-dithiothreitol

Fig. 1. Kinetics of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase reactions in *Drosophila* larvae

The ornithine decarboxylase assay (a) contained 0.1 ml of supernatant from homogenates of 72 ± 3 h *Drosophila* larvae in a total volume of 0.25 ml of assay medium (42 mM-Tris/HCl, pH 7.1, 0.04 mM-pyridoxal phosphate, 5 mM-dithiothreitol and 0.1–1.5 mM-L-ornithine with a constant specific radioactivity of $0.5 \mu\text{Ci}$ of DL-[1- ^{14}C]ornithine/ μmol). The *S*-adenosylmethionine decarboxylase assays (b) contained 0.1 ml of supernatant from a homogenate of 72 ± 6 h larvae in 0.25 ml of assay medium (100 mM-sodium phosphate buffer, pH 7.0, 0.04 mM-pyridoxal phosphate and variable substrate at a constant specific radioactivity of $0.5 \mu\text{Ci}$ of *S*-adenosyl-L-[1- ^{14}C]methionine/ μmol) and (c) contained 0.1 ml of supernatant from homogenates of 40 ± 3 h larvae in a total volume of 0.25 ml of assay medium supplemented with 0–2.0 mM-putrescine.

to the assay and homogenization medium increased the activity 2–3-fold. Similar observations have been made about the enzyme from *Cecropia* (Wyatt *et al.*, 1973) and rat prostate (Jänne & Williams-Ashman, 1971). As in these tissues, *Drosophila* ornithine decarboxylase has a pyridoxal phosphate requirement.

The variation in enzyme activity from 72 h larvae with increasing L-ornithine concentration was determined. An L-ornithine concentration of 1 mM was required to saturate the enzyme (Fig. 1) and the apparent K_m for the enzyme was determined to be 0.27 mM by double-reciprocal plots. The K_m for *Cecropia* ornithine decarboxylase was found to be 0.2 mM (Wyatt *et al.*, 1973). Variable *S*-adenosylmethionine decarboxylase activity was also present in *Drosophila* larvae. Phosphate buffer was used during homogenization and assay because Tris/HCl was found to inhibit enzyme activity by 30–40%. This has also been observed with *S*-adenosylmethionine decarboxylase from the rat prostate (Pegg & Williams-Ashman, 1969). Under the conditions of the assay used, enzyme activity was linear with respect to incubation time and protein concentration.

The variation in *S*-adenosylmethionine decarboxylase activity with increasing substrate concentration was determined (Fig. 1). The saturating concentration of substrate was 0.2 mM and the apparent K_m was 0.035 mM. This compares very well with the K_m for the

enzyme in rat tissues: 0.035 mM for rat liver (Feldman *et al.*, 1972), 0.05 mM for rat prostate (Pegg & Williams-Ashman, 1969) and 0.052 mM for rat brain (Schmidt & Cantoni, 1973).

Putrescine has been shown to stimulate the activity of *S*-adenosylmethionine decarboxylase purified from rat prostate (Pegg & Williams-Ashman, 1969) and from rat liver (Feldman *et al.*, 1972); 2 mM-putrescine was found to increase the enzyme activity in 40 h larvae 15–20-fold (Fig. 1). Putrescine concentrations of up to 8.0 mM did not yield any greater increase. The apparent K_m for putrescine was determined to be 0.25 mM, which compared with the K_m of 0.33 mM calculated for rat liver *S*-adenosylmethionine decarboxylase (Feldman *et al.*, 1972).

Drosophila larvae aged 24–96 h were assayed for decarboxylase activity (Table 1). Ornithine decarboxylase activity was very high in 24 h larvae occurring at more than 10-fold the enzyme activity reported for *Cecropia* pupae after injection of ecdysone (Wyatt *et al.*, 1973). The enzyme activity was still elevated in 48 h larvae, but by 72 h had fallen to 20–25% of its 24 h value at which it remained in 96 h larvae.

S-Adenosylmethionine decarboxylase activity assayed without exogenous putrescine was also greatest in 24 and 48 h larvae and fell by 30–40% in 72 and 96 h *Drosophila* larvae (Table 1). The putrescine-stimulated enzyme activity was 7–20 times the activity in the absence of putrescine and similar to

Table 1. Variation in ornithine decarboxylase and *S*-adenosylmethionine decarboxylase activities during the development of *Drosophila* larvae

Drosophila larvae of the indicated ages (± 4 h) were washed, homogenized, and the enzyme activities were assayed as indicated in the text. Activity is expressed as pmol of CO₂/h per μ g of protein. *S*-Adenosylmethionine decarboxylase activity was determined in the presence and in the absence of 2mM-putrescine.

Age (h)	Ornithine decarboxylase activity	<i>S</i> -Adenosylmethionine decarboxylase activity	
		–Putrescine	+Putrescine
24	7.27	0.46	0.59
48	5.21	0.50	10.02
72	1.71	0.18	1.30
96	1.79	0.21	1.60

the high *S*-adenosylmethionine decarboxylase activity observed in the sea urchin, *Strongylocentrotus purpuratus* (Manen & Russell, 1973).

Spermidine concentrations are highest in young larvae and decrease 6–7-fold between 24 and 96h of development (Herbst & Dion, 1970). On the other hand, the concentration of putrescine is maintained at a low concentration throughout larval development. The observation that both decarboxylase activities were highest during early development and lowest in the older larvae suggests that the synthesis of putrescine and spermidine are co-ordinately controlled. Since putrescine concentrations are relatively low at 24–48h when ornithine decarboxylase activity is high, the size of the putrescine pool is probably limited by its utilization for spermidine synthesis.

S-Adenosylmethionine decarboxylase activity in 24h larvae was not stimulated by putrescine to the same extent as that in the older larvae (Table 1). The enzyme activity was only 30% higher in the 24h larvae when assayed in the presence of 2mM-putrescine. Unlike the *S*-adenosylmethionine decarboxylase in tissues of the rat (Pegg & Williams-Ashman, 1969; Feldman *et al.*, 1972), the enzyme activity in *Physarum polycephalum* is not stimulated by putrescine at any stage of development (Mitchell & Rusch, 1973). *Drosophila* larvae, however, show a differential or biphasic stimulation of *S*-adenosylmethionine decarboxylase by putrescine, possibly indicative of the presence of isoenzymes in different developmental stages. This suggests that, in early stages of development, maximum rates of spermidine

synthesis could be attained in spite of the low concentrations of putrescine available in the larvae. The maintenance of a high cellular content of spermidine could be a significant factor in the rapid synthesis of RNA during early development (Herbst *et al.*, 1973).

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