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Arginine Decarboxylase and Putrescine Oxidase in Ovaries of *Pisum sativum* L.[1]

Changes during Ovary Senescence and Early Stages of Fruit Development

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Enzymatic activities involved in putrescine metabolism in ovaries of *Pisum sativum* L. during ovary senescence and fruit set were investigated. Accumulation of putrescine was observed during incubation of extracts from gibberellic acid-treated unpollinated ovaries (young developing fruits) but not in extracts from untreated ovaries (senescent ovaries). Extracts from pea ovaries showed arginine decarboxylase (ADC) activity, but ornithine decarboxylase and arginase activity were not detected. ADC activity decreased in presenescent ovaries and increased markedly after induction of fruit set with gibberellic acid. Increases in ADC activity were also observed with application of other plant growth substances (benzyladenine and 2,4-dichlorophenoxyacetic acid), after pollination, and in the slender (la cry^s) pea mutant. By contrast, putrescine oxidase activity increased in presenescent ovaries but did not increase during early fruit development. All of these results suggest that ADC and putrescine oxidase are involved in the control of putrescine metabolism. Ovary senescence is characterized by the absence of putrescine biosynthesis enzymes and increased levels of putrescine oxidase and fruit development by an increase in ADC and a constant level of putrescine oxidase.

Several lines of evidence have implicated a role for polyamines in fruit development (Evans and Malmberg, 1989; Egea-Cortines and Mizrahi, 1991). Evidence comes from: (a) experiments of exogenous application of polyamines during fruit set or initial steps of fruit development, (b) changes in their endogenous levels during that process, (c) changes in activity of enzymes involved in polyamine biosynthesis, and (d) experiments with specific inhibitors of biosynthetic enzymes. During initial steps of fruit development changes in the activity of ODC (EC 4.1.1.17) and/or ADC (EC 4.1.1.19), enzymes involved in putrescine biosynthesis, have been found in tomato (*Lycopersicon esculentum*) (Heimer et al., 1979; Cohen et al., 1982; Teitel et al., 1985), *Phaseolus vulgaris* (Palavan and Galston, 1982), mandarin orange Citrus reticulata (Nathan et al., 1984), tobacco (*Nicotiana tabacum*) (Slocum and Galston, 1985), avocado (*Persea americana*) (Apelbaum, 1986; Kushad et al., 1988), and apple (*Malus domestica*) (Biasi et al., 1991).

In tomato and tobacco, a correlation between increases in ODC activity and cell division (or active growth) has been observed, suggesting that ODC is the primary enzyme in the regulation of putrescine biosynthesis during initial fruit growth. A clear role for ADC has still to be identified, however. In general, it has been suggested that changes in ODC may regulate cell division in actively growing tissues, whereas ADC may regulate cell extension and secondary metabolic processes such as alkaloid biosynthesis (Tiburcio et al., 1990). Putrescine levels are not only dependent on ADC and ODC but also on other metabolic pathways and transport. Putrescine levels can also be affected by (a) the activity of enzymes that regulate the availability of Orn and Arg as substrates, such as L-Arg amidinohydrolase (arginase, EC 3.5.3.1) or OTC (EC 2.1.3.3), which can supply Orn from Arg or vice versa, (b) catabolic enzymes, mainly putrescine oxidase (EC 1.4.3.10), (c) interconversion of free putrescine with its conjugates, and (d) utilization of putrescine as substrate for spermidine biosynthesis and for secondary metabolism, such as polyamine-derived alkaloids. With regard to fruit development some work has been done on the determination of conjugated polyamines (Egea-Cortines et al., 1993), but putrescine catabolism has received no attention.

In unpollinated pea (*Pisum sativum* L.) ovaries, the total amount of endogenous polyamines (i.e. putrescine, spermidine, and spermine) increases after GA_3 treatment but remains nearly constant or decreases slightly in untreated ovaries (Carbonell and Navarro, 1989). Development of unpollinated pea ovaries shows two well-defined steps. The first is characterized by continuous growth, with an increase in length and fresh weight, that ends around 2 d p.a. The second is characterized by a senescence process that shows degenerative alterations in endocarp and mesocarp structures (Vercher et al., 1987; Vercher and Car-

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Abbreviations: ADC, arginine decarboxylase; DFMA, α-DL-difluoromethylarginine; DFMO, α-DL-difluoromethylornithine; ODC, ornithine decarboxylase; OTC, ornithine transcarbamylase; p.a., post anthesis; PCA, perchloric acid.

bonell, 1991). Treatment of the ovaries with plant growth regulators (i.e. auxin, GA, and cytokinin) during the first step induces parthenocarpic fruit development (García-Martínez and Carbonell, 1980), although only GA_3 treatment results in fruits with final size and shape that are nearly identical to fruits generated by pollination of the ovaries (Carbonell and García-Martínez, 1985). In pea ovaries, cell division apparently stops before the end of its growth, and fruit growth is mainly sustained by enlargement of the mesocarp and differentiation of the endocarp (Vercher et al., 1984, 1987).

Previous work showed an increase in the total amount of putrescine, spermidine, and spermine in pea ovaries after induction of fruit development with plant growth regulators (Carbonell and Navarro, 1989) and a high OTC/carbamoyl phosphate synthetase ratio, indicative of active Arg synthesis (García-España et al., 1989). In this work, we have investigated the role of some enzymes of putrescine metabolism, including ADC, ODC, arginase, and putrescine oxidase, and of putrescine conjugates in early stages of fruit development and during ovary senescence in peas.

MATERIALS AND METHODS

Plant Material

Ovaries and Fruits

Pea (*Pisum sativum* L. cv Alaska) and slender mutant (la cry^s) plants were grown as previously described (Carbonell and García-Martínez, 1985). Only the first and/or the second flowers in each plant were used for experiments. Unpollinated ovaries were obtained by emasculation of the flowers (petals and stamens were removed) 2 d before anthesis. Parthenocarpic fruit development of unpollinated ovaries was induced by application of 20 μL of 0.33 mM GA_3 (Fluka, Buchs, Switzerland), 0.44 mM BA (Fluka), or 0.045 mM 2,4-D (Fluka). All the solutions contained 0.1% Tween 80. Treatments were carried out on the day of anthesis (d 0) or 2 d p.a. Ovaries and fruits were collected at different stages of development, weighed, and immediately homogenized for enzymatic assays or frozen in liquid nitrogen and stored at −80°C for determination of conjugated polyamines.

Germinating Seeds and Growing Roots

Pea cv Alaska seeds were washed with 1% (w/v) NaClO, rinsed several times with deionized water, and germinated in darkness in Petri dishes containing moist filter paper. Germinating seeds and growing roots were collected either 2 or 4 d after germination, respectively, and used immediately for extraction of enzymatic activities.

Incubation of Pea Ovary Extracts

GA_3-treated and untreated ovaries were homogenized at 4°C in 5 volumes of 100 mM potassium phosphate buffer, pH 7.0, containing 10 mM β-mercaptoethanol, 10 μM leupeptin, 150 μM pyridoxal phosphate, and 50 μg mL^{-1} 1,6-diaminohexane dihydrochloride (Aldrich, Buchs, Switzerland) as internal standard for putrescine quantification. Homogenates were centrifuged at 15,000*g* for 10 min at 4°C. Supernatants were used directly for incubation at 30°C. Additions of DFMA or DFMO (Marion Merrell Dow, Inc., Cincinnati, OH) were made just before incubation. Addition of radiolabeled substrates was as follows: 0.6 μCi of L-[U-^{14}C]Arg (Amersham, 305 mCi mmol^{-1}) or 0.5 μCi of L-[U-^{14}C]Orn (New England Nuclear, 257 mCi mmol^{-1}) in a final volume of 270 μL. At different times, 50-μL aliquots were removed, mixed with 1 volume of cold 0.4 N $HClO_4$, and centrifuged at 15,000*g* for 10 min at 4°C. Putrescine in the supernatant was derivatized and quantified by HPLC as described below.

Extraction and Assay for ADC and ODC

Ovaries or roots were homogenized in 5 to 10 volumes of 150 mM Tris-HCl, pH 8.0, with 10 mM β-mercaptoethanol, 10 μM leupeptin, and 150 μM pyridoxal phosphate and centrifuged at 20,000*g* for 15 min at 4°C. The supernatant was used for ADC and ODC assays. ADC and ODC activities were assayed according to Birecka et al. (1985) by measuring the labeled CO_2 liberated from L-[U-^{14}C]Arg (Amersham, 305 mCi mmol^{-1}) and L-[1-^{14}C]Orn (Amersham, 56 mCi mmol^{-1}), respectively. One hundred microliters of enzymatic extract were mixed with 50 μL of substrate (including 0.10–0.20 μCi of labeled compound) to give a final concentration of 10 mM Arg or 50 mM Orn. The amount of protein was 100 to 250 μg per assay. The reaction was carried out at 30°C for 40 min in tapered vials bearing a Whatman No. 1 paper disk impregnated with 20 μL of 4 N KOH. The reaction was stopped by injection of 200 μL of cold 15% (w/v) TCA. After 30 min at 30°C the vials were opened, filters were air dried, and radioactivity was determined in a liquid scintillation counter. All assays were duplicated. Controls were carried out using either homogenization buffer or TCA-treated extracts instead of enzymatic extracts. Reactions progressed linearly for 1 h and for protein concentration.

Extraction and Assay for Arginase

Arginase activity was extracted and assayed colorimetrically as described by Ruiter and Kollöffel (1982) by measuring the urea released in an assay mixture containing 225 mM L-Arg and 2 mM $MnCl_2$. Extracts were preactivated for 10 min at room temperature, and reactions were carried out for 30 min at 25°C. Assays were triplicated for pea extracts as well as for urea standards.

Extraction and Assay for Putrescine Oxidase

Putrescine oxidase activity was determined by a modification of the method of Okuyama and Kobayashi (1961) according to Flores and Filner (1985), using [1,4-^{14}C]putrescine. Pea ovaries were homogenized at 4°C in 7 to 15 volumes of 100 mM potassium phosphate buffer, pH 7.0, with 1 mM DTT, 2% (w/v) cross-linked PVP, and 2 mM EDTA. Homogenates were centrifuged at 20,000*g* for 10 min at 4°C. Forty microliters of the supernatant were mixed with 240 μL of homogenization buffer (without PVP), 10

μL of catalase (5 μg μL^{-1}), 6 μL of 50 mM putrescine, and 3 to 5 μL of [1,4-^{14}C]putrescine (0.15–0.25 μCi, Amersham, 108 mCi $mmol^{-1}$). Protein amount was 50 to 100 μg per assay. Incubations were carried out for 20 min at 37°C and stopped with 300 μL of 4 N NaOH. Δ-[^{14}C]Pyrroline was extracted with 1 mL of toluene. Two aliquots of 200 μL were recovered, and radioactivity was measured as described above. All assays were duplicated. Controls were carried out using homogenization buffer. Reactions progressed linearly for 40 min and for protein concentration.

Free and Conjugated Putrescine Determination

Putrescine was separated by dansylation of extracts and HPLC chromatography and quantified using 1,6-diaminohexane as internal standard following the procedure previously described (Carbonell and Navarro, 1989). For determination of [^{14}C]putrescine, a flow-scintillation counter was used after fluorimeter detection. Conjugated putrescine was measured after acid hydrolysis of pea ovary extracts for 24 h at 110°C according to Tiburcio et al. (1985).

Protein Determination

Protein concentration in the enzymatic extracts was determined according to Bradford (1976) using BSA fraction V (Sigma) as standard.

RESULTS

Free and Conjugated Putrescine in Unpollinated Pea Ovaries

Figure 1 shows the time course of free and PCA-soluble conjugated putrescine content in untreated and GA_3-treated unpollinated ovaries. No significant amounts of PCA-soluble conjugated putrescine were detected, and a slight decrease in free putrescine was detected in untreated, unpollinated ovaries during the presenescence (d 1 and 2 p.a.) and senescence of the ovaries (d 3 and 4 p.a.). After treatment of unpollinated ovaries with GA_3, a rapid and significant increase in the amount of free putrescine was observed during early parthenocarpic fruit development. No significant amounts of conjugated putrescine were detected 24 h after the treatment, and only a low amount (20% of total putrescine) could be detected 48 h after the treatment. No significant amounts of PCA-insoluble conjugated putrescine were detected in any of the samples (data not shown).

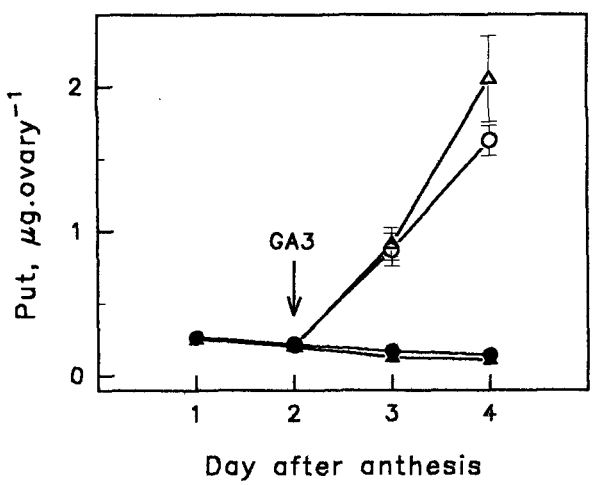

Figure 1. Time course of free putrescine in GA_3-treated (○) and untreated (●) unpollinated ovaries and free plus PCA-soluble conjugated putrescine in GA_3-treated (△) and untreated (▲) unpollinated ovaries. Mean ± SE, $n = 5$.

Table I. *Effect of inhibitors of ADC and ODC activity on the accumulation of putrescine in extracts from GA_3-treated ovaries*

Unpollinated ovaries were treated on d 2 and collected on d 3 p.a. Values represent the level of putrescine produced after 45 min of incubation in the presence of different concentrations of DFMA and DFMO, inhibitors of ADC and ODC activity, respectively. The concentration of putrescine at time 0 was 12 ± 1 μg g^{-1} fresh weight. Values are from a single experiment. Mean ± SE, $n = 3$.

Inhibitor Concentration	Putrescine			
	DFMA		DFMO	
mM	*μg g^{-1} fresh weight*	*%*	*μg g^{-1} fresh weight*	*%*
0	150 ± 7	100	150 ± 7	100
2	54 ± 5	36	139 ± 6	93
4	46 ± 2	31	138 ± 10	92
6	36 ± 3	24	134 ± 6	89
8	33 ± 2	22	147 ± 9	98

Synthesis of Putrescine in Incubated Extracts from Pea Ovaries

Accumulation of putrescine was observed during the incubation of extracts from GA_3-treated unpollinated pea ovaries (approximately 200 μg h^{-1} g^{-1} fresh weight), whereas no change in the amount of putrescine was observed in extracts from untreated ovaries (approximately 10 μg g^{-1} fresh weight). The production of putrescine in extracts from GA_3-treated ovaries was significantly reduced when incubation was carried out in the presence of 2 mM DFMA, an inhibitor of ADC activity (Table I). The presence of DFMO, an inhibitor of ODC activity, did not affect the production of putrescine even at relatively high concentrations. Synthesis of radiolabeled putrescine was observed when L-[U-^{14}C]Arg was added to the extract, but no labeled putrescine was detected from L-[U-^{14}C]Orn (Fig. 2). The synthesis of radiolabeled putrescine was also significantly inhibited (approximately 97%) by 2 mM DFMA.

ADC, ODC, and Arginase

Extracts from GA_3-treated and untreated, unpollinated pea ovaries showed a large difference in ADC activity at d 3 p.a. (Table II). ADC activity was 10 times greater in the former. Around 90% of the activity in extracts of GA_3-treated ovaries was inhibited by 2 mM DFMA and was not affected by DFMO. In extracts from untreated ovaries the low level of ADC activity was not inhibited either by 2 mM DFMA or by DFMO (data not shown). The K_m value for L-Arg was 1.5 mM. For the determination of K_m, the high

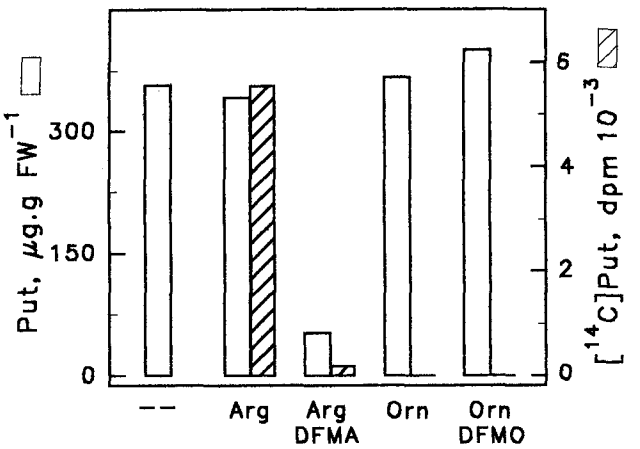

Figure 2. Synthesis of [^{14}C]putrescine from labeled substrates and effect of inhibitors of ADC and ODC activity in incubated (40 min at 30°C) extracts of GA_3-treated unpollinated ovaries. Ovaries were treated on d 2 and collected on d 3 p.a. – –, No additions; Arg, 0.6 μCi L-[U-^{14}C]Arg; Arg DFMA, 0.6 μCi L-[U-^{14}C]Arg plus 2 mM DFMA; Orn, 0.5 μCi L-[U-^{14}C]Orn; Orn DFMO, 0.5 μCi of L-[U-^{14}C]Orn plus 2 mM DFMO. The concentration of putrescine at time 0 was 30 μg g^{-1} fresh weight. Values are from a single experiment.

level of endogenous Arg in the extract, approximately 0.8 mM (V. Rubio, unpublished data), was taken into account. Optimum pH showed a broad range between 7 and 8. When pyridoxal phosphate was not included in the extraction buffer, the presence of 0.1 mM pyridoxal phosphate in the mixture assay enhanced ADC activity by 45%. ODC and arginase activities were not detected in extracts of pea ovaries, but assay with the same protocols showed ODC activity in young roots after 4 d of germination, and arginase showed activity in seeds after 2 d of germination (Table II).

Putrescine Oxidase

Putrescine oxidase activity was detected in GA_3-treated and in untreated, unpollinated ovaries (Table II). Activity was inhibited approximately 70% in the presence of 1 mM aminoguanidine, a competitive inhibitor of diamine oxidase, in assays that were not preincubated (Yanagisawa et al., 1981). The addition of 1 mM NAD^+ after 20 min of incubation effected a decrease in Δ-[^{14}C]pyrroline. This result indicates the presence of pyrroline dehydrogenase activity in GA_3-treated and in untreated, unpollinated pea ovaries. This enzyme catalyzes the conversion of Δ-[^{14}C]pyrroline into γ-[^{14}C]aminobutyric acid (Flores and Filner, 1985). Accumulation of this latter compound was not tested.

Changes in ADC and Putrescine Oxidase Activity in Unpollinated Ovaries and in Developing Fruits

The weight of unpollinated pea ovaries did not show large changes p.a. However, treatment of unpollinated ovaries with GA_3, either at d 0 or at d 2 p.a., promoted dramatic increases in their weight, associated with parthenocarpic fruit development (Fig. 3A). The total amount of ADC activity in untreated, unpollinated pea ovaries showed a decrease that resulted in a very low value at d 2 p.a. This low value was nearly constant in senescent ovaries and was not inhibited by 2 mM DFMA (Fig. 3B). However, in GA_3-treated ovaries ADC activity increased transiently during the first step of fruit growth and showed a peak at d 3 or 4 p.a. for treatments made on d 0 or 2 p.a., respectively. The increase was observed independently of the day of treatment but was higher for ovaries treated on d 0 rather than on d 2 p.a.

The time course for the total amount of putrescine oxidase activity in untreated, unpollinated ovaries showed an increase in presenescent ovaries with a maximum at d 2 p.a. When ovaries entered into senescence, the activity decreased (Fig. 3C). In GA_3-treated ovaries, putrescine oxidase activity increased after the treatment. The profiles of changes in putrescine oxidase activity and weight of the ovaries and fruits were similar to each other (Fig. 3, A and C) and different from that of changes in ADC activity (Fig. 3B).

In untreated, unpollinated ovaries, protein concentration showed a broad peak in presenescent ovaries (between d 0

Table II. *Activity of some enzymes involved in putrescine biosynthesis and catabolism in different pea organs*

Unpollinated ovaries were treated on d 2 p.a. GA_3-treated and untreated ovaries were collected on d 3 p.a. Germinated seeds and roots were used 2 and 4 d after germination, respectively. Values are expressed in nmol h^{-1} mg^{-1} protein. Mean ± SE, $n \geq 3$.

Activity	Plant Organ			
	Ovary		Germinated seed	Root
	Untreated	GA_3 treated		
ADC	59 ± 32	611 ± 87	—[a]	—
ODC	<1	<1	—	23 ± 5
Arginase	<75	<75	322 ± 125	—
Putrescine oxidase	322 ± 16	220 ± 20	772 ± 20	—

[a] —, Not assayed.

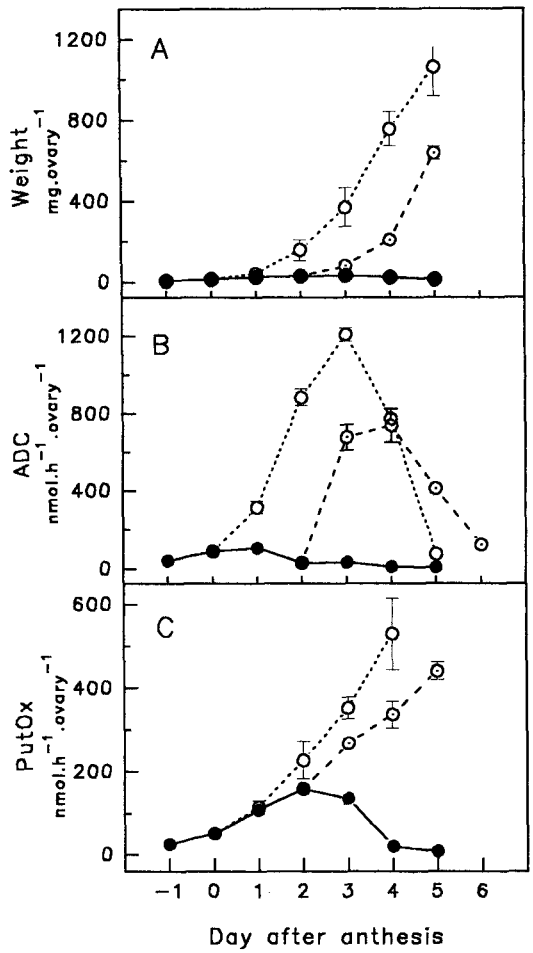

Figure 3. Time course of weight (A), ADC activity (B), and putrescine oxidase activity (C) of untreated and GA_3-treated unpollinated ovaries. ●, Untreated ovaries; ○, GA_3-treated ovaries on d 0 p.a.; ⊙, GA_3-treated ovaries on d 2 p.a. Mean ± SE, $n \geq 3$.

and 2 p.a.) and a marked decrease in senescent ovaries (d 3 and 4 p.a.) (Fig. 4A). In presenescent ovaries, ADC specific activity decreased to a very low level and no further change was observed in senescent ovaries (Fig. 4B). GA_3 treatment of the ovaries resulted in a decrease in protein concentration when the treatment was carried out on d 0 and had no effect (compared with untreated ovaries) on d 2 p.a. By contrast, ADC specific activity showed a slight transient increase after treatment on d 0 and a sharp increase after treatment on d 2 p.a. Significant increases in specific activity could be detected as early as 2 h after the treatment (data not shown), just when the first increase in pea fruit length induced by treatment could also be detected (Estruch and Beltrán, 1991).

The specific activity of putrescine oxidase increased in untreated ovaries until the beginning of senescence and decreased after d 3 p.a. once senescence was established (Fig. 4C). In GA_3-treated ovaries the specific activity showed an effectively constant level.

When parthenocarpic fruit development was induced by treatment of the unpollinated ovaries with other plant growth substances, such as BA or 2,4-D (Fig. 5), the level of ADC activity increased in a manner similar to that seen with GA_3 in the first 12 h. However, 24 h after the treatment, a higher activity was observed in GA_3-treated ovaries compared with the other treatments. This may be associated with differences in ovary length and weight. ADC activity was also measured in self-pollinated Alaska ovaries as well as in the slender mutant (Table III). This mutant, which shows a saturated response to GAs, produces parthenocarpic fruits. ADC activity increased during the first 2 d p.a. in a manner similar to that observed with Alaska wild-type peas. At d 2 p.a., ADC specific activity was very similar in both Alaska and slender pea fruits.

DISCUSSION

Metabolism of Putrescine in Pea Ovaries and Young Fruits

Investigations of the pathways that could affect the metabolism of putrescine in pea ovaries indicate that its biosynthesis is via ADC, that putrescine oxidase is involved in

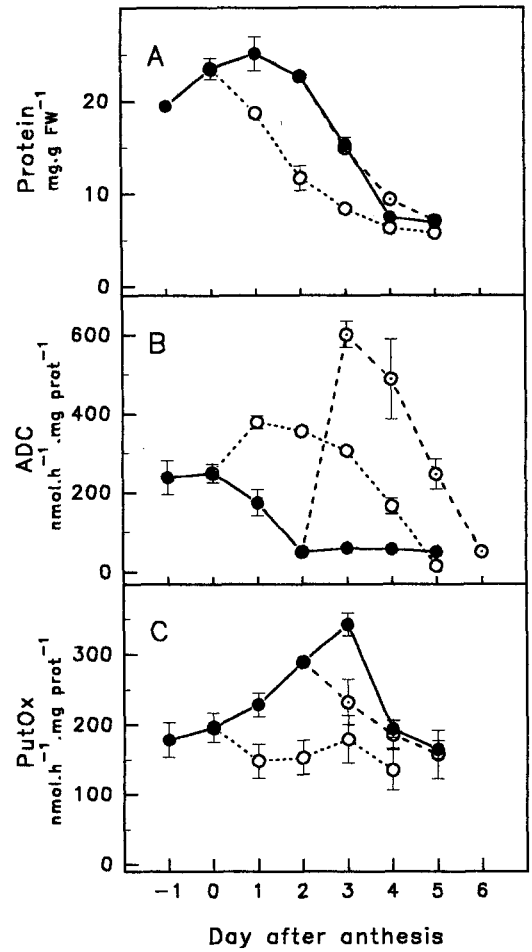

Figure 4. Time course of protein (A), ADC specific activity (B), and putrescine oxidase specific activity (C) of untreated and GA_3-treated unpollinated ovaries. ●, Untreated ovaries; ○, GA_3-treated ovaries on d 0 p.a.; ⊙, GA_3-treated ovaries on d 2 p.a. Mean ± SE, $n \geq 3$.

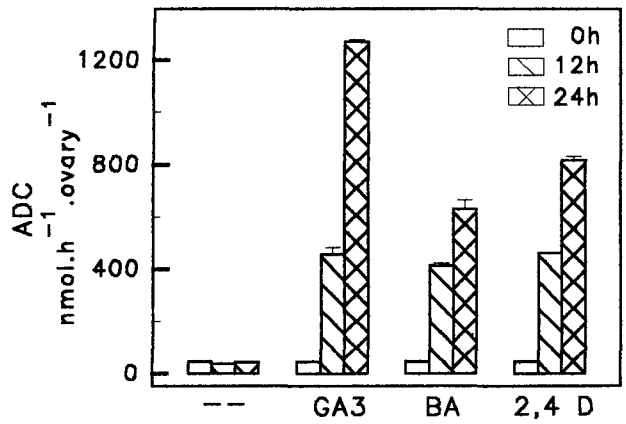

Figure 5. ADC activity in unpollinated ovaries after treatment with different plant growth substances. Unpollinated ovaries were treated on d 2 p.a. Treated and untreated ovaries were collected 12 and 24 h later (d 3 p.a.). – –, Untreated ovaries. Weights of untreated ovaries and ovaries treated with GA_3, BA, and 2,4-D, collected at d 3 p.a., were 38, 113, 76, and 105 mg ovary^{-1}, respectively. Values are from a single experiment.

its catabolism, and that interconversion with conjugates is not significant.

The absence of ODC is correlated with the absence of cellular division during fruit development. As described by Vercher et al. (1984), pea fruit development was due mainly to an enlargement of mesocarp cells, which are about 80% of the pod volume, and differentiation of endocarp cells. The absence of ODC and the low level of putrescine in pea fruits, compared with spermidine and spermine (Carbonell and Navarro, 1989), contrasts with the presence of ODC and the high level of free and conjugated putrescine in tomato fruits (Egea-Cortines et al., 1993). This difference between pea and tomato fruits is correlated with differences in their development; in peas no cell divisions are associated with fruit growth, whereas in tomato active cell division is associated with rapid fruit growth (Heimer et al., 1979; Teitel et al., 1985). The absence of arginase, the high Arg/Orn ratio, and the high activity of OTC (García-España et al., 1989) can be correlated with the role of Arg as source of nutrients for seed development in pea pods (Pate and Flinn, 1977). In pea ovaries and fruits putrescine is mostly in the free form. No significant amounts of PCA-soluble and -insoluble conjugates were detected. In developing fruits, the relative amount of putrescine conjugates reached 20% of the total amount of putrescine, indicating that conjugates are neither a source for putrescine at very early stages of fruit growth nor a major pathway in its catabolism.

ADC and Ovary Senescence/Fruit Development

Increases in ADC and ODC activity have been detected in early stages of fruit development in several species (reviewed by Egea-Cortines and Mizrahi, 1991). All of the reports show the presence of both activities except in avocado (Apelbaum, 1986). The ratio of ADC/ODC varies considerably according to the species, tissue, and physiological state (Smith, 1985), so it is difficult to identify the role of both enzymes in fruit development. However, it has been reported that high ODC activity is associated with fruit growth and active cell divisions, as in tomato (Heimer, 1979), tobacco (Slocum and Galston, 1985), and avocado (Kushad et al., 1988). Similar patterns for ODC and ADC were found in pear by Biasi et al. (1991), and values ranging between 2 and 8 were reported for ADC/ODC ratio in mandarin orange in early fruit growth (Nathan et al., 1984). Biasi et al. (1991) and Teitel et al. (1985) also associated low ADC and/or ODC activity with lack of cell division and with fruit growth due to cell enlargement. Our results show that in pea ovaries, senescence is associated with an absence of putrescine biosynthetic enzymes and that the increase in fruit growth rate is linked to an increase in ADC activity. As discussed before, no cell divisions are involved in pea fruit growth.

Putrescine Oxidase

To the best of our knowledge, no work on the presence of putrescine oxidase in fruits has been reported. The changes observed in putrescine oxidase and ADC in ovary senescence and pea fruit development suggest a coordination in the level of biosynthetic and catabolic enzymes of putrescine. At the beginning of ovary senescence, a lack of putrescine biosynthetic enzymes, a high putrescine oxi-

Table III. *ADC activity and weight of pea ovaries at early stages of fruit development in Alaska and in slender la cry^s mutant*

Flowers of Alaska were not manipulated and fruit set was induced by self-pollination. Flowers of slender mutant were emasculated 2 d before anthesis.

	Alaska			Slender (la cry^s)		
	Activity	Activity	Weight	Activity	Activity	Weight
	nmol h^{-1} mg^{-1} protein	*nmol h^{-1} ovary^{-1}*	*mg ovary^{-1}*	*nmol h^{-1} mg^{-1} protein*	*nmol h^{-1} ovary^{-1}*	*mg ovary^{-1}*
Day −1	—[a]	—	—	112 ± 5	20 ± 1	9.3
Anthesis	174 ± 11	58 ± 4	12.9	—	—	—
Day +1	395 ± 2	215 ± 1	21.4	250 ± 6	181 ± 5	20.4
Day +2	420 ± 20	446 ± 21	71.2	421 ± 16	1242 ± 48	120.8

[a] —, Not assayed.

dase, and the presence of pyrroline dehydrogenase activity suggest the utilization of putrescine for recycling C and N. Succinate and Ala would be synthesized from γ-aminobutyric acid and pyruvate (Flores and Filner, 1985). In early fruit development, putrescine oxidase specific activity is restored to a constant level, while a sharp increase in ADC is observed. Increases in the total amount of spermidine and spermine observed in fruit development induced by GA_3 (Carbonell and Navarro, 1989) suggest that putrescine might be used as substrate for spermidine biosynthesis. S-Adenosylmethionine decarboxylase has been detected in pea seedlings (Icekson et al., 1985), but spermidine synthase activity remains to be demonstrated in peas.

GAs and ADC Activity in Peas

Effects of GA_3 on ADC activity in peas were described by Dai et al. (1982). GA_3 treatment induced a marked increase in ADC activity in light-grown dwarf Progress pea seedlings and a reversal of the red-light-induced inhibition of ADC activity in etiolated Alaska pea epicotyls. These results and the absence of effect on ODC activity suggested a role for GAs in the control of polyamine biosynthesis via ADC activity but no relationship between ODC and growth rate in peas. The reversal of photoinhibition of elongation in Alaska and the promotion of elongation in Progress pea would be dependent on the same mechanism. As described before, our results show a similar pattern for changes in ADC activity during the induction of cell enlargement by GA_3. In different tissues of peas, it can be concluded that one of the effects of exogenous GAs is to induce increases in ADC activity that precede enlargement of cells in actively growing tissues without cell division. Results in the slender pea mutant, which shows a saturated response to GAs, also support this conclusion.

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