Relation of Polyamine Biosynthesis to the Initiation of Sprouting in Potato Tubers

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ABSTRACT

The polyamines putrescine, spermidine, and spermine and their biosynthetic enzymes arginine decarboxylase, ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase are present in all parts of dormant potato (Solanum tuberosum L.) tubers. They are equally distributed among the buds of apical and lateral regions and in nonbuds tissues. However, the breaking of dormancy and initiation of sprouting in the apical bud region are accompanied by a rapid increase in ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities, as well as by higher levels of putrescine, spermidine, and spermine in the apical buds. In contrast, the polyamine biosynthetic enzyme activities and titer remain practically unchanged in the dormant lateral buds and in the nonbud tissues. The rapid rise in ornithine decarboxylase, but not arginine decarboxylase activity, with initiation of sprouting suggests that ornithine decarboxylase is the rate-limiting enzyme in polyamine biosynthesis. The low level of polyamine synthesis during dormancy and its dramatic increase in buds in the apical region at break of dormancy suggest that polyamine synthesis is linked to sprouting, perhaps causally.

The PA,2 Put, Spd, and Spm are present throughout the microbial, animal, and plant worlds (3, 7). In microbial and plant cells, Put is derived either from arginine via ADC and the intermediate Agm, or from ornithine by ODC. In mammalian cells, Put synthesis occurs only by the latter pathway. The Put thus formed is converted successively to Spd and Spm through propylamine group transfer from SAM mediated by SAMDC in all the above types of cells (3, 11). These amines have been implicated in several important processes involved in cell growth and development, especially those involving nucleic acids (3, 7, 11, 27).

Both the activity of PA biosynthetic enzymes and PA titer have been reported to increase dramatically during rapid growth in many plant systems, such as germinating seeds of Zea, Pisum, Triticum, and Tragopogon (30), developing seedlings of Phaseolus (4) and Lathythus (21, 22), ovaries of tomato, and rapidly dividing tobacco cells in suspension culture (12), during crown gall-tumor development (6) and embryogenesis of carrot suspension cells (18, 19). Although little is known about the activities of PA biosynthetic enzymes in dormant tissues such as tubers and senescencing plant parts, contents of PA in these systems are extremely low (1, 16). We have observed that activities of PA biosynthetic enzymes and PA titer decrease during aging and senescence of oat leaves (17). Furthermore, exogenous application of the PA or their precursors amino acids retards senescence of leaves in several monocotyledonous and dicotyledonous plants (16) and of protoplasts from oat leaves (2, 10, 14, 15). PA appear to inhibit senescence by preventing Chl, protein, and RNA breakdown in leaves (16) and by increasing macromolecular synthesis and mitotic activity in protoplasts (15). The antisenescence properties of PA and their correlation with cell proliferation and differentiation lend support to the contention that they act as growth factors (3, 5, 11). Despite these observations, the relative roles of individual PA biosynthetic enzymes in dormant and actively dividing plant tissues is not well understood. We have, therefore, examined the activities of ADC, ODC, and SAMDC and the endogenous levels of Put, Spd, and Spm in dormant and actively growing tissues of potato tubers.

MATERIALS AND METHODS

Plant Materials. Idaho Russet baking potatoes (Solanum tuberosum L.) (U.S. No. 1) were purchased from the local supermarket. The tubers were firm, with no sign of sprouting, and were therefore regarded as dormant. Medium-size tubers were selected and allowed to sprout by storing them in the dark at room temperature for about 2 months. Samples of tissue were taken from dormant, initial, and advanced (profuse) sprouted tubers. Three areas from the same tuber showing different sprouting activity were selected for sampling (Fig. 1): (A) bud tissue in the apical region (hereafter referred to as apical buds) which developed sprouts; (B) bud tissue from the lateral region (hereafter referred to as lateral buds) which remained dormant throughout the storage period; and (C) nonbud tissue which does not develop sprouts. Tissue sections (7 mm in diameter, 3 mm in length) containing the skin and outer cortex were removed. During the periods when sprouting occurred, the sprouts were excised with the apical bud tissue and assayed together. Six to eight tubers were used for each experiment and slices of each selected area were randomized. Suitable amounts were selected for determining ADC, ODC, and SAMDC activities and PA titer.

Extraction and Measurements

Polyamine Biosynthetic Enzymes. For each extraction, approximately 5 g (9 discs) of tissue was homogenized in chilled mortars with 1.5 ml of 100 mm phosphate buffer at pH 7.6. The homogenates were centrifuged at 26,000g for 15 min at 4°C and the resulting clear supernatant fractions were assayed for enzyme activities.

Enzyme assays were done by modification of conventional methods (8, 18, 24) in 12 × 75 mm polystyrene culture tubes sealed with polyethylene caps. A filter paper disc (6 mm diameter) impregnated with 50 μl 2 N KOH was supported on a 22-gauge syringe needle through the cap and used to trap the 14CO2 liberated. ADC activity was determined by measuring the release of 14CO2 from the substrate, l-[U-14C]arginine. The reaction mixture

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2 Abbreviations: PA, polyamine; Put, putrescine; Spd, spermidine; Spm, spermine; ADC, arginine decarboxylase; Agm, agmatine; ODC, ornithine decarboxylase; SAM, S-adenosyl-L-methionine; SAMDC, S-adenosyl-L-methionine decarboxylase.
Plant biologists measured the activity of ODC in dormant potato tubers. The activity was determined by measuring the rate of ODC activity, which was found to be lower in dormant tubers compared to sprouting tubers. This suggests that ODC activity is a key factor in the transition from dormancy to sprouting. The enzyme activities were measured using a spectrophotometric assay, and the results were analyzed using statistical software. The data showed that ODC activity increases significantly during the sprouting process, indicating that ODC is an important enzyme in this process. The findings have implications for understanding the molecular mechanisms of sprouting and could have applications in agricultural science.
higher activities of ODC than ADC in developing ovaries of tomato and in the exponential growth phase of tobacco cells in suspension cultures. In animal systems also, ODC is considered the key enzyme in PA biosynthesis (3, 7). Our observations with the potato tuber, together with those of Heimer et al. (12) suggest that either ODC or ADC can act as the rate-limiting enzyme for PA biosynthesis in different actively growing plant tissues.

Polyamine Titer. Measurements of PA levels show that Put, Spd, and Spm are present in tissues from dormant and sprouting potato tubers (Table I). Cadaverine was not detected either by HPLC or TLC. In dormant tubers, PA titer in apical buds, lateral buds, and nonbud tissues is essentially the same (Table I). However, at break of dormancy accompanying initiation of sprouting, PA in the apical buds increased sharply, and like the enzyme activities, continued to increase with progressive sprouting. Spm increased more than three times, Spd and Put about two times, and Put about 1.5 times the initial values at dormancy. At profuse sprouting, the increase in Spm titer was the highest. Levels of Spm in the apical buds increased by more than 10 times, while Spd and Put increased by about three and two times, respectively, when compared with the initial levels (Fig. 4). Furthermore, the HPLC profiles of PA show that peaks of the individual PA are well resolved and that PA titer increases with progressive sprouting.

Table 1. Polyamine Content in Progressive Stages of Sprouting Potato Tuber

<table>
<thead>
<tr>
<th>PA</th>
<th>Type of Tissue</th>
<th>Dormant</th>
<th>Initial Sprouting</th>
<th>Profuse Sprouting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agm</td>
<td>Apical buds</td>
<td>40.4 ± 2.1</td>
<td>65.3 ± 3.1</td>
<td>181.9 ± 8.7</td>
</tr>
<tr>
<td></td>
<td>Lateral buds</td>
<td>40.4 ± 2.1</td>
<td>50.6 ± 1.7</td>
<td>55.6 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>Nonbud tissue</td>
<td>20.8 ± 2.0</td>
<td>32.8 ± 1.7</td>
<td>37.8 ± 6.6</td>
</tr>
<tr>
<td>Put</td>
<td>Apical buds</td>
<td>4.5 ± 0.7</td>
<td>6.4 ± 0.1</td>
<td>9.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Lateral buds</td>
<td>4.5 ± 0.7</td>
<td>4.4 ± 0.3</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Nonbud tissue</td>
<td>6.0 ± 0.0</td>
<td>4.5 ± 0.2</td>
<td>4.7 ± 0.4</td>
</tr>
<tr>
<td>Spd</td>
<td>Apical buds</td>
<td>7.7 ± 1.7</td>
<td>14.5 ± 1.6</td>
<td>20.7 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Lateral buds</td>
<td>7.7 ± 1.7</td>
<td>9.6 ± 0.0</td>
<td>9.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Nonbud tissue</td>
<td>9.0 ± 0.4</td>
<td>8.5 ± 0.6</td>
<td>7.3 ± 0.1</td>
</tr>
<tr>
<td>Spm</td>
<td>Apical buds</td>
<td>1.0 ± 0.2</td>
<td>3.4 ± 0.5</td>
<td>10.2 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Lateral buds</td>
<td>1.0 ± 0.2</td>
<td>2.1 ± 0.0</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Nonbud tissue</td>
<td>0.6 ± 0.1</td>
<td>1.0 ± 0.3</td>
<td>2.3 ± 0.1</td>
</tr>
</tbody>
</table>

*±SE; n = 6 to 12.
ODC is the rate-limiting enzyme in Put synthesis in potato tubers. The high localized PA titer in advanced sprouting and the low titer in dormant tubers suggest that PA, like the well-known hormones ABA, gibberellins, and cytokinins, can act as growth factors. PA probably differ from hormones, however, in being relatively immobile intercellularly (Young and Galston, in preparation). ABA levels generally increase with dormancy, whereas gibberellins and cytokinins increase with break of dormancy. Such changes in endogenous levels of these hormones have been well established in seeds, and vegetative and flower buds, which like the potato tuber, undergo a period of dormancy. In the potato tuber also, dormancy is associated with low endogenous levels and sprouting with high levels of growth promoting hormones (13, 29). The increased PA synthesis during potato tuber sprouting observed here suggests that PA are also involved in the break of dormancy, perhaps as second messengers. The action of exogenous PA in preventing senescence of excised leaves (1, 16), protoplasts (2), and chloroplast thylakoids (20) support their proposed physiological role.

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