Disappearance of Porphobilinogen Deaminase Activity in Leaves Before the Onset of Senescence

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ABSTRACT

The activity of porphobilinogen deaminase was measured in young and senescent or mature leaves of pepper (Capsicum annuum), and poinsettia (Euphorbia pulcherrima). Whereas high activity was found in the crude extracts of the young leaves, almost no activity was found in the extracts of senescent or mature leaves. The decrease in deaminase activity was not due to the presence of an isolatable inhibitor. By purifying the crude enzyme extracts from leaves of different ages on DEAE-cellulose columns it was shown that the decrease in deaminase activity was due to a real decrease in the amount of enzyme. Fruiting also decreased porphobilinogen deaminase activity. Several kinetic constants of the C. annuum deaminase were determined.

MATERIALS AND METHODS

PBG was obtained by synthesis (9). All other reagents were commercial products of analytical grade. All of the solvents used were previously purified and distilled. DEAE-cellulose was obtained from Eastman Kodak and used after treatment by the method of Peterson and Sober (14).

Plant Material. Leaves from C. annuum L., grown either in a greenhouse or in the field, were collected. Leaves from E. pulcherrima Willd. grown in a garden were harvested in the morning and used within 30 min. When C. annuum leaves were used, the first and second leaves starting from the apex were called young leaves, and the first three leaves from the bottom upward were called senescent leaves. Equivalent leaves from several plants were used. None of the leaves were yellowing yet. Equivalent leaves from fruiting plants were also used. When E. pulcherrima leaves were used, the first and the second leaves from the top downward (small size and light green), were collected and called young leaves. The fourth and fifth leaves from the top downward (large size and dark green) were collected and called mature leaves. Equivalent leaves from several branches of the same tree were used.

Enzyme Preparation. The deribbed leaves were cut into small pieces, and thoroughly ground with a pestle in an ice-cold mortar until no fibrous residue was left. The grinding medium (3 ml/g fresh weight) consisted of a 10 mm Tris-HCl buffer solution (pH 7.4) and sand. The homogenate was filtered through several layers of nylon cloth, and centrifuged at 20,000 g for 15 min. All operations were performed at 4 C. The supernatant was used as enzyme source either for direct activity measurements, or for further enzyme purifications. No improvement of the PBG deaminase activity was found when the above mentioned grinding medium was supplemented with either 5 mm mercaptoethanol, PVP (5%, w/v) or nicotine (0.1%, v/v). Protein was estimated by the method of Lowry (13), and Chl concentration by the method of Arnon (1).

PBG Deaminase and Uroporphyrinogen III Cosynthase Assay. Unless otherwise indicated, the incubation mixture contained, in a final volume of 150 μl: 15 μmol phosphate buffer (pH 7.4), 13 nmol of PBG, and enzyme. When uroporphyrinogen III cosynthase was assayed, purified wheat germ PBG deaminase was added to the incubation mixture in order to form 0.7 to 1.0 nmol total uroporphyrin. Incubations were usually run at 37 C for 60 min; blanks omitting either enzyme or substrate were simultaneously run. PBG deaminase was assayed by either substrate (PBG) consumption, or product (uroporphyrinogen) formation. PBG was measured with Ehrlich's reagent (2% w/v p-dimethylaminobenzaldehyde in glacial acetic acid—HClO4 (84:16, v/v) at 552 nm (6). Uroporphyrinogens were estimated as uroporphyrins as described elsewhere (6). Uroporphyrin isomers were estimated by the method of Falk and Benson (5).

RESULTS

Effect of Aging and Fruiting on PBG Deaminase Activity. The effect of senescence or maturation on the activity of PBG deami-
nase in leaves of *C. annuum* and *E. pulcherrima* can be seen in Table I. In both, the highest activity was found in the young leaves. Almost no activity was detected in the crude extracts of the senescent leaves of *C. annuum*, and very low activity was found in the mature leaves of *E. pulcherrima*. The total amount of Chl and proteins was the same in the young and in the senescent or mature leaves. Fruiting also decreased the activity of deaminase in *C. annuum* (Table I). The activity of PBG deaminase was higher in the extracts of *E. pulcherrima*.

To eliminate the possibility that the observed decrease in the deaminase activity in the older leaves was due to the presence of an inhibitor or to a masking substance, the extracts of the senescent leaves were incubated together with those of the young leaves. No meaningful inhibition in the PBG deaminase activity of the young leaves was detected. To confirm that the decrease in the deaminase activity was due to the aging process, a purification of the enzyme was carried out. It was known from previous work (6) that wheat germ PBG deaminase was eluted with 0.2 M NaCl from a DEAE-cellulose column. The leaf enzyme behaved in an analogous manner, and a typical elution profile from *C. annuum* young leaves is depicted in Figure 1. All of the PBG deaminase activity was eluted with 0.2 M NaCl, as was the cause with the wheat germ enzyme (6).

The four crude leaf extracts of *C. annuum* obtained at the different physiological conditions (Table I), were purified following the procedure described above. The results (Fig. 2) indicate a very low deaminase activity in the senescent and fruiting leaves. In order to detect the activity in the senescent leaves, the incubations had to be carried out for 2 h at 42°C. The amount of protein eluted with 0.2 M NaCl was lower in the young leaves than in those of *C. annuum*, as calculated per g fresh leaf weight, or per mg protein.

When the leaves of *C. annuum* grown in the field were examined, the activity of the deaminase in the younger leaves was found to be 70% higher than the activity obtained from those grown in the greenhouse. The activity in the senescent leaves of the field-grown plants was also higher, and amounted to about 10% of the activity of the young leaves (compare with *E. pulcherrima* results in Table I).

All five leaves of a young *C. annuum* plantula were found to have a good activity of the deaminase, but the first leaf from the top was found to have the highest activity. The addition of either

![Figure 1](https://www.plantphysiol.org/)

Fig. 1. DEAE-cellulose elution profile of PBG-deaminase from *C. annuum* young leaves. Crude extract (7 ml) prepared as described under "Materials and Methods" was applied on a DEAE-cellulose column (2 × 18 cm), and eluted stepwise. Fractions of 5 ml were collected. Activity was assayed as described, using 100 μl of enzyme. Incubations were carried out at 37°C for 60 min.

**TABLE I**

Effect of aging and fruiting on porphobilinogen deaminase activity

Enzymatic activity was measured in the crude extracts as described in Materials and Methods. An amount of enzyme equivalent to 30 mg of fresh leaves was used in all cases. The extracts of *C. annuum* were prepared from leaves of plants grown in a greenhouse; the protein content of the extracts varied from 3.6 mg/ml to 6.6 mg/ml.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>LEAVES</th>
<th>HOMOGENIZED WITH</th>
<th>ENZYMATIC ACTIVITY</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>PBG CONSUMED</td>
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<td></td>
<td></td>
<td></td>
<td>nmoles</td>
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<tr>
<td><em>C. annuum</em></td>
<td>Young</td>
<td>Buffer</td>
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<tr>
<td></td>
<td>Senescent</td>
<td>Buffer</td>
<td>0.27</td>
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<td></td>
<td>Young</td>
<td>Buffer</td>
<td>1.8</td>
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<tr>
<td></td>
<td>fruiting plant</td>
<td>Buffer</td>
<td>-</td>
</tr>
<tr>
<td><em>E. pulcherrima</em></td>
<td>Young</td>
<td>Buffer</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>Young</td>
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<td>7.9</td>
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<tr>
<td></td>
<td>Mature</td>
<td>Buffer</td>
<td>7.0</td>
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<tr>
<td></td>
<td>Mature</td>
<td>Buffer + PVP</td>
<td>1.1</td>
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<td></td>
<td>+ nicotine</td>
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</table>
The activity uroporphyrinogen III cosynthase in the young and senescent leaves of *C. annuum* was the same. When the crude extracts of the senescent leaves were supplemented with wheat germ deaminase, they formed the same amount of uroporphyrinogen III as the young leaves.

**Properties of Pepper Leaf PBG Deaminase.** The activity of PBG-deaminase from the young leaves of pepper increased with time and enzyme concentration. The increase with time was linear up to 30 min and was followed by a rate enhancement (Fig. 3). The enzymic activity increased with temperature as was the case with the deaminase of other origins (6). At 25°C its activity was 48% of the activity at 37°C (4.5 nmol of PBG consumed under the assay conditions), while at 45°C its activity was 168% of the activity at 37°C. A $K_m$ of 6.6 μM was calculated for uroporphyrinogen formation.

A typical pH optimum curve is shown in Figure 4. Within the same pH range, the activity peaks were strongly dependent on the buffer used. These variations were not observed with the wheat germ enzyme, although the activities of both the pepper leaf and wheat germ deaminase were similar.

**DISCUSSION**

There is a negative correlation between the activity of PBG deaminase and the age of the leaf. Deaminase activity is highest in the young leaves. After Chl formation reaches a maximum, the activity of deaminase strongly decreases. The disappearance of deaminase activity is not due to the presence of an inhibitor which could be removed by a DEAE-cellulose purification or by Seph-
and fruiting is in some cases considered to be a first signal of senescence (12). If senescence is considered as a highly ordered and programmed series of physiological events, rather than a random running down of the life processes, then it is conceivable that the interruption of Chl biosynthesis in the leaf precedes its degradation. If it is accepted that Chl build-up is mainly dependent on deaminase activity, the latter should continue as long as an active Chl synthesis is needed. It is known that photosynthetic activity decreases before senescence starts, and Chl breakdown does not occur until much later. The disappearance of deaminase activity in leaves when the Chl content is still high entirely supports the observations (15) that the decrease in the rate of Chl synthesis controls the latter's level during senescence.

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FIG. 4. Effect of pH and different buffers on PBG deaminase activity. Incubation mixture contained in a final volume of 100 \(\mu\)l: 13 nmol PBG, 25 \(\mu\)l enzyme from C. anumum young leaves (7 mg fresh weight, 0.12 mg protein), and 10 \(\mu\)mol of the different buffers. Incubations were carried out at 37 C for 1 h. Uroporphyrins formed were measured as described (6). (●—●): Phosphate buffer; (○—○): Tris-maleic; (▲—▲): barbital buffer.

The strong decrease in deaminase activity precedes the onset of senescence. Young leaves of fruiting plants also show this decrease