Aspects of [8-\(^{14}\)C]Benzyaminopurine Metabolism in *Phaseolus vulgaris*

ANGelo RAMINA

Institute of Pomology, University of Padova, 35100 Padova, Italy

ABSTRACT

*Phaseolus vulgaris* L. plants were supplied through the root with [8-\(^{14}\)C]Benzyaminopurine (\(^{14}\)CBA). Collections of root, apex, and leaves were made 8 and 48 hours after labeling; ethanolic extracts of tissues were purified and subjected to thin layer chromatography on silica gel and/or cellulose powder. From [\(^{14}\)CBA at least two different metabolites originate, the BA riboside and ribotide. The balance among \(^{14}\)CBA, the ribosome, and the ribotide changed in time. The \(^{14}\)CBA riboside was detected as the only labeled compound in the xylem sap collected from the stem. The biological function of BA riboside and ribotide is discussed.

In a previous paper (6), it was shown that when BA\(^{2}\) is supplied through the root, translocation occurs mainly as compounds different from BA; that, with other data, provided evidence for a BA metabolism which might occur in the roots. Results of attempts to identify some of these BA metabolites are presented here.

MATERIALS AND METHODS

Plant Material. *Phaseolus vulgaris* L. seedlings were grown in sand from seeds, under continuous light (intensity 1,500 ft-c at plant top), at 25 C and 70% RH. When the cotyledonary leaves were fully expanded the root system was washed and the plants transferred for a 2-hr period to a nutrient solution containing [8-\(^{14}\)C]BA (Amersham/Searle, 50 mCi/mol) at 3.2 \times 10^{-7} M. After labeling, the roots were rinsed and transferred to an unlabeled medium. Collections of plant parts—root, leaves, and apex (0.25 cm of the upper portion of the stem plus terminal bud)—were made 8 and 48 hr from the end of [8-\(^{14}\)C]BA application.

Extraction of [8-\(^{14}\)C]BA. The procedure was basically that followed by Syöño and Torrey (9). Plant tissues were extracted three times with 80% ethanol, each for 2 hr in freezer (−19 C). The extracts were combined and concentrated under reduced pressure below 35 C. Removal of the ethanol, the aqueous phase was adjusted to pH 3 with 2 N HCl and extracted with methylene chloride three times. The residual aqueous phase was adjusted to pH 8 with 2 N NaOH and extracted with 1-butanol three times. The three butanol extracts were combined and taken to dryness in vacuo. More than 90% of the original radioactivity was recovered in the butanol fraction; insignificant radioactivity was detected in the methylene chloride fraction and the water fraction.

RESULTS AND DISCUSSION

Distribution of radioactivity of the butanol fraction after TLC in solvent system A (Fig. 1) showed two peaks located at \(R_R\) 0.1 to 0.2 and 0.5 to 0.8. Radioactivity in zone 0.5 to 0.8 in solvent system A rechromatographed in solvent system C (Fig. 2) resulted in two peaks. The peak with the higher \(R_R\) (0.9) corresponded to the mobility of BA riboside, while that with the lower \(R_R\) (0.5-0.6) moved with BA.

The purified activity in zone 0.9 in solvent system C (Fig. 2) was eluted with methanol and divided into two equal portions. One aliquot was hydrolyzed with 0.1 M HCl at 100 C for 6 min. After hydrolysis the solution was made alkaline (pH 8) with NaOH and extracted with 1-butanol. The other portion was used as control. After hydrolysis the activity at zone 0.9 in solvent system C moved to the lower zone (0.5-0.6) corresponding to BA (Fig. 3). These results provided evidence for the possibility that the radioactivity in zone 0.9 was BA riboside.

Radioactivity in zones 0.1 and 0.2, because of its low mobility in solvent system A, according to Syöño and Torrey (9), was suspected to be a nucleotide. To confirm this possibility, these regions were scraped off the plates, eluted with \(^{14}\)H\(_2\)O, and treated with alkaline phosphatase. The reaction mixture consisted of 0.1 ml eluted extract, 0.05 ml 0.1 M MgCl\(_2\), 0.4 ml 0.1 M Tris buffer (pH 8.5), and 0.07 mg chicken intestine alkaline phosphatase (8). This mixture was incubated for 3 hr at 37 C. A control was treated similarly, except that the boiled enzyme was used. After the 3-hr reaction period, the mixture was extracted with 1-butanol and chromatographed in solvent system B. After treatment with alkaline phosphatase, the radioactivity moved to \(R_R\) 0.5 (Fig. 4), corresponding to BA riboside; reaction with boiled enzyme did not change mobility of the \(^{14}\)C-labeled material.

The radioactivity of xylem sap, after TLC in solvent system A was located at \(R_R\) 0.5 to 0.7 (Fig. 5a). By rechromatography of

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2 Abbreviations: BA: benzylaminopurine.
This zone in solvent system C on cellulose powder plates, the radioactivity moved to Rf 0.9, corresponding to the mobility of BA riboside (Fig. 5b). Further evidence for the possibility that the radioactivity in zone 0.9 was BA riboside was provided by acid hydrolysis and rechromatography in the same solvent system.

Results shown in Table I indicate that the balance among BA and its riboside and ribotide changes in time. At root level the [14C]BA showed a net decrease in time, while the BA riboside and ribotide had an opposite tendency; 48 hr after [14C]BA supply, more than 50% of the radioactivity was present as BA ribotide. The time course analysis of extracts of apex and leaves indicated an increase of BA and BA riboside, and a decrease of BA ribotide. In the xylem sap, no differences were found in the time course analysis; chromatograms showed that BA acropetally translocated almost exclusively in the xylem form. These results are consistent with the findings reported by Gordon et al. (5) for added [3H]zeatin in radish, although the same authors found zeatin glucoside as a major storage form. Since a conclusive identification of BA riboside and ribotide would entail more rigorous methodology than chromatography (e.g. mass spectrometry), we can not exclude the possibility that some of the measured radioactivity was due to glucoside rather than riboside of BA.

These data indicate that [14C]BA is metabolized at root level; at least two different compounds are formed, the BA riboside and...
Tab. I - Distribution of radioactivity (cpm) among BA, and its riboside and ribotide in extracts of root, apex, and leaves, and in the xylem sap, 8 and 48 hr after $^{14}C$-BA supply.

<table>
<thead>
<tr>
<th>hr after $^{14}C$-BA supply</th>
<th>root</th>
<th>apex</th>
<th>leaves</th>
<th>xylem sap</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>BA</td>
<td>Bärts</td>
<td>BA</td>
<td>Bärts</td>
</tr>
<tr>
<td>104,850</td>
<td>48,420</td>
<td>46,830</td>
<td>42,320</td>
<td>84,720</td>
</tr>
<tr>
<td>48</td>
<td>28,470</td>
<td>63,450</td>
<td>108,230</td>
<td>72,150</td>
</tr>
</tbody>
</table>

Although the biological importance of cytokinin phosphorylation is unknown, there is evidence indicating direct or indirect functions of cytokinin ribotides on intracellular transport and cell division (2, 3, 7). Also there are reports of incorporation of small quantities of cytokinin base into polynucleotides (1, 4, 10) indicating perhaps a cytokinin nucleotide function in RNA metabolism.

LITERATURE CITED