PURINE SALVAGE IN COTYLEDONS OF GERMINATING LUPIN SEEDS

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1. Introduction

Purine metabolism is much less elucidated in plants [1,2] than in animal tissues. In contrast to animal tissues, in plants adenosine is hydrolyzed to adenine and ribose by adenosine nucleosidase (EC 3.2.2.7.) found in leaves of various species [3–7], as well as in the cotyledons of yellow lupin seedlings [8]. For the cotyledons, the highest adenosine nucleosidase activity has been observed on day 4–5 of seed germination [9]. However, as Brown found [10,11] in legume seeds, adenine does not accumulate in their cotyledons at any stage of germination. This could suggest that the activity of purine salvage pathways is relatively high. The aim of this work was to study changes in the activity of enzymes of the purine salvage pathways in cotyledons of germinating lupin seeds.

In animals, two ways of purine salvage are postulated:

(i) The one-step way catalyzed by purine phosphoribosyltransferases (EC 2.4.2.7. and EC 2.4.2.8.);
(ii) The two-step way dependent on purine nucleoside phosphorylase (EC 2.4.2.1.) and nucleoside kinases (EC 2.7.1.20 and 2.7.1.73.) or nucleoside phosphotransferase (EC 2.7.1.77).

Some of these enzymic activities have been studied in germinating wheat embryos, but only up to the second day of germination [12].

Abbreviations: PRPP, 5-phosphoribosyl-1-pyrophosphate; R-1-P, ribose-1-phosphate; POPOP, 1,4-di-2-phenyloxazyl/benzene; PPO, 2,5-diphenyloxazole

2. Materials and methods

2.1. *Plant material*

Seeds of yellow lupin (*Lupinus luteus* cv. Express) were germinated on cotton wool at 22°C in day light.

2.2. *Experimental procedure*

Cotyledons for extract preparation were collected from non-germinating seeds and from seeds after 1,2,3,4,5 and 6 days of germination. The extracts were prepared by grinding 10 cotyledons in a chilled mortar with 10 ml 50 mM Tris–HCl buffer (pH 8.0), containing 10% (v/v) glycerol. The homogenate was centrifuged at 20 000 × *g* for 20 min and the supernatant was used as the source of enzymes.

2.3. *Assays*

Adenine, hypoxanthine and guanine phosphoribosyltransferase activities were measured in an incubation mixture containing in 100 μl final volume: 100 mM Tris–HCl buffer (pH 8.0), 1.5 mM PRPP, 5 mM $MgCl_2$, 0.1 mM [8-^{14}C]adenine or hypoxanthine or guanine (5 mCi/mmol), respectively, and supernatant corresponding to 0.1 mg protein. To take into account the increase in hydrolase activities during seed germination [13], the activities of purine phosphoribosyltransferases were calculated as the sum of radioactivities of AMP and adenosine, or IMP and inosine, or GMP and guanosine. In addition, the degradation of PRPP by the supernatant obtained from the cotyledons of germinating seeds was estimated according to [14]. It was found that under the conditions used for the purine phosphoribosyltransferases assay only 12% of PRPP was decomposed.

Adenosine and inosine phosphorylase activities were measured in 100 μl incubation mixture containing: 100 mM Tris–HCl buffer (pH 8.0), 1.5 mM R-1-P, 0.1 mM [8-^{14}C]adenine or hypoxanthine (5 mCi/mmol) and supernatant corresponding to 0.1 mg protein. The possibility of R-1-P degradation by non-specific phosphatases under the experimental conditions was checked by the method in [15]. R-1-P was not hydrolized by phosphatases during 9 min incubation with the supernatant. In measurements of the catabolic activity of inosine phosphorylase, the 100 μl incubation mixture contained: 100 mM Tris–HCl buffer (pH 8.0), 2 mM KH_2PO_4, 0.1 mM [8-^{14}C]inosine and supernatant containing 0.1 mg protein.

Adenosine kinase activity was measured in 100 μl of the incubation mixture containing: 100 mM Tris–HCl buffer (pH 8.0), 10 mM ATP, 5 mM $MgCl_2$, 0.1 mM [8-^{14}C]adenosine (5 mCi/mmol) and supernatant containing 0.1 mg protein.

Nucleoside phosphotransferase activity was measured in 100 μl incubation mixture containing 100 mM Tris–HCl buffer (pH 8.0), 10 mM AMP, 0.1 mM [8-^{14}C]inosine (5 mCi/mmol) and supernatant containing 0.1 mg protein.

Adenosine and adenine deaminase activities were measured in 100 μl incubation mixture containing 100 mM Tris–HCl buffer (pH 8.0), 0.1 mM [8-^{14}C]-adenosine or adenine (5 mCi/mmol) and supernatant containing 0.1 mg protein.

Inosine nucleosidase activity was measured in 100 μl final volume containing 100 mM Tris–HCl buffer (pH 8.0), 0.1 mM [8-^{14}C]inosine and supernatant corresponding to 0.1 mg protein.

The samples were incubated at 30°C for 3 min, 6 min or 9 min, depending on the level of the enzyme activity during germination. The reaction was stopped by addition of HCOOH to 2 M final conc. After centrifugation, supernatant samples were spotted onto cellulose thin-layer sheets (5 μl) or Whatman 3 MM paper (50 μl) with appropriate carriers. Chromatograms were developed in 5% aqueous Na_2HPO_4 [4] the spots were localized under ultraviolet light and their radioactivity counted in a liquid scintillation spectrometer with 0.01% POPOP and 0.5% PPO in toluene. The results presented are averages of duplicate determinations in two experiments: average deviation from the mean was <10%.

3. Results and discussion

Extracts from yellow lupin seeds exhibited all enzyme activities of the one-step purine salvage pathway: adenine phosphoribosyltransferase (A-PRTase), hypoxanthine phosphoribosyltransferase (H-PRTase) and guanine phosphoribosyltransferase (G-PRTase). Of the two-step pathway of purine salvage, only the enzymes converting nucleosides into nucleotides were detected, whereas purine nucleoside phosphorylase activity was absent throughout germination. This suggests that the free bases are reutilized mainly via the one-step salvage pathway. On the other hand, nucleosides could be salvaged by nucleoside kinase, or nucleoside phosphotransferase, both enzymes being present in the cotyledons. No activities of inosine hydrolase (EC 3.2.2.2.), adenosine and adenine aminohydrolases (EC 3.5.4.4. and EC 3.5.4.2.) were detected in the cotyledons throughout germination. Activities of purine phosphoribosyltransferases in the cotyledons decreased markedly during germination. The activity of A-PRTase dropped by ~75% after 4 days of germination (fig.1A). During the same period, the activities of H-PRTase and G-PRTase decreased by ~50% (fig.1B). The activity of A-PRTase exceeded that of H-PRTase and G-PRTase, especially at the beginning of germination. This has also been reported for wheat embryos [12]. The activities of H-PRTase and G-PRTase, as well as their changes during germination, were similar (fig.1B). It may be concluded that, like animal tissues, the plant material also contains a single enzyme which salvages both hypoxanthine and guanine. The adenosine kinase activity declined during germination similarly to that of the enzymes of the one-step purine salvage pathway (fig.2A). However, at the beginning of germination, the activity of adenosine kinase was higher than that of both above-mentioned enzymes, but it decreased faster to an undetectable level, after 4 days of germination. However, this result has to be considered with caution, because of the high activity of adenosine nucleosidase found in the cotyledons on the second day of germination [9]. In contrast to changes in the activities of H-PRTase and G-PRTase, the activity of nucleoside phosphotransferase increased markedly after 2 days of germination (fig.2B), and after 6 days it was >50-times higher than that in imbibed seeds. It is of interest that the nucleoside phosphotransferase activity

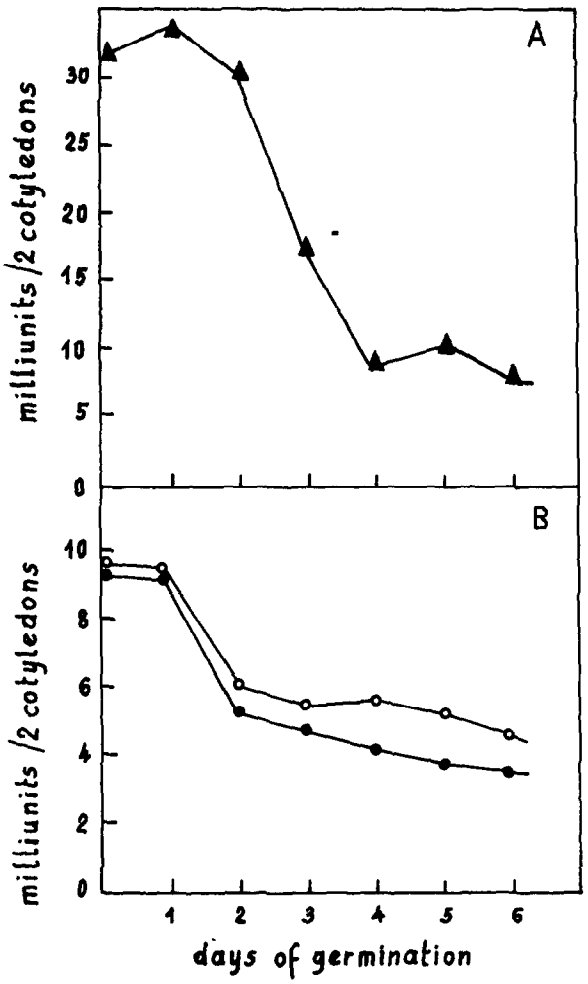

Fig.1. Changes in activities of purine phosphoribosyltransferases in cotyledons during germination of lupin seeds: (A) adenine phosphoribosyltransferase (▲); (B) hypoxanthine phosphoribosyltransferase (●) and guanine phosphoribosyltransferase (○). The assay conditions are described in section 2. One milliunit of activity represents the amount of enzyme converting one nanomole of substrate per minute under the incubation conditions

began to increase rapidly at the time of a drop in the activities of all other enzymes of both purine salvage pathways: A-PRTase, H-PRTase, G-PRTase and adenosine kinase. It seems that nucleoside phosphotransferase is the most important enzyme of purine salvage in the cotyledons during the later period of germination. The absence of purine nucleoside phosphorylase in cotyledons and the fact that inosine is not the substrate for adenosine nucleosidase [8] suggest that inosine is not catabolized any further and that nucleoside phosphotransferase plays an important role in inosine reutilization.

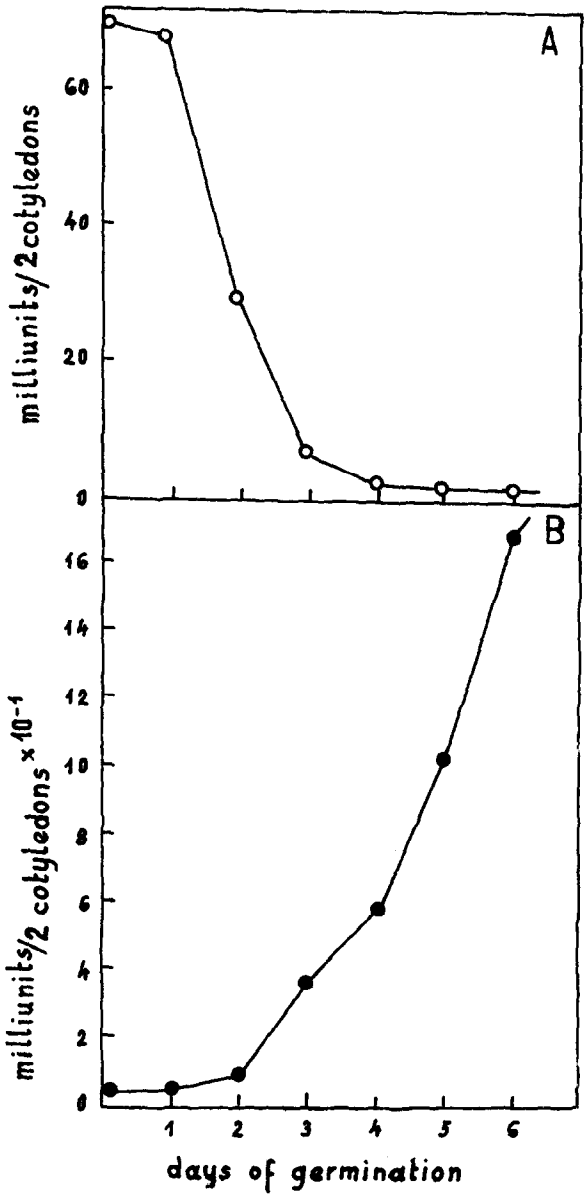

Fig.2. Changes in activities of adenosine kinase (A) and nucleoside phosphotransferase (B) in cotyledons during germination of lupin seeds. The assays conditions are described in section 2. One milliunit of activity represents the amount of enzyme converting one nanomole of substrate per minute under the incubation conditions.

The drop in the activities of A-PRTase and adenosine kinase can be correlated with the decrease in AMP, ADP and ATP levels [17] and RNA content [18] reported for pea cotyledons. On the other hand, the present data show that the increase in the activity of adenosine nucleosidase in lupin cotyledons [9] is not accompanied by enhanced activity of the enzymes taking part in the salvage of adenine compounds. The absence of adenine accumulation during germination can not be explained by the action of adenine deaminase which has been detected in various plants [2], because adenine deaminase was not found in the cotyledons. Therefore, A-PRTase activity seems to be responsible for the absence of adenine accumulation during germination.

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References

[1] Brown, E. C. (1975) Biochem. Soc. Trans. 3, 1199–1202.
[2] Suzuki, T. and Takahashi, E. (1977) Drug Metab. Rev. 213–242.
[3] Miller, G. W. and Evans, H. J. (1955) Plant Physiol. 34 S, 37.
[4] Roberts, D. W. A. (1956) J. Biol. Chem. 222, 259–270.
[5] Mazelis, M. (1959) Plant Physiol. 24, 153–158.
[6] Mazelis, M. and Creveling, R. K. (1963) J. Biol. Chem. 238, 3358–3361.
[7] Page, O. T. and Clark, M. C. (1968) Can. J. Bot. 46, 979–985.
[8] Guranowski, A. and Schneider, Z. (1977) Biochim. Biophys. Acta 482, 145–158.
[9] Guranowski, A. and Pawełkiewicz, J. (1978) Planta 139, 245–247.
[10] Brown, E. G. (1963) Biochem. J. 88, 498–504.
[11] Brown, E. G. (1965) Biochem. J. 95, 509–514.
[12] Price, C. E. and Murray, A. W. (1969) Biochem. J. 115, 129–133.
[13] Matile, Ph. (1975) in: Cell Biology Monographs, vol. 1, pp. 134–138, Springer-Verlag, Wien/New York.
[14] Henderson, J. F. and Khoo, M. K. Y. (1965) J. Biol. Chem. 240, 2349–2357.
[15] Barankiewicz, J. and Henderson, J. F. (1977) Biochem. Med. 17, 45–53.
[16] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[17] Brown, A. P. and Wray, J. L. (1968) Biochem. J. 108, 437–444.
[18] Beevers, L. and Guernsey, F. S. (1966) Plant Physiol. 41, 1455–1458.