THE INFLUENCE OF NO_3^- ON PARTICULATE 6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY IN PEA ROOTS

M. J. EMES, H. ASHIHARA+ and M. W. FOWLER

Department of Biochemistry, The University, Sheffield S10 2TN, England and *+Department of Biology, Faculty of Science, Ochanomizu University, Tokyo, Japan*

Received 23 July 1979

1. Introduction

We have shown that the pentose phosphate pathway enzymes are present within both the cytosolic and plastid fractions of pea roots [1,2]. Data has been presented which suggests that a major function of the pentose phosphate pathway in non-photosynthetic plant cells is to provide the NADPH necessary for nitrite reductase (EC 1.6.6.4). Within non-photosynthetic plant cells, two isoenzymes of 6-phosphogluconate dehydrogenase (EC 1.1.1.44) have been demonstrated [4,5], one of which may be located in plastids [2,6,7].

The aim of this was to ascertain whether or not the increased 6-phosphogluconate dehydrogenase activity concomitant with the induction of the enzymes of nitrate assimilation [8] is confined to one species, or is a more general property of the total 6-phosphogluconate dehydrogenase activity of the tissue. The approach adopted was to induce nitrate assimilation in the roots of intact seedlings of *Pisum sativum*, to measure the activity of the iso-enzymes of 6-phosphogluconate dehydrogenase after separation by column chromatography and to compare the data with that for intact plastids isolated by density gradient centrifugation. Confirmation of the presence of iso-enzymic forms of 6-phosphogluconate dehydrogenase was also sought by gel electrophoresis.

Address correspondence to: Dr M. W. Fowler

2. Materials and methods

Purified enzymes, co-factors and substrates were purchased from either Boehringer (Mannheim) or Sigma London Chemical Co. Pea seeds (*Pisum sativum* L. var. Kelvedon Wonder) were purchased from Suttons Seeds, Torquay, England, 1976 harvest.

Roots of 5 day old seedling peas were obtained as in [8]. Intact plastids were purified by sucrose density gradient centrifugation [2] and the three fractions containing the highest activity of plastid marker enzymes pooled. 6-Phosphogluconate dehydrogenase was assayed in plastids from roots incubated in 10 mM potassium nitrate for 8 h [8] and compared with control experiments in which roots were incubated in the absence of nitrate [8]. Protein was determined as in [9]. Iso-enzymes of 6-phosphogluconate dehydrogenase were separated as in [5].

Disc electrophoresis of enzyme preparations followed [10]. Particulate and soluble forms of 6-phosphogluconate dehydrogenase were obtained by differential centrifugation of root extracts for 30 min at 10 000 × g. The resuspended pellet was washed once then lysed by a combination of osmosis and mechanical rupture. Aliquots of the particulate and supernatant fractions were then run on 7% polyacrylamide gels. 6-Phosphogluconate dehydrogenase (activity) was located by staining for enzyme activity [10].

3. Results and discussion

In [5] we demonstrated the presence of two iso-

Elsevier/North-Holland Biomedical Press

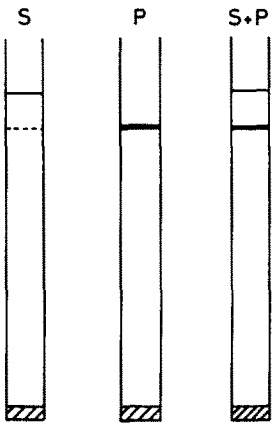

Fig.1. Distribution of 6-phosphogluconate dehydrogenase on 7% polyacrylamide gels: S, soluble fraction; P, particulate fraction; S+P, mixture of the two.

enzymes of 6-phosphogluconate dehydrogenase in pea roots using column chromatography. This observation has been confirmed in the present work using gel electrophoresis (fig.1). While only one band of activity was observed for the plastid fraction, two bands of activity were noted from supernatant preparations. The lower band moves to an equivalent position in the electrophoretogram as that seen for the plastid located activity and presumably results from the release of 6-phosphogluconate dehydrogenase from broken plastids.

To ascertain the effect of the induction of nitrate assimilation on cytosolic and plastid-located 6-phosphogluconate dehydrogenase activity we first incubated roots in nitrate for 8 h then separated the plastids by density gradient centrifugation (table 1).

The data show a significant increase in plastid 6-phosphogluconate dehydrogenase activity. Because of the use of BSA in the extraction medium the specific activity of cytosolic 6-phosphogluconate dehydrogenase could not be estimated, however, this did not interfere with measurement of the plastid enzymic activity as BSA was not included in the sucrose gradients. It is of interest to compare the increased enzyme activity seen in the plastid fraction in the present work with the very small increase in total 6-phosphogluconate dehydrogenase activity concomitant with the induction of nitrate assimilation observed [8]. A possible explanation of this is that although the increase in activity of the plastid enzyme is substantial (47%) its activity relative to the total 6-phosphogluconate dehydrogenase activity is low [2] and is consequently masked by it.

That the effect of the induction of nitrate assimilation is principally on the plastid located 6-phosphogluconate dehydrogenase was confirmed by the use of column chromatography. Separation of the two forms of the enzyme [5] of whole root extracts clearly shows an increase in the activity of the plastid enzyme but not of the cytosolic one. (The intracellular location of these two peaks of activity has been confirmed [5] (fig.2).)

The studies indicate a close association between the onset of nitrate assimilation and increased plastid 6-phosphogluconate dehydrogenase activity. That there is a specific relationship between the plastid enzyme and nitrate assimilation is reinforced by two observations. (i) Recent studies have shown the enzymes of nitrite assimilation to be plastid treated [11]; (ii) the lack of response of cytosolic 6-phosphogluconate dehydrogenase to the induction of nitrate assimilation as seen here. The pentose phosphate pathway has been suggested to provide the reducing equivalents, as NADPH, for the reduction of nitrite to ammonia in plant cells [12]. The location of a form of 6-phosphogluconate dehydrogenase within the plastid and its specific positive response to nitrate assimilation induction adds further support to that hypothesis. Such a spatial and dynamic co-operation

Table 1
Activity of 6-phosphogluconate dehydrogenase in plastids of pea roots after incubating intact roots in 10 mM potassium nitrate for 8 h

	Distilled water	NO_3^-	% change
Enzyme activity (mU/mg protein)	194.4 ± 27.5	285.8 ± 26.7	47

Results are quoted as the mean ± SE of 5 separate experiments

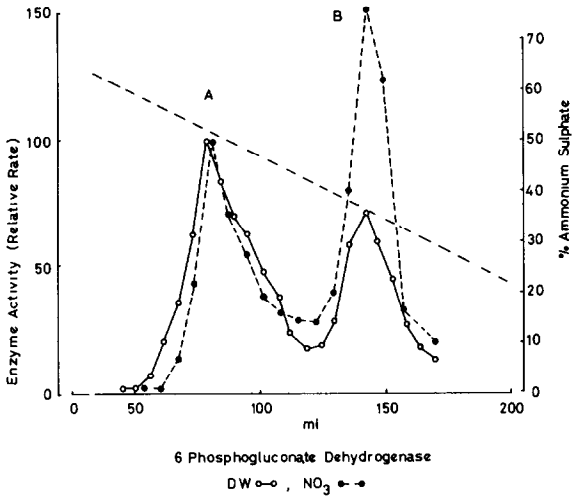

Fig.2. Separation of isoenzymes of 6-phosphogluconate dehydrogenase after ammonium sulphate fractionation on a celite column: peak (A), cytosolic enzyme; peak (B), plastid enzyme.

between the pentose phosphate pathway and nitrite reductase is likely to be of advantage to the cell in vivo, removing the need for transport of reducing equivalents across an organelle membrane, thereby ensuring a closely coupled supply of electrons as long as sufficient hexose phosphate is available for oxidation.

Acknowledgements

M. J. E. thanks the Science Research Council (UK) for a postgraduate studentship. H. A. thanks the British Council for financial support. M. W. F. thanks the Royal Society for a Special Investigations Grant.

References

[1] Emes, M. and Fowler, M. W. (1978) Biochem. Soc. Trans. 6, 203–205.
[2] Emes, M. and Fowler, M. W. (1979) Planta 145, 287–292.
[3] Stepan-Sarkissian, G. and Fowler, M. W. (1978) Int. J. Biochem. 9, 817–822.
[4] Simcox, P. D. and Dennis, D. T. (1978) Plant Physiol. 62, 287–290.
[5] Ashihara, H. and Fowler, M. W. (1979) Int. J. Biochem. in press.
[6] Miflin, B. J. and Beevers, H. (1974) Plant Physiol. 53, 870–874.
[7] Simcox, P. D., Reid, E. E., Canvin, D. T. and Dennis, D. T. (1977) Plant Physiol. 59, 1128–1132.
[8] Sarkissian, G. S. and Fowler, M. W. (1974) Planta 119, 335–349.
[9] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[10] Muto, S. and Uritani, I. (1972) Plant Cell Physiol. 13, 931–936.
[11] Emes, M. and Fowler, M. W. (1979) Planta 144, 249–253.
[12] Fowler, M. W. (1975) New Phytol. 75, 461–478.