TISSUE LOCALIZATION OF BARLEY (*HORDEUM VULGARE*) GLUTAMINE SYNTHETASE ISOENZYMES

A. F. MANN, P. A. FENTEM and G. R. STEWART
Department of Botany, The University, Manchester M13 9PL, England

Received 9 December 1979

1. Introduction

The presence of multiple molecular forms of glutamine synthetase has been reported recently in soybean hypocotyl tissue [1], and green leaf tissue of barley [2] and rice [3]. The two forms (designated GS_I, GS_{II}) present in barley leaf tissue can be separated by ion-exchange chromatography and while they have similar molecular weights they exhibit different pH optima and respond differently to thiol reactive ligands [2]. Subcellular localization studies indicate GS_I is localized in the cytosol while GS_{II} is present in chloroplasts [2]. Etiolated leaf tissue was found to contain only GS_I and the enzyme of barley seeds and roots was found to elute as a single peak of activity from DEAE–Sephacel which corresponded to the GS_I of light-grown leaves [2]. This suggests that GS_I is present in all tissues while GS_{II} is exclusively a leaf isoenzyme.

We report here the occurrence of GS_{II} in other photosynthetically active tissues of barley and that while the catalytic properties of GS_I and GS_{II} from green leaf tissue differ, those of GS_I from leaf tissue are similar to those of the root enzyme.

2. Materials and methods

Details for the growth of barley plants (var. Golden Promise) have been described in [2]. Material from mature flowering plants was obtained from plants grown in the University Botanic Garden. The extraction, assay and ion-exchange chromatography of glutamine synthetase were as in [2]; the coupled spectrophotometric assay used in kinetic studies was that of [4]. Amino acid analyses were done as in [5]. Chlorophyll was determined by the method in [6] and protein by the Lowry procedure [7]. Nitrate was determined as in [8].

3. Results and discussion

It is clear from the results presented in table 1 that GS_{II} is present in tissues other than the green leaves of barley. Extracts prepared from stem, glumes and awns exhibited both GS_I and GS_{II} activity although the relative amount of GS_{II} in the glumes was small (30%) compared with that in leaf tissue (70–80%). It is striking that the only part of the developing seed which was found to have GS_{II} activity was the pericarp testa, that is the photosynthetically active tissue of the developing seed (see [9]). Stem tissue immediately below the inflorescence contained chlorophyll and exhibited predominantly GS_{II} activity (70%). In contrast stem tissue immediately above the flag leaf node, which was enclosed by the flag leaf sheath and was largely devoid of chlorophyll, exhibited only GS_I activity. This is consistent with our findings [2] that etiolated leaf tissue exhibited only GS_I activity and suggests a close relationship between the photosynthetic activity of different tissues and the occurrence of GS_{II}.

Furthermore it is evident (table 1) that as the flag leaf becomes senescent and loses chlorophyll there is also a decrease in glutamine synthetase activity which is for the most part accounted for by a decrease in GS_{II}. The apparent relationship between photosynthetic capacity and GS_{II} activity is indicative that this isoenzyme is localized in the chloroplasts of other green tissues as well as those of barley leaves.

The GS_I type activity of green tissues elutes from

Table 1
Tissue localization of glutamine synthetase isoenzymes

Tissue	Total GS activity[a] (μmol . min^{-1} . g fresh wt^{-1})	% Activity	
		GS_I	GS_{II}
Mature seed	3.4	100	
Root	8.5	100	
Primary leaf	24.4	30	70
Flag leaf (2.60[b])	68.0	22	78
Flag leaf (1.64)	32.5	36	64
Flag leaf (0.54)	16.7	60	40
Stem (below inflorescence)	28.0	37	63
Stem (above flag leaf node)	7.0	100	
Embryo	28.0	100	
Endosperm	0.4	100	
Pericarp-testa	19.0	85	15
Glumes	5.0	70	30
Awns	39	21	79

[a] Transferase activity
[b] Figures in brackets are mg chl . g fresh wt^{-1}

DEAE–Sephacel at the same ionic strength as the single form of the enzyme present in non-green tissue, suggesting they may be the same isoenzymic forms of glutamine synthetase. Consistent with this view are observations on the catalytic properties of leaf GS_I and the root enzyme (table 2). The app. K_m values for glutamate, ammonia and ATP and the pH optima of GS_I and the root enzyme are very similar (table 2). The app. K_m values for ammonia and ATP of GS_{II} are similar to those found for GS_I. The app. K_m values for glutamate of GS_{II} are however markedly different. The Lineweaver-Burk plot for glutamate was found to be non-linear and yielded 2 app. K_m values (1.0, 20.0 mM). A similar negative co-operativity has been reported for GS_{II} of rice leaves [3].

Table 2
Kinetic characteristics of glutamine synthetase isoenzymes

	pH optimum	Apparent K_m (mM)		
		NH_4^+	ATP	Glutamate
Root	7.0	0.05	0.5	5.9
Leaf GS_I	7.0	0.05	0.6	5.4
Leaf GS_{II}	7.5	0.05	0.6	1.0; 20.0

Kinetic characterizations were carried out on partially purified preparations

Table 3
The influence of nitrogen nutrition on glutamine synthetase isoenzymes

Nitrogen source	Shoot	Root		Root
	Glutamine Synthetase Activity[a] (μmol . min^{-1} . g fresh wt^{-1})	% Activity		Glutamine Synthetase Activity (μmol . min^{-1} . g fresh wt^{-1})
		GS_I	GS_{II}	
0.1 mM NO_3	1.3	25	75	0.42
5.0 mM NO_3	1.3	23	77	0.45
20.0 mM NO_3	1.3	24	76	0.27
1 mM NH_4	1.6	20	80	0.28
Minus nitrogen	1.1	38	62	0.41

[a] Activity determined by synthetase reaction

The occurrence of GS_{II} in green tissue is consistent with its localization in chloroplasts. It is interesting that glutamine synthetase has been reported in root plastids of pea seedlings [10,11]. An investigation of the localization of GS_I of barley roots would seem worthwhile in view of the cytoplasmic localization of leaf GS_I [2]. It has been suggested that cytoplasmic glutamine synthetase is however involved in the re-assimilation of ammonia released in the photorespiratory nitrogen cycle or other deamination reactions [12]. The presence of GS_I activity in root tissue actively assimilating ammonia (see tables 3,4) indicates that it must also function in the primary assimilation of ammonia. The results in table 3 also show that neither the form nor concentration of available nitrogen have much influence on either total glutamine synthetase activity of root and shoot or on the relative proportions of GS_I and GS_{II} in leaf tissue. In nitrate assimilating plants the nitrate ion and, to a lesser extent, glutamine are exported from root to shoot (table 4) and this suggests that both root GS_I and shoot GS_{II} could participate in the primary assimilation of ammonia derived from nitrate. Glutamine and asparagine are the principal compounds exported by the roots of ammonia assimilating plants and this suggests that primary ammonia assimilation occurs exclusively in the roots and that GS_{II} of such plants can have little if any role in primary ammonia assimilation. The catabolism of asparagine will however release ammonia and since high concentrations of asparagine have been reported in chloroplasts [13] it is possible that GS_{II} could function in the re-assimilation of ammonia. Alternatively the maintenance of GS_{II} activity in plants where its role in primary ammonia assimilation would seem redundant may reflect the necessity to retain an ammonia detoxification mechanism in order to prevent the build up of ammonia concentrations in the chloroplasts which would otherwise uncouple photophosphorylation [14].

Table 4
Composition of bleeding sap from barley plants grown on nitrate or ammonia

Compound	mM in sap	
	1 mM NO_3	0.04 mM NH_4
NO_3	25	–
NH_4	1	1
Glutamine	3	27
Asparagine	0.5	11
Amino acids	1.0	7

Plants were grown in a continuous flow system and sap was collected over the initial 60 min after decapitation

Acknowledgements

This work was supported by a grant from the Agricultural Research Council. P.A.F. was supported by an SRC-CASE award. Stimulating discussion with B. J. Miflin and P. J. Lea is gratefully acknowledged.

References

[1] Stasiewiz, S. and Dunham, V. L. (1979) Biochem. Biophys. Res. Commun. 87, 627–634.
[2] Mann, A. F., Fentem, P. A. and Stewart, G. R. (1979) Biochem. Biophys. Res. Commun. 88, 515–521.
[3] Guiz, C., Hirel, B., Shedlofsky, G. and Gadal, P. (1979) Plant Sci. Lett. 15, 271–278.
[4] Stewart, G. R. and Rhodes, D. (1977) New Phytol. 79, 257–268.
[5] Ahmad, I., Larher, F. and Stewart, G. R. (1979) New Phytol. 82, 671–678.
[6] Arnon, D. I. (1969) Plant Physiol. 24, 1–15.
[7] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[8] Mann, A. F., Hucklesby, D. P. and Hewitt, E. J. (1979) Planta 146, 83–89.
[9] Duffas, C. M. and Rosie, R. (1978) Plant Physiol. 61, 570–576.
[10] Miflin, B. J. (1974) Plant Physiol. 54, 550–555.
[11] Emes, M. J. and Fowler, M. W. (1979) Planta 144, 249–253.
[12] Keys, A. J., Bird, I. F., Cornelius, M. J., Lea, P. J., Wallsgrove, R. M. and Miflin, B. J. (1978) Nature 275, 741–743.
[13] Chapman, D. J. and Leech, R. M. (1979) Plant Physiol. 63, 567–572.
[14] Givan, C. K. (1978) Phytochemistry 18, 375–382.