Vol. 114, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 29, 1983 Pages 604-611

FOUR ISOFORMS OF GLUTAMINE SYNTHETASE IN LIGHT-GROWN SOYBEANS

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Received June 10, 1983

Summary: Glutamine synthetase (GS) activity recovered from linear sucrose gradients was associated with the cytosol of cells isolated from etiolated soybean hypocotyls whereas light-grown tissue contained increased GS activity localized in both the cytosol and chloroplasts. DEAE-cellulose chromatography indicated two GS isoforms in etiolated hypocotyls whereas light-grown hypocotyls and primary leaves contained four isoforms. Only one GS isoform was recovered from both etiolated and light-grown cotyledons.

Isoforms of glutamine synthetase (GS, E.C. 6.3.1.2.) have been isolated from numerous flowering plants including soybean (1,2), barley (3), rice (4-6), pea (7,8), pumpkin (9), peanut (10) and sorghum (11). In several of these plants, etiolated tissue contained one chromatographically distinct isoform whereas light-grown plants contained two isoforms (3,5,8). The subcellular localization of these GS activities has demonstrated the existence of a cytosol isoform in etiolated tissue while both cytosol and chloroplast isoforms are present in light-grown tissues.

Because of the important role of GS in nitrogen assimilation (12,13) and the presence of two isoforms previously reported in hypocotyls of etiolated soybean (1), we have investigated the influence of light on the appearance and subcellular localization of GS isoforms in several soybean tissues. This paper reports the presence of four isoforms of GS in light-grown hypocotyls and leaves. However, only one isoform was found in both light-grown and etiolated cotyledons.

0006-291X/83 $1.50

Materials and Methods

Plant material: Soybean seeds (Glycine max, variety Harosoy 63) were obtained from William Boyd, Phelps, New York, and stored in a cold room (2°C) prior to use. The seeds were repeatedly rinsed, then soaked, planted in moist vermiculite and watered with tap water as necessary. Seedlings were germinated in an environmental chamber (29°C) for four, six or eight days. Light-grown seedlings received an average light intensity of 47.11 $\mu E\ s^{-1}m^{-2}$ as measured with a Li-Cor Quantum/Photometer, (Model LI-185, Lambda Instrument Corporation) with constant illumination from two 40-watt cool-white fluorescent bulbs. Six day-old light-grown seedlings were germinated in the dark for four days prior to illumination.

Enzyme isolation: Primary leaves without petioles, cotyledons without the epicotyl and 2-3 cm sections of the hook region of the hypocotyl were used as sources of enzyme. The isolation and purification of GS was as described elsewhere (1). The volume of homogenization buffer used was 2:1 (volume/g fresh weight) for primary leaves and cotyledons, whereas hypocotyls were homogenized in a 1.4:1 ratio of buffer. After the enzyme preparation was desalted using Sephadex G-50 chromatography, it was applied to a DEAE-cellulose column (1.8 x 30 cm) previously equilibrated with homogenization buffer. The column was then washed with 50 ml of the same buffer and enzyme activity was eluted with a linear KCl gradient (total volume of 200 ml). Three ml fractions were collected and monitored for protein (280 nm) and for GS activity.

Linear sucrose gradients: Plastids were isolated on linear sucrose gradients from six day-old light-grown and etiolated hypocotyls. Tissue was minced in one volume of grinding buffer (60% sucrose in homogenization buffer, pH 7.5; several drops of Antifoam B) and then ground using an iced mortar and pestle. After filtering through four layers of cheesecloth, 10 ml of the homogenate were carefully layered on top of a linear sucrose gradient comprised of (from bottom to top): 4 ml of 60% (w/v) sucrose in 0.2 M imidazole-HCl (pH 7.5), 18 ml of a linear sucrose gradient (50-25% w/v) in the same buffer, followed by 3 ml of 25% sucrose in the same buffer. The gradients were centrifuged in a Beckman Ultracentrifuge, Model L3-50, in a SW-27 rotor (4°C) for 5 min at 4000 rpm, followed by 10 min at 10,000 rpm. Five ml fractions were assayed for GS activity and chlorophyll (14).

Assay procedure: GS activity was determined by the transferase assay (15), with slight modification (1). GS activity was expressed as μmoles hydroxamate formed/10 min at 37°C with reference to a standard curve obtained with γ-glutamyl hydroxamate (Sigma Chemical Company).

Results

Initial experiments indicated a 2-fold increase in GS activity (μmoles of product/g dry weight) in two-day old etiolated tissues following two days of continual illumination (data not shown). Because of this observation and the fact that GS compartmentalization has been shown in other plants (3,5,8),

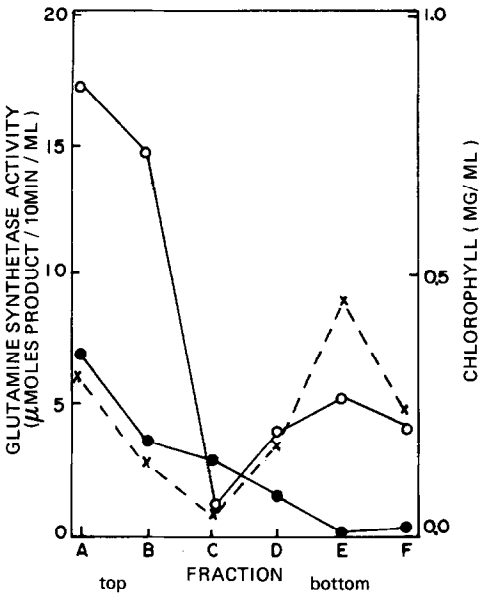

Figure 1. Distribution of GS activity in soybean cells. GS activity (μmoles/ml) was recovered in fractions collected from linear sucrose gradients of 6 day-old etiolated (●—●) and light-grown (○—○) soybean hypocotyl homogenates. Localization of a chloroplast rich fraction was monitored by chlorophyll (X---X).

experiments were performed to determine the subcellular localization of GS in soybean.

Linear sucrose gradients demonstrated the presence of GS activity associated with intact chloroplasts and in the supernatant fractions of light-grown hypocotyl homogenates (Fig. 1). Thirty two percent of the chlorophyll and 72% of the GS activity was recovered in the supernatant fractions A-C (representing ruptured chloroplasts and cytosol). Sixty eight percent of the chlorophyll and 28% of the GS activity were recovered in chloroplast fractions D-F. Because of the presence of chlorophyll in the supernatant fractions (A-C), GS from the chloroplast may also be present in these fractions. Overall recovery of chlorophyll and GS was 95% and 93% respectively. When homogenates of six day-old dark-grown hypocotyl were applied to sucrose gradients, only soluble (cytosol) activity was detected with 85% of the GS activity recovered from the gradients

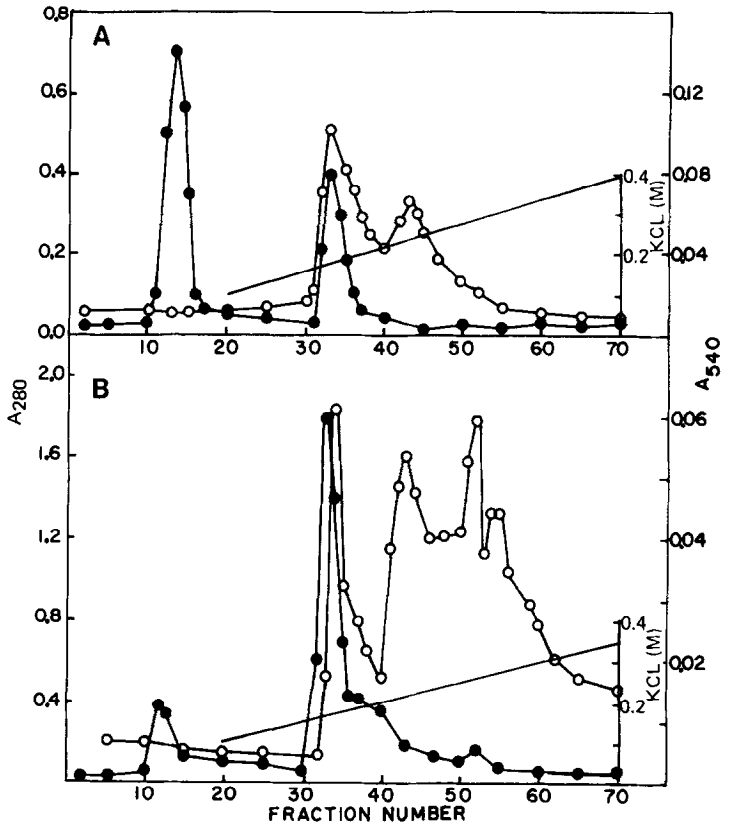

Figure 2. DEAE-cellulose chromatography of GS activity from etiolated (A) and light-grown hypocotyls (B). The enzyme was purified from 4 day-old etiolated or light-grown hypocotyls (constant illumination) and eluted with a linear KCl gradient as described in Materials and Methods. Fractions were monitored for protein at 280 nm (●—●) and GS activity at 540 nm (○—○).

(Fig. 1). These results indicate the presence of GS in both the cytosol and chloroplasts of light-grown hypocotyl, while etiolated seedlings only demonstrated cytosol GS activity.

Because of the observed GS compartmentalization, we investigated whether the two isoforms present in dark-grown hypocotyl (1) were also present in light-grown tissue. As previously reported (1), purification of enzyme preparations from four-day old dark-grown hypocotyls on DEAE-cellulose, using a linear KCl gradient, resulted in two major peaks of GS activity (Fig. 2A). These fractions, referred to as Peak I and II, eluted from the column with 0.18 and 0.23 M KCl respectively. Four fractions

TABLE 1

ELUTION PROPERTIES OF GS ISOFORMS

Peak	KCl (M)		% Total GS Activity	
	Etiolated	Light	Etiolated	Light
I	0.18	0.18	100	100
II	0.23	0.23	66	88
III	N.D.*	0.27	N.D.	97
IV	N.D.	0.28	N.D.	71

GS isoforms were recovered by DEAE-52 cellulose chromatography of enzyme 4 day-old etiolated (Figure 2A) and light-grown (Figure 2B) soybean hypocotyls. The molarity of KCl reflects the fraction in which maximal GS activity was recovered. Percent total activity has been normalized with Peak I representing 100%.

* N.D. = not detected.

containing GS activity (Peaks I-IV) were recovered from light-grown hypocotyls (Fig. 2B) and eluted at 0.18, 0.23, 0.27 and 0.28 M KCl respectively. The similar elution properties and percent distribution of the isoforms are summarized in Table 1. In both light and dark-grown hypocotyls, Peak I and II were recovered, but Peaks III and IV were only present in light-grown tissue, supporting the hypothesis of light-mediated induction of enzyme systems. Moreover, the four isoforms isolated from primary leaves of eight day-old illuminated seedlings eluted from DEAE-cellulose at the same salt concentrations as Peaks I-IV of light-grown hypocotyls (data not shown).

Because four isoforms were detected in hypocotyls and primary leaves, it was important to determine whether these isoforms were present in all green tissue. Elution of enzyme preparations of four day-old dark grown cotyledons from DEAE-cellulose resulted in only one fraction of GS activity (Fig. 3A) This fraction eluted at 0.18 M KCl which is identical to that of Peak I from both light and dark-grown hypocotyls (Fig. 2).

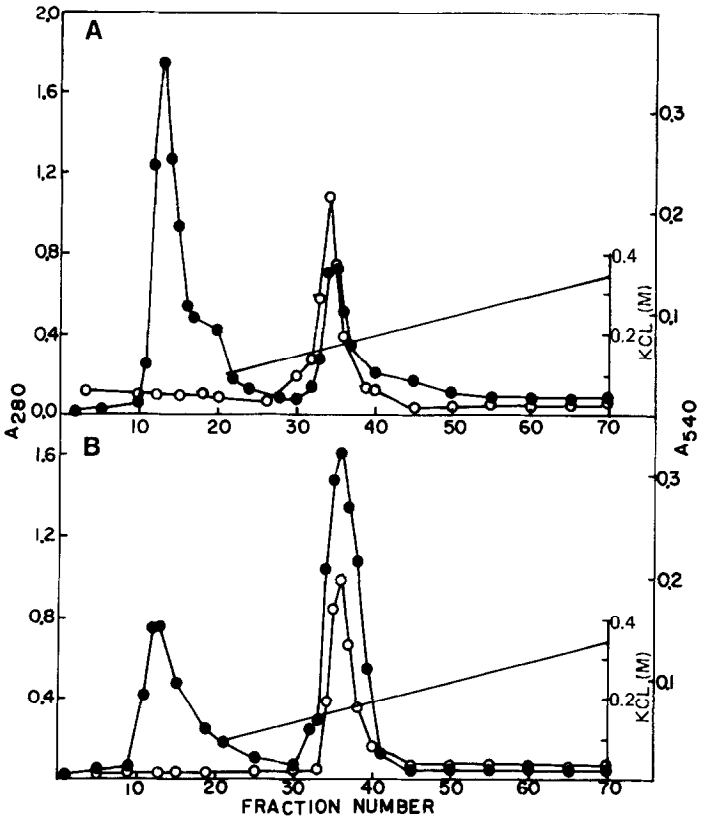

Figure 3. DEAE-cellulose chromatography of GS activity from etiolated (A) and light-grown cotyledons (B). The enzyme was purified from 4 day-old etiolated or light-grown cotyledons (constant illumination) and eluted with a linear KCl gradient as described in Materials and Methods. Fractions were monitored for protein at 280 nm (●—●) and GS activity at 540 nm (○—○).

Only one peak of GS activity was recovered from four day-old light-grown cotyledons (Fig. 3B) and eluted at the same salt concentration as observed in dark-grown cotyledons. The presence of only one GS isoform in both light and dark-grown cotyledons suggests that the transcriptional control of these isoforms is tissue specific, and the chloroplast enzymes may not be present (or functional) at this stage of development.

Discussion

Exposure of plants to light may result in an increase in enzyme activity either as a result of photoactivation (17) or

enzyme induction (18). Recent studies have shown a rapid (five minutes or less) two-fold increase or decrease of GS activity when cultures of Anabaena cylindrica were illuminated or placed in the dark (19). These findings parallel the rapid conformational changes of enzyme or enzyme-substrate complexes associated with photoactivation/deactivation rather than enzyme induction.

Increased GS activity in flowering plants that have been illuminated does not follow the pattern observed in A. cylindrica. For example, significant increases of GS activity were observed in barley (3), sorghum (11) and pea leaves (8) which required hours, if not weeks of illumination. We have found similar slow increases in GS activity in illuminated soybean seedlings, suggesting enzyme induction concomitant with chloroplast development. However, the data do not preclude the involvement of photosynthetic metabolites in the photoactivation of enzyme systems.

This paper reports four GS isoforms in light-grown leaves and hypocotyls of soybean seedlings, two of which were not present in dark-grown tissue. Based on the subcellular localization of these isoforms, Peaks I and II appear to be cytosol enzymes whereas Peaks III and IV are found in chloroplasts. The increased number of isoforms may be the results of enzyme induction concomitant with organelle development (i.e., chloroplasts). These data are the first observation of multiple GS activities in both subcellular compartments.

The absence of multiple GS isoforms in both etiolated and light-grown cotyledons indicates that the mechanisms involved in the appearance of GS isoforms is controlled differently in this tissue or that the isoforms are absent or nonfunctional at this stage of development.

Acknowledgement

We thank Dr. Wayne Yunghans for helpful discussions during the course of this research. We also wish to express our appreciation to Dr. John Bryan for critical comments.

References

1. Stasiewicz, S. and Dunham, V. L. (1979) Biochem. Biophys. Res. Commun. 87, 627-634.
2. McMaster, B. J. and Dunham, V. L. (1981) Plant Sci. Lett. 21, 41-50.
3. Mann, A. F., Fentem, P. A., and Stewart, G. R. (1979) Biochem. Biophys. Res. Commun. 88, 515-521.
4. Guiz, C. B., Hirel, B., Shedlofsky, G., and Gadal, P. (1979) Plant Sci. Lett. 15, 271-277.
5. Hirel, B. and Gadal, P. (1980) Plant Physiol. 66, 619-623.
6. Iyer, R., Tuli, R., and Thomas, J. (1981) Arch. Biochem. and Biophys. 209, 628-636.
7. Wallsgrove, R. M., Lea, P. J., and Miflin, B. J. (1979) Plant Physiol. 63, 232-236.
8. Hirel, B. and Gadal, P. (1981) Z. Pflanzen Physiol. Bd. 102, 315-319.
9. Evstigneeva, Z. G., Pushkin, A. V., Solovieva, N. A., Golova, T. V., and Kretovich, W. L. (1981) Mol. Cell. Biochem. 40, 123-125.
10. Winter, H. C., Powell, G. K., and Dekker, E. E. (1982) Plant Physiol. 69, 41-47.
11. Hirel, B. and Gadal, P. (1982) Physiol. Plant. 54, 69-74.
12. Miflin, B. J. and Lea, P. J. (1976) Phytochemistry 15, 873-885.
13. Miflin, B. J. and Lea, P. J. (1977) Ann. Rev. Plant Physiol. 28, 299-329.
14. Arnon, D. I. (1949) Plant Physiol. 24, 1-15.
15. Woolfolk, C. A., Shapiro, B., and Stadtman, E. R. (1966) Arch. Biochem. Biophys. 116, 177-192.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-276.
17. Smith, K. C. (1981) In The Science of Photobiology, K. C. Smith, ed., pp. 397-417, Plenum Press, New York.
18. Beevers, L. (1976) Nitrogen metabolism in plants, pp. 199-259, Elsevier Publishing Company, New York.
19. Rowell, P., Sampaio, M. J. A. M., Ladha, J. K., and Stewart, W. D. P. (1979) Arch. Microbiol. 120, 195-200.