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ENZYMES OF CARBOHYDRATE METABOLISM IN DEVELOPING GRAINS OF HIGH LYSINE BARLEY MUTANT*

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Key Word Index—*Hordeum vulgare*; Gramineae; barley; high lysine mutant; Notch-2; NP 113; grain development; sucrose synthetase; hexokinase; phosphoglucoisomerase; phosphoglucomutase.

Abstract—Activities of UDP(ADP)-sucrose synthetase, hexokinase, phosphoglucoisomerase and phosphoglucomutase have been studied in both a high lysine mutant barley, Notch-2 and its parent NP 113 during development. The Notch-2 mutant had higher average activities of UDP(ADP)-sucrose synthetase, hexokinase and phosphoglucomutase and lower activity on a grain basis of phosphoglucoisomerase than NP 113. This reflected the decreased dry matter in the mutant grain. In general, the average activities of hexokinase and phosphoglucomutase per grain did not differ significantly between Notch-2 and NP 113. It is suggested that the lower level of phosphoglucoisomerase in Notch-2 compared with NP 113 would limit the synthesis of glucose 6-phosphate, which in turn would result in reduced starch synthesis.

INTRODUCTION

Concerted efforts to improve the quality of cereal proteins have resulted in the isolation of several high lysine mutants in barley [1], maize [2] and sorghum [3]. Unfortunately, all these mutants, including the high lysine barley mutant Notch-2 [4], have lower grain yield than the respective parent strains or commercial varieties.

Notch-2 mutant grain has lower dry matter accumulation compared with its parent NP113 [5]. Lower synthesis rather than degradation of starch appears to be mainly responsible for lower dry matter accumulation in the mutant as amylase activity in developing grains of NP 113 and Notch-2 did not differ significantly [5]. Starch synthesis in the Notch-2 mutant is not limited at the level of the supply of soluble sugars [6]. In our earlier study [7], Notch-2 grains were found to have higher average activities of starch phosphorylase and ADPG(UDPG)-pyrophosphorylase and lower activities of ADPG(UDPG)-starch synthetase than NP 113 grains during development [7].

Sucrose in the grain is cleaved by sucrose synthetase leading to the formation of UDPG(ADPG) and fructose. Fructose thus released may be converted to glucose 1-phosphate and finally to starch by successive action of hexokinase, phosphoglucoisomerase, phosphoglucomutase and starch phosphorylase. Any constraint in this pathway would also limit starch accumulation. Therefore, in this study the activities of sucrose synthetase, hexokinase, phosphoglucoisomerase and phosphoglucomutase in developing grains of NP 113 and its high lysine mutant Notch-2 barley have been investigated.

RESULTS

Sucrose synthetase

Developmental changes in the activity of ADP-sucrose synthetase and UDP-sucrose synthetase are shown in Fig. 1. UDP-sucrose synthetase activity per grain increased until day 24 where by a sharp decline at day 31 in both NP 113 and Notch-2 followed (Fig. 1a). Specific activity of UDP-sucrose synthetase also increased up to day 24 in both varieties and declined thereafter at day 31 (Fig. 1c). Activity per g dry wt remained fairly constant until about day 24 and then decreased at maturity (Fig. 1b). ADP-sucrose synthetase activity followed the developmental pattern similar to that for UDP-sucrose synthetase (Fig. 1d, e, f). Both UDP- and ADP-sucrose synthetase activities per grain were higher in Notch-2 than in NP 113 grain. On a dry wt basis the mutant Notch-2 grain had higher activities than NP 113 until day 31.

Hexokinase

Hexokinase activity per grain as well as on a dry wt basis was similar in both NP 113 and Notch-2 grains during development (Fig. 2a, b). The activity per grain increased rapidly until day 24 and then declined slightly at day 31 in both NP 113 and Notch-2 grains. Hexokinase activity per grain did not differ much between Notch-2 and NP 113 during development. The activity per g dry wt was higher in Notch-2 than its parent NP 113 at all stages except at day 10 (Fig. 2b). Specific activity of hexokinase followed a developmental pattern similar to that obtained on a per grain basis, except that the increase in specific activity continued (Fig. 2c). The specific activity did not differ between NP 113 and Notch-2 grains during early development, whereas during later stages it was higher in Notch-2 than NP 113.

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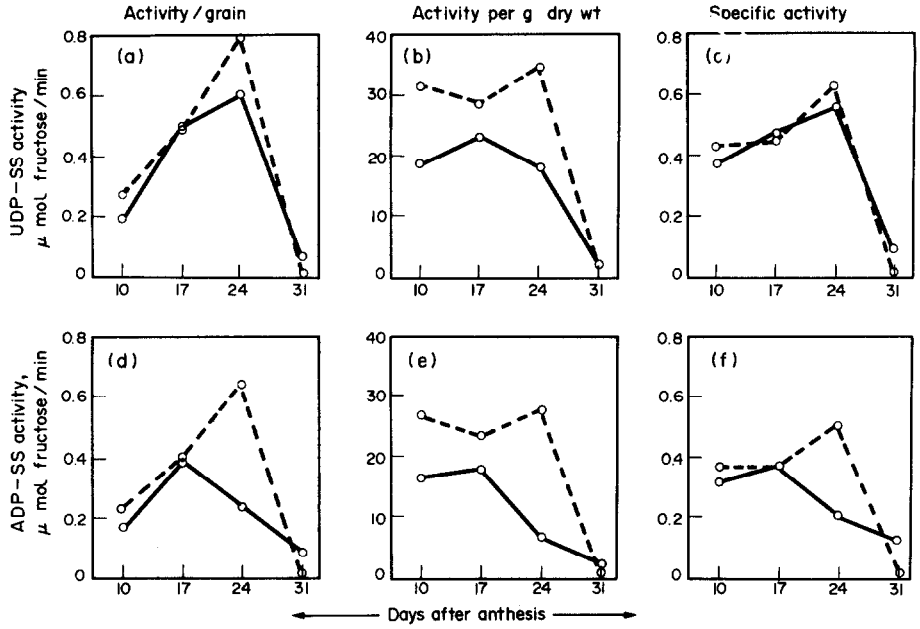

Fig. 1. Activity per grain, per g dry wt and specific activity of ADP(UDP)-sucrose synthetase in developing grains of NP 113 (——) and Notch-2 barley (– – –).

Phosphoglucoisomerase

Phosphoglucoisomerase activity per grain, on a dry wt basis, and the specific activity in developing grains of NP 113 and its mutant Notch-2 are shown in Fig. 2 (d,e,f). Activity per grain showed a peak between days 17 and 24. Throughout development, phosphoglucoisomerase activity per grain was lower in Notch-2 than in NP 113 (Fig. 2d). Activity per g dry wt decreased throughout development in NP 113 while in Notch-2 it increased at day 17 and decreased thereafter (Fig. 2e). The activity was higher in NP 113 at day 10 and in Notch-2 at day 17 whereas at days 24 and 31 the activity did not differ much between NP 113 and Notch-2. As a result, the average activity on a dry wt basis during development was nearly the same in both. Specific activity of phosphoglucoisomerase was lower in Notch-2 compared with NP 113 up to day 24 (Fig. 2f).

Phosphoglucomutase

Phosphoglucomutase activity per grain increased up to day 17 in Notch-2 and day 24 in NP 113 (Fig. 2g), whereas it decreased rapidly at day 31 in both. The activity per grain was higher in Notch-2 at day 17 and in NP 113 at day 31, whereas at other stages the activity did not differ significantly between NP 113 and Notch-2 grains. Phosphoglucomutase activity per g dry wt decreased during development both in NP 113 and Notch-2 (Fig. 2h). Activity during development, on a dry wt basis, was higher in Notch-2 except at day 31 when it was slightly lower when compared with NP 113. Specific activity of phosphoglucomutase did not differ between NP 113 and Notch-2 grains at days 10 and 31 (Fig. 2i). At days 17 and 24 it was higher in Notch-2 compared with NP 113.

DISCUSSION

Sucrose translocated from the vegetative parts to the developing grain, or synthesized photosynthetically in the ears itself, serves as the primary precursor for the synthesis of starch in cereal grains [8]. Accumulation of starch in Notch-2 mutant is not limited by the level of supply of sucrose. Further, the Notch-2 mutant grain has higher activity of ADPG(UDPG)-pyrophosphorylase and starch phosphorylase compared with NP 113 grain [7]. However, ADPG(UDPG)-starch synthetase activities are relatively lower in Notch-2 mutant [7].

During development, average activities per grain of both UDP-sucrose synthetase and ADP-sucrose synthetase were slightly higher in Notch-2 mutant compared with the parent NP 113, thus ruling out any possibility of constraints in Notch-2 at this level.

Fructose, one of the products of sucrose cleavage, in sucrose–starch transformation may be converted into glucose 1-phosphate by the successive action of hexokinase, phosphoglucoisomerase and phosphoglucomutase. Phosphoglucoisomerase activity per grain was lower in Notch-2 than in NP 113 during grain development, indicating the isomerization reaction to be one of the sites at which starch synthesis in the mutant grain may be regulated. Also in high lysine Opaque-2 maize endosperm, Joshi *et al.* [9] have suggested that a reduced level of phosphoglucoisomerase and starch synthetase might be responsible for reduced starch synthesis in this mutant compared with the normal maize endosperm. Activities of hexokinase and phosphoglucomutase do not differ substantially in the mutant Notch-2 grain and NP 113 during development. However, a lower level of the substrate glucose 6-phosphate, as a consequence of lower activity of phosphoglucoisomerase, will tend to result in relatively lower level of the product glucose 1-phosphate. This may subsequently result in lower synthesis of starch by the action of starch phosphorylase, which is believed to catalyse *de novo* synthesis of primer molecules for further chain lengthening and branching of these molecules [10].

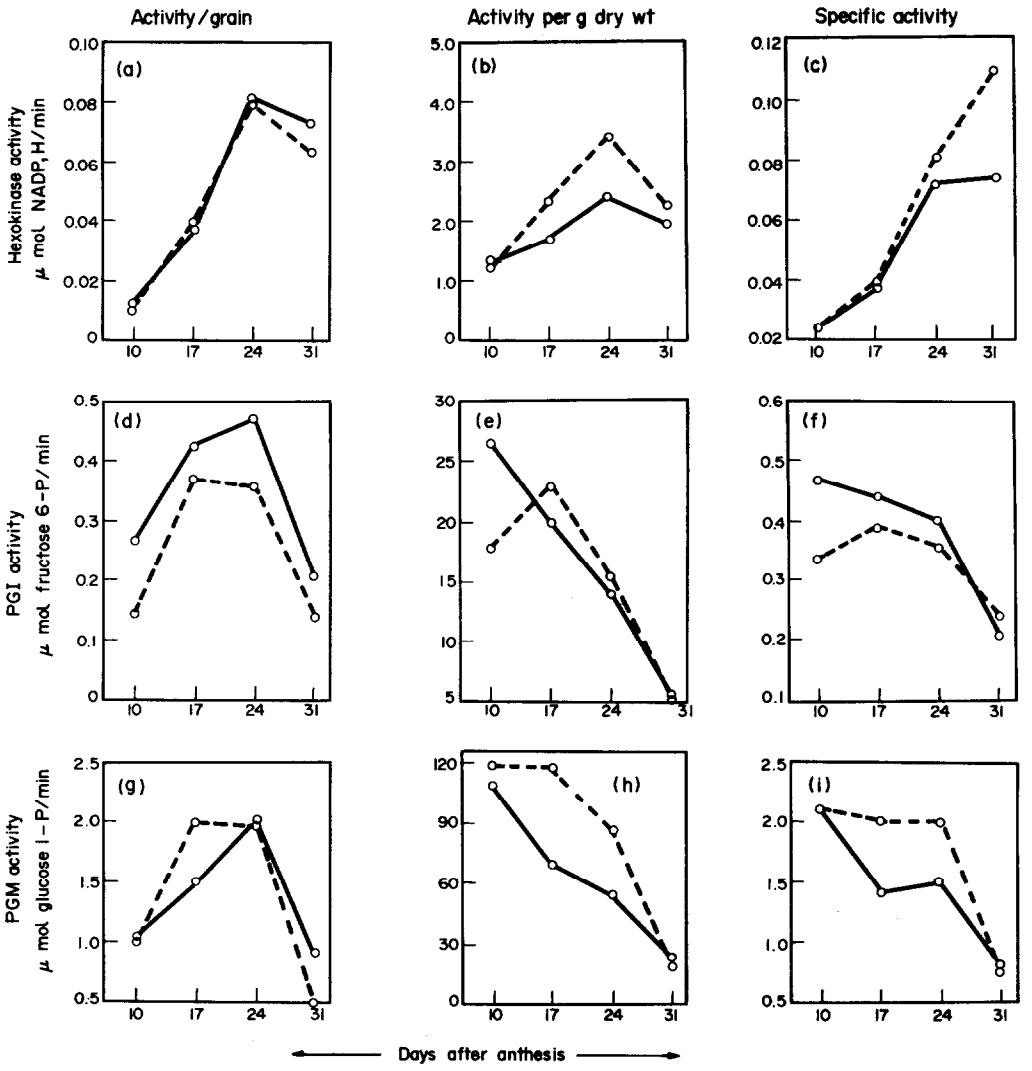

Fig. 2. Activity per grain, per g dry wt and specific activities of hexokinase (a, b, c), phosphoglucoisomerase (d, e, f), phosphoglucomutase (g, h, i) in developing grains of NP 113 (——) and Notch-2 barley (– – –).

EXPERIMENTAL

Plant material. NP 113 and Notch-2 barley plants were grown in pots in the winter (rabi) season of the crop year 1977–1978. The ears were harvested at 10, 17, 24 and 31 days after anthesis, the grains dehusked and stored in liquid N_2 until further use.

Enzyme extraction. Hexokinase, phosphoglucoismerase and phosphoglucomutase were extracted by hand-grinding grains in liquid N_2 with 50 mM Tris–Cl buffer (pH 7.6). Tris–Cl buffer (50 mM, pH 7.3) containing 0.1 mM EDTA, 0.1 mM DTT and 1% PVP was used for extraction of sucrose synthetase. The homogenates were centrifuged at 25 000 *g* for 20 min at 4° and the supernatants decanted and used for assay of enzymes.

Enzyme assay. ADP(UDP)-sucrose synthetase: The activity was assayed according to [11]. The reaction mixture in a final vol. of 0.9 ml contained sucrose, 250 μmol; ADP(UDP) (pH 7.0), 2.5 μmol; NaF, 5 μmol; and enzyme extract, 100 μl. Assay was carried out at 37° for 30 min. Fructose released was measured by Nelson's reagent [12] against the control, in which UDP was omitted.

Hexokinase: The activity was assayed by coupling the product glucose 6-phosphate with glucose 6-phosphate dehydrogenase reaction. The reaction mixture contained HEPES buffer (pH 7.5), 120 μmol; glucose, 5 μmol; ATP, 10 μmol; $MgSO_4$, 10 μmol; enzyme prepn, 100 μl; glucose 6-phosphate dehydrogenase, 2 units; NADP, 2, μmol, and H_2O to a final vol. of 0.7 ml [13]. Glucose was omitted in the control set.

Phosphoglucoisomerase: The activity was assayed by incubating the enzyme prepn with glucose 6-phosphate and measuring the formation of fructose 6-phosphate according to [14]. The reaction mixture in a final vol. of 0.1 ml contained HEPES buffer (pH 8.0), 100 μmol; glucose 6-phosphate, 4 μmol and enzyme prepn 50 μl [13]. It was incubated at 30° for 5 min. In the control the enzyme was omitted.

Phosphoglucomutase: Assay for phosphoglucomutase was based on [13]. The reaction mixture contained HEPES buffer (pH 7.4), 60 μmol; $MgSO_4$, 5 μmol; cysteine, 5 μmol; enzyme prepn, 100 μl; glucose 1-phosphate, 4 μmol; and H_2O to a final vol. of 1 ml. Incubation was carried out at 30° for 5 min. The contents after incubation were heated in a boiling H_2O bath for 10 min and cooled. Inorganic phosphate was determined [15] in a suitable aliquot, after centrifugation, against a control in which 1 ml 5 N H_2SO_4 was added before addition of the enzyme prepn to the reaction mixture.

Soluble protein was estimated as given in [16]. For all enzymes two independent extractions were carried out for each sample and then analysed again in duplicate. The values reported in this study are an average of values for two independent extractions which agreed closely.

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REFERENCES

1. Munck, L. (1972) *Symposium on Seed Protein* (Inglett, G. E., ed.) p. 144. AVI, Westport, Conn., U.S.A.
2. Mertz, E. T., Bates, L. S. and Nelson, O. E. (1964) *Science* **145**, 279.
3. Axtell, J. D., Van Scoyos, S. W., Christensen, P. J. and Ejeta, G. (1979) *International Symposium on Seed Protein Improvement in Cereals and Grain Legumes*. I.A.E.A., Vienna.
4. Bansal, H. C. (1970) *Curr. Sci.* **39**, 494.
5. Sen, K. and Mehta, S. L. (1980) *Phytochemistry* **19**, 1323.
6. Batra, V. I. P. (1979) Ph.D. thesis, P.G. School, IARI, Delhi.
7. Batra, V. I. P. and Mehta, S. L. (1981) *Phytochemistry* **20**, 635.
8. Porter, H. K. (1962) *Annu. Rev. Plant Physiol.* **13**, 303.
9. Joshi, S., Lodha, M. L. and Mehta, S. L. (1980) *Phytochemistry* **19**, 2305.
10. Khan, A. A. (1978) *The Physiology and Biochemistry of Seeds*, p. 447. Elsevier, New York.
11. Avigad, G. and Milner, Y. (1966) *Methods in Enzymology* (Neufeld, E. F. and Ginsburg, V., eds.) Vol. VIII, p. 341. Academic Press, New York.
12. Nelson, N. (1944) *J. Biol. Chem.* **153**, 375.
13. Tsai, C. Y., Salamini, F. and Nelson, O. E. (1970) *Plant Physiol.* **46**, 299.
14. Roe, J. H. (1934) *J. Biol. Chem.* **107**, 15.
15. Fiske, C. H. and Subbarow, Y. (1925) *J. Biol. Chem.* **66**, 375.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.