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# Repression of the Plastidic Isoenzymes of Aldolase, 3-Phosphoglycerate Kinase, and Triosephosphate Isomerase in the Barley Mutant "albostrians"

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## ABSTRACT

**White leaves of the mutant line albostrians and green leaves of the wild-type cultivar Salome of barley (*Hordeum vulgare* L.) were screened for the presence of plastidic and cytosolic isoenzymes of sugar-phosphate metabolism. Isoenzyme separation was achieved by anion-exchange chromatography on Fractogel TSK DEAE-650(S). The mutant tissue had a markedly reduced level of plastidic 3-phosphoglycerate kinase, triosephosphate isomerase, and aldolase activity. In contrast, the activity of plastidic glucosephosphate isomerase, fructose 1,6-bisphosphatase, 6-phosphogluconate dehydrogenase, starch phosphorylase, and ADP-glucose pyrophosphorylase was in the same range as in wild-type leaf tissue. The activity of the corresponding cytosolic isoenzymes (including UDP-glucose pyrophosphorylase) showed essentially no differences in mutant and wild type. The same trend was observed in dark-grown mutant and wild-type leaves. Interestingly, the total activity levels of all isoenzymes were about the same when comparing dark-grown and light-grown mutant or wild-type plants. From these data, it is concluded that mutant leaves exhibit a selective decrease of a subgroup of plastidic isoenzymes associated with the Calvin cycle.**

The nuclear gene albostrians in barley (*Hordeum vulgare* L.) induces plastome mutations that become phenotypically expressed as pure white leaves or white stripes in otherwise green leaves (14). Plastids of white tissue lack 70S ribosomes and, as a consequence, are unable to synthesize proteins that are encoded by chloroplast DNA, such as the large subunit of Rubisco (6, 17). Despite this defect, the ribosome-deficient plastids are able to multiply (3, 31), to replicate their DNA (31), and to import nuclear gene-encoded proteins (33). Obviously, these important functions depend solely on genes residing in nuclear DNA. The genes involved must be active and expressed at levels comparable to those of green, Wt[1] leaves, because the amounts of plastids and plastid DNA are nearly the same in green and white leaves.

However, some nuclear genes are influenced by the plastid mutation. It was shown previously that the small subunit of Rubisco (6) and the Calvin cycle enzymes GAP DH and PRK are absent or present in only very low amounts in the mutant compared with green, Wt leaves (7, 13). From these results, it was concluded that chloroplasts may control the expression of some but not all nuclear genes. During the last several years, evidence has accumulated to indicate that the expression of certain light-induced nuclear genes is influenced by the developmental stage of the plastids. A plastid signal was postulated to control the transcription of such genes (for review, see refs. 2 and 34). It has been shown that the presence of undifferentiated plastids leads to a low level of mRNA for the small subunit of Rubisco and light-harvesting Chl protein in cells of white tissue of albostrians barley (16).

The white Mt plastids also pose the physiological problem that they are capable of accumulating starch in dark-grown leaves (13). Therefore, in contrast to some of the Calvin cycle enzymes (6, 7), the enzymes for starch biosynthesis must be functional in the plastids. In particular, the lack of some Calvin cycle enzymes raises the question of which part of gluconeogenesis is functional in the Mt plastids.

Thus, our goal was to determine which of the sugar-phosphate-metabolizing isoenzymes are present, reduced, or absent in albostrians Mt cells and which enzymes could contribute to the biosynthesis of starch. In addition, the question of whether or not the cytosolic isoenzymes of sugar-phosphate metabolism would be affected by the albostrians mutation was addressed. Finally, it was interesting to know to what extent light, by morphogenetic means, interferes with the expression of the nuclear DNA-encoded plastidic and cytosolic isoenzymes of sugar-phosphate metabolism. Irradiation with white or morphogenetic light via phytochrome action has previously been shown to enhance the activity of one group of plastidic isoenzymes (including GAP DH and aldolase), but not of a second (including GPI, PGM, and 6PG DH) nor any of the corresponding cytosolic isoenzymes in etiolated spinach and mustard seedlings (9, 20, 27, 30).

[1] Abbreviations: Wt, wild type; GAP DH, glyceraldehyde 3-phosphate dehydrogenase; PRK, phosphoribulokinase; GPI, glucosephosphate isomerase; PGM, phosphoglucomutase; 6PG DH, 6-phosphogluconate dehydrogenase; 3-PGK, 3-phosphoglycerate kinase; G6P DH, glucose 6-phosphate dehydrogenase; TPI, triosephosphate isomerase; FBPase, fructose 1,6-bisphosphatase; UDPG, uridine diphosphoglucose; PP'ylase, pyrophosphorylase; P'ylase, phosphorylase; ADPG, adenosine diphosphoglucose; Mt, mutant; oPP-Pathway, oxidative pentose phosphate pathway.

## MATERIALS AND METHODS

### Materials

Seeds of barley (*Hordeum vulgare* L.) were germinated and grown for 8 d in soil. Plants were kept either in daylight (400–600 $\mu$E $m^{-2}$ $s^{-1}$; 16-h photoperiod) or in constant darkness at 20°C. Seeds of Wt plants (cv Salome) were obtained from the Saat- und Pflanzengut (Potsdam-Rehbrücke, Germany), whereas seeds of the mutant line albostrians 4205 (14) were provided by Dr. G. Künzel, Institute of Genetics and Horticulture Research, Gatersleben, Germany.

### Isoenzyme Separation

Five grams of leaf material from 8-d-old plants were homogenized with 40 mL grinding buffer (10 mM potassium phosphate, 20 mM 2-mercaptoethanol, pH 8.6) in a Waring Blendor at full speed for 3 × 20 s at 4°C. The homogenate was squeezed through two layers of Miracloth and centrifuged for 20 min at 40,000*g*. The supernatant was readjusted to pH 8.6 with 5 M KOH and the conductivity was lowered to <3 mS by diluting with 20 mM 2-mercaptoethanol, pH 8.6. The solution was then applied to a Fractogel TSK DEAE-650(S) (Merck, Darmstadt, Germany) column (1 × 10 cm) equilibrated with grinding buffer. Proteins were eluted with a 200-mL gradient of 0 to 0.3 M KCl in grinding buffer. The flow rate was 2 mL $min^{-1}$. Fractions of 2 mL were collected and measured for salt concentration and enzyme activities. The activities recovered from the column were usually between 70 and 90% (and at least 55%) of the loaded activities.

### Assays

Enzyme activities were assayed at 20°C and changes in absorbance at 340 nm were monitored with an Eppendorf photometer (Netheler and Hinz, Hamburg, Germany). Aldolase (EC 4.1.2.13) (19), 3-PGK (EC 2.7.2.3) (18), GPI (EC 5.3.1.9) (28), G6P DH (EC 1.1.1.49), 6PG DH (EC 1.1.1.43) (29), PGM (EC 2.7.5.1) (23), TPI (EC 5.3.1.1) (35), FBPase (EC 3.1.3.11) (21), and UDPG PP'ylase (EC 2.7.7.9) (32) were assayed by published procedures. The activity of starch P'ylase (EC 2.4.1.1) was assayed in the direction of glucose 1-phosphate formation from starch in an end-point assay. The reaction mixture contained soluble starch (10 mg/mL), 25 mM $KH_2PO_4$, 25 mM imidazole, pH 7.0, and 0.2 mL of enzyme solution (4–15 $\mu$g protein) in a final volume of 0.4 mL. After incubation for 18 h at 30°C, the samples were boiled for 5 min to stop the reaction. The samples were centrifuged for 5 min at 12,000*g* and aliquots (0.1 mL) of the supernatant were used for the estimation of glucose 1-phosphate in a coupled enzymic assay according to Mateyka and Schnarrenberger (22). ADPG PP'ylase (EC 2.7.7.28) was determined in a modified assay according to Sowokinos (32). The reaction mixture contained 0.2 mL of 1 mM ADPG, 5 mM $MgCl_2$, 10 mM DTT, 3 mM PPi, 160 mM glycylglycine, pH 7.5, and 0.2 mL of enzyme solution (12–20 $\mu$g protein). After incubation for 2 h at 30°C, the samples were boiled for 5 min and then centrifuged at 12,000*g* for 5 min. Aliquots (0.1 mL) of the supernatant were used for the coupled enzymic assay.

ford (8). Salt concentration was determined by conductivity measurements.

### Identification of Chloroplast Isoenzymes

To determine the intracellular compartmentation of the isoenzymes, intact chloroplasts were isolated by differential centrifugation (28). Isolated chloroplasts were suspended in grinding buffer (see above), sonicated for 5 × 10 s at 0°C, and centrifuged for 10 min at 18,000*g*. Soluble proteins were applied to a Fractogel-DEAE column as described above.

## RESULTS

To analyze the occurrence of cytosol- and plastid-specific isoenzymes of sugar-phosphate metabolism in Wt and Mt barley leaves, we have separated these isoenzymes by anion-exchange column chromatography on Fractogel-DEAE. Figure 1 shows the isoenzyme activity profile for aldolase and 6PG DH from Wt and Mt leaves as examples representative for all studies. Profiles were obtained for plants grown either in the light or in the dark and for isolated Wt chloroplasts. Experiments were done independently two to four times. Absolute and relative activities varied between the experiments, but the general trend was always the same. The results of one representative experiment are shown. The elution profiles of the plastidic and cytosolic isoenzymes were very similar to previously published results from other plant tissues (19, 22, 23, 26, 29, 32).

It is evident from the data in Figure 1 that plastidic aldolase activity was strongly, but not totally, reduced, whereas the cytosolic aldolase was not markedly affected by the mutation. For 6PG DH, both the plastidic and cytosolic isoenzymes showed little difference between the Mt and Wt. Light had no distinct effect on total and relative activities.

The isoenzymes of five additional enzymes, including 3-PGK, TPI, FBPase, GPI, and P'ylase were separated. For G6P DH and PGM, a resolution of isoenzymes could not be achieved. In spinach, ADPG PP'ylase and UDPG PP'ylase are known to be plastid- and cytosol-specific enzymes, respectively (25, 32). Our data confirm this finding for the major activities of the two enzymes in barley leaves. However, we detected a second isoenzyme of UDPG PP'ylase that contributes <1% and about 4% of the total activity in light-grown and dark-grown plants, respectively. Due to the low activity, its intracellular location could not be determined.

The total isoenzyme activities in extracts from Wt and Mt leaves, as recovered from the Fractogel columns, are compiled in Table I. When the relative isoenzyme activities of light-grown (16 h light, 8 h darkness) Wt and Mt leaves (Fig. 2A) are compared, it is apparent that the activity of the Calvin cycle-associated plastidic isoenzymes of aldolase, 3-PGK, and TPI was <15% in the mutant. The corresponding cytosolic isoenzymes were little, if any, affected by the mutation. For FBPase, GPI, 6PG DH, and P'ylase, the activity of the plastidic and cytosolic isoenzymes showed only minor variations between Wt and Mt leaves. The activities of G6P DH and PGM, for which no isoenzymes could be resolved, appeared to be not affected by the mutation, because parallel experiments showed that these differences were within the limits

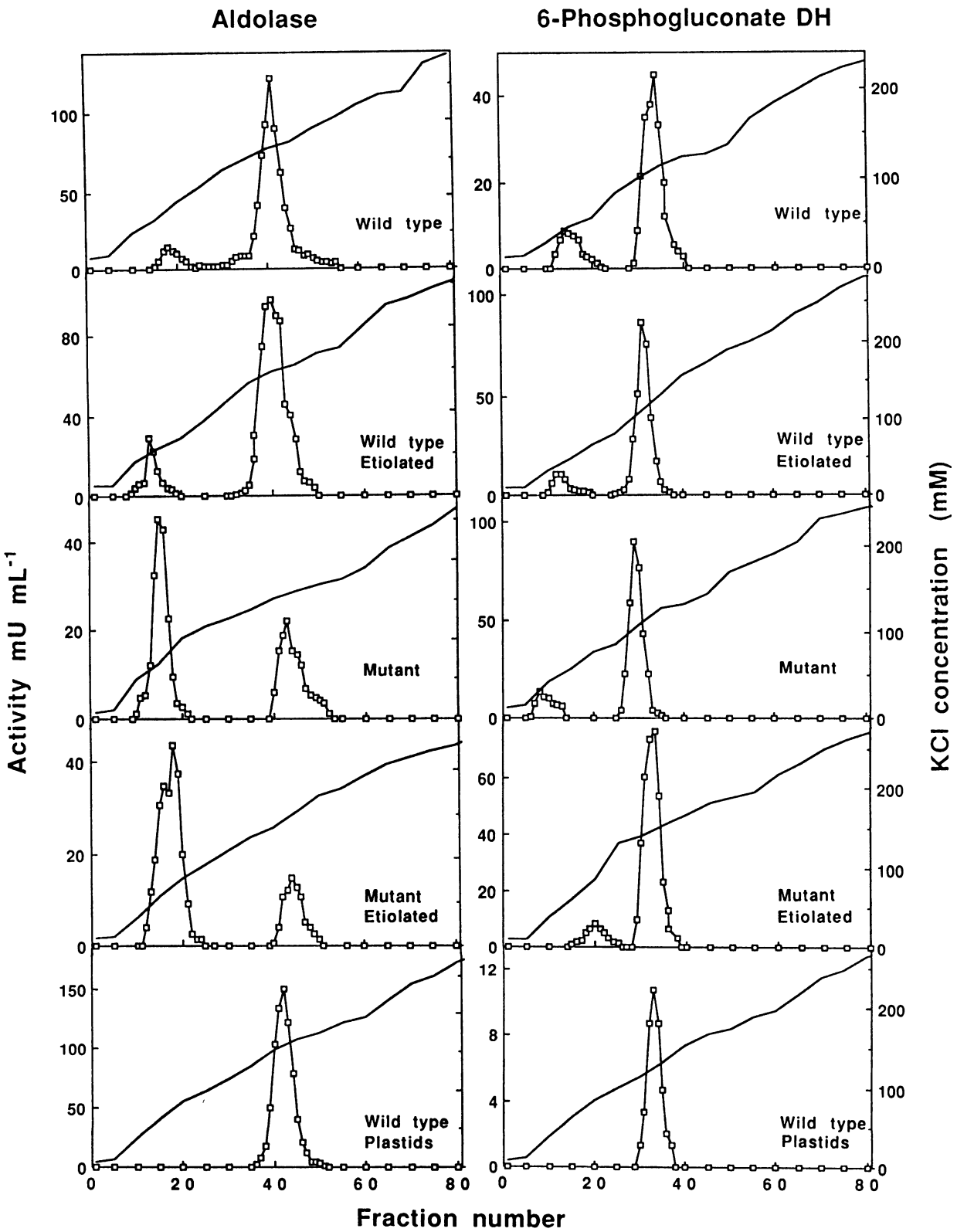


**Figure 1.** Elution pattern of isoenzymes of aldolase and 6-P-gluconate dehydrogenase after chromatography on Fractogel-DEAE. Note that the plastidic aldolase is repressed by the albostrians mutation, whereas the plastidic 6PG DH is not. To identify the plastidic isoenzymes, a soluble extract from isolated Wt chloroplasts was fractionated under the same conditions as for total soluble leaf protein. (Activity in munit/mL = nmol $mL^{-1}$ $min^{-1}$).

**Table I.** *Total Enzyme Activity Recovered from Fractogel-DEAE Columns*

| Enzyme | Light-Grown Plants | | Dark-Grown Plants | |
|---|---|---|---|---|
| | Wt | Mt | Wt | Mt |
| | *μmol/min* | | | |
| Aldolase | 1.9 | 0.66 | 1.6 | 0.69 |
| 3-PGK | 54 | 14 | 63 | 11 |
| TPI | 115 | 55 | 171 | 64 |
| FBPase | 0.65 | 0.56 | 0.63 | 0.47 |
| G6P DH | 0.71 | 1.0 | 0.56 | 0.51 |
| PGM | 19 | 15 | 10 | 6 |
| GPI | 12 | 6 | 9 | 5 |
| 6PG DH | 0.62 | 0.92 | 0.86 | 0.91 |
| P'ylase | 0.041 | 0.058 | 0.051 | 0.078 |
| UDPG PP'ylase | 0.027 | 0.027 | 0.037 | 0.039 |
| ADPG PP'ylase | 0.056 | 0.044 | 0.065 | 0.060 |

of experimental error. The same was observed for ADPG PP'ylase and UDPG PP'ylase. Light seems to have little or no effect on the measured enzyme activities. In general, the absolute (Table I) and relative activities (Fig. 2B) of the cytosolic and plastidic isoenzymes of dark-grown seedlings were found to be similar to activities determined in extracts from leaves of seedlings grown under a 16:8 h light-dark regimen.

## DISCUSSION

We observed relatively low activities of one group of plastidic isoenzymes of sugar-phosphate metabolism (3-PGK, TPI, and aldolase) in the Mt tissue of both light-grown and dark-grown plants. Previously, a similar observation was reported for this Mt concerning Rubisco (6, 16), PRK, and NADP-dependent GAP DH (7). The activity of the other plastidic isoenzymes were not affected markedly by the plastome mutation. These findings suggest a specific and strong

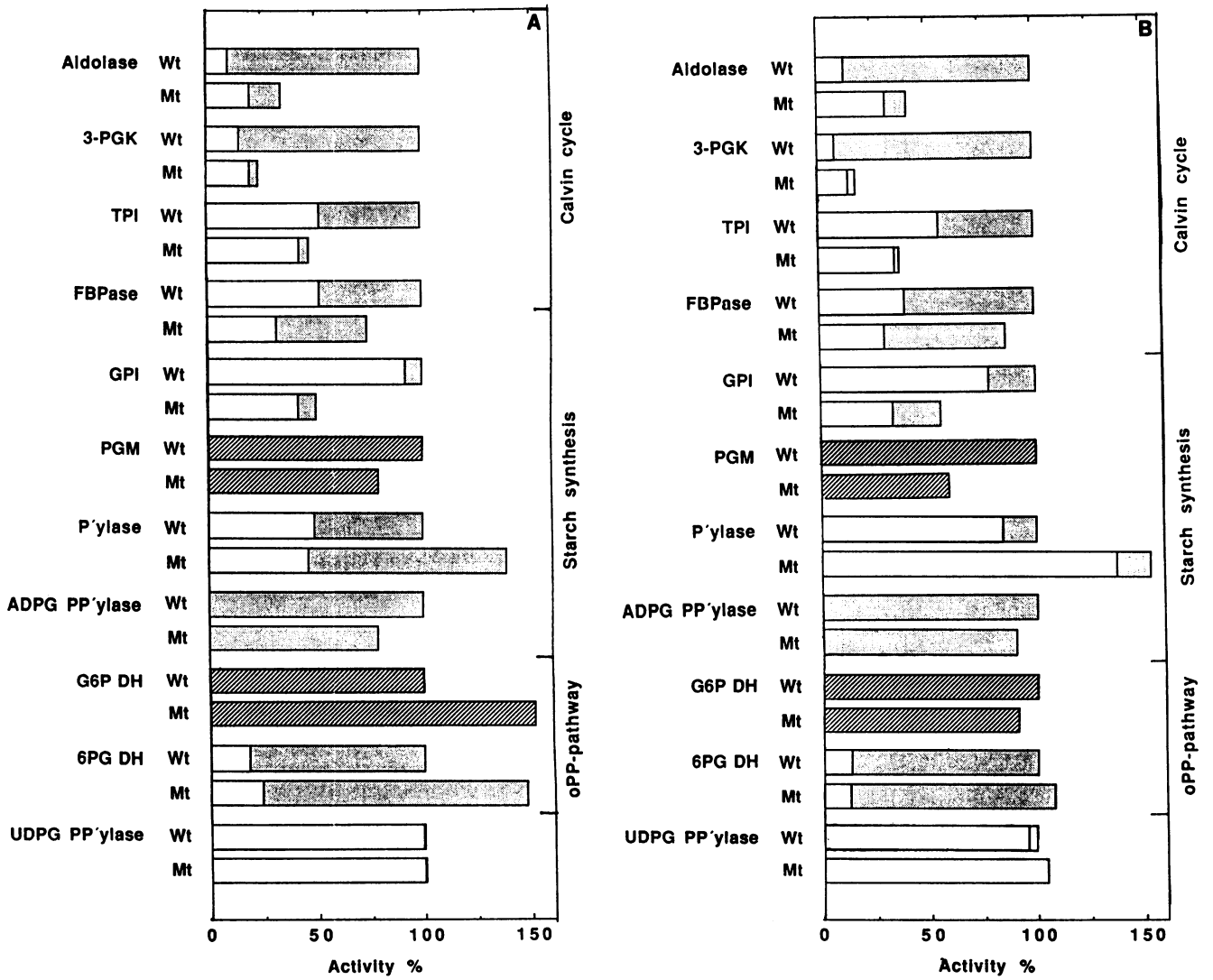


**Figure 2.** Relative enzyme activities in fractionated extracts from light-grown (A) and dark-grown (B) leaves of Wt and Mt seedlings. Total activity in Wt (see Table I) was taken as 100%. □, Cytosolic activity; □, plastidic activity; ▨, cytosolic and plastidic activity were not separated.

pertubation of Calvin cycle enzymes. An exception is the plastidic FBPase, which participates in the Calvin cycle but is not markedly affected. In contrast with the other Calvin cycle enzymes, this enzyme is also involved in plastidic starch synthesis. Also, the other studied enzymes of starch metabolism (GPI, P'ylase, and ADPG PP'ylase) and enzymes of the oPP-Pathway (G6P DH and 6PG DH) are either not affected or exhibit a slightly increased activity in white leaves. The corresponding cytosolic isoenzymes are virtually not influenced by the plastome mutation. Considering that light-harvesting Chl protein (6, 16) and coupling factor (4) are also decreased, one may postulate that predominantly photosynthetic chloroplast functions (plastid- and nuclear-encoded) are repressed by the plastome mutation and that other nuclear-encoded plastid functions are not affected. Other functions affected little, if any, by the mutation include plastid division (3, 31), DNA replication (31), plastid protein import (33), enzymes of Chl-biosynthesis (W. Hess, B. Fiedler, T. Börner, unpublished data), ferredoxin-NADP oxidoreductase (1), nitrite reductase (5), the oPP-Pathway, and starch-metabolizing enzymes (this study). The only exception is nitrate reductase, a cytosolic, nonphotosynthetic enzyme that is absent in the Mt (5).

In previous reports on the regulation of isoenzymes of sugar-phosphate metabolism, it was observed (27) that the plastidic isoenzymes of one group of activities, *i.e.* NADP-dependent GAP DH and aldolase (9, 20, 30), were reduced in etiolated cotyledons of the dicots spinach, radish, and mustard, and enhanced by light via phytochrome action. The plastidic isoenzymes of another group (GPI, PGM, and 6PG DH) were not greatly influenced by light. The corresponding cytosolic isoenzymes were neither decreased in the dark nor modulated by light (9, 20, 30). The light-dependent expression pattern characteristic of dicotyledonous plants seems to be different in monocots, *e.g.* in rye (11) and in barley leaves (this study). Although the activities of PRK and GAP DH were clearly light-inducible in Wt and Mt barley (7), no distinct effect of light could be observed in the case of aldolase, 3-PGK, and TPI (Table I, Fig. 2).

The albostrians Mt is also remarkable from a physiological point of view because the Chl-deficient, photosynthetically inactive Mt cells are able to accumulate starch in the dark (13). In the present paper, we provide evidence that all enzymes (except starch synthase, which was not assayed) leading from/to FBPase to/from starch are present in the Mt with similar activities as in Wt cells. Therefore, it is concluded that the participating enzymes are synthesized in the cytosol as previously proposed (13). However, the question remains of which reaction sequence is used in the Mt plastids for starch biosynthesis. Carbon import into chloroplasts has been shown to be facilitated by 3-P-glycerate and dihydroxyacetone-phosphate via the phosphate translocator (15). In amyloplasts, glucose 6-phosphate as well as triose phosphates are transported by the phosphate translocator (15). The question of which translocator activity is responsible for starch biosynthesis in the Mt plastids is intriguing. Alternatively, a glucose 6-phosphate translocator might not be postulated if the remaining activity of the Calvin cycle isoenzymes is sufficient to sustain rates of starch biosynthesis from triose phosphates in the albostrians Mt.

One of the most characteristic features of the albostrians Mt is the lack of 70S chloroplast ribosomes. The same effect can also be induced by growing rye seedlings at an elevated temperature of 32°C (10). This treatment causes a 72% repression in the specific activity of plastidic NADP-dependent GAP DH (26) and a total absence of Rubisco from the isolated plastid fraction (12). Other plastidic isoenzymes such as G6P DH, 6PG DH, ADPG PP'ylase, starch synthase, and starch P'ylase were not affected by heat treatment, whereas 3-PGK, GPI, PGM, and hexokinase were two- to fourfold higher in heat-treated than in control leaves (26). This stands in contrast with the barley Mt, in which not only Rubisco and GAP DH, but also four other Calvin cycle enzymes are repressed. Therefore, the mechanisms in rye and barley appear to be different. It remains to be determined what are the molecular causes for the differential regulation of activity of plastidic and cytosolic isoenzymes of sugar-phosphate metabolism in the abostrians Mt.

A chloroplast signal was postulated to control the expression of nuclear genes that encode plastid proteins that were found at very low levels in white leaves of the albostrians barley Mt and norflurazon-treated plants (2, 24, 34). The results reported in this paper are in agreement with the view that the nuclear genes encoding the enzymes of the Calvin cycle, except FBPase, are under the control of such a developmental signal. Furthermore, the data indicate that this signal is mainly important for the expression of those genes that encode proteins necessary only in green, photosynthetically active chloroplasts, and not in other types of plastids, such as those found in nongreen tissue.

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