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RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE AND PHOSPHOENOLPYRUVATE CARBOXYLASE ACTIVITY IN BARLEY AND ITS VIRESCENS MUTANT

Teresa J. Barankiewicz*, Radovan B. Popovic and Saul Zalik
Department of Plant Science, University of Alberta
Edmonton, Alberta, Canada, T6G 2E3

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SUMMARY

The most pronounced difference in carboxylase activities between Gateway barley and its virescens mutant occurred in seedlings grown in the light for 4 days. At this stage seedlings of Gateway had 11 times more ribulose-1,5-bisphosphate carboxylase and 18 times higher activity per gram fresh weight than the mutant. Although phosphoenolpyruvate carboxylase represented only 8% of the sum of the two carboxylase activities for the normal it accounted for 70% in the mutant. It represented 17% and 30% of the activity in 8-day dark-grown seedlings of the normal and mutant respectively but accounted for only 3% and 11% after 8 days in light. The high activity of phosphoenolpyruvate carboxylase in young seedlings of the mutant grown in light suggests an adaptation within the mutant during the period when the amount of RuBP-carboxylase is low.

INTRODUCTION

The virescens mutant of Gateway barley represents a single nuclear gene mutation which manifests itself by low pigment levels, poorly developed chloroplasts and less protein than the normal for greening seedlings (1,2,3). It has also been found that the lag in chlorophyll synthesis in the mutant seedlings is accompanied by much lower levels of the main soluble leaf protein (Fraction 1 protein).

In etiolated and greening leaves of a number of C_3 species it has been found that PEP-carboxylase plays a role in CO_2 fixation (4,5,6,7). Since the development of the virescens mutant resembles greening of etiolated plants in light, it provides a means of examining the development of CO_2 fixation under conditions where a delay in greening

*Present address: Department of Plant Metabolism, Institute of Botany, University of Warsaw, Warsaw, Poland.
Abbreviation - PVP: polyvinylpyrrolidone.

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is caused by factors other than the absence of light. Therefore this study was undertaken to determine the quantity and activity of RuBP-carboxylase and PEP-carboxylase in light and dark-grown seedlings of normal Gateway barley and its virescens mutant. In addition to characterization of the mutant in relation to these two carboxylases it was thought that the results might elucidate the role of PEP-carboxylase in this C_3 plant.

MATERIALS AND METHODS

Enzyme Preparation

Barley (Hordeum vulgare L.) cv. Gateway and its virescens mutant were grown in trays of vermiculite kept moist with nutrient solution in growth cabinets under continuous light of 600 ft-c or in darkness at 21°C. Leaf tissue was ground in a chilled mortar with pestle using acid washed sand in the medium which consisted of 0.01 M EDTA; 0.01 M KCl; 0.001 M $MgCl_2$; 10 mM DTT and 0.15 M Tris-HCl buffer pH 7.5 (8). The ratio of tissue to extraction medium was 1:2 (w/v). The leaf homogenate was squeezed through two layers of Miracloth and the filtrate was centrifuged for 5 min at 2000x*g* followed by centrifugation at 35000x*g* for 30 min. The final supernatant was used in the estimation of PEP-carboxylase activities.

RuBP-carboxylase was also prepared by the one-step method essentially as described by Goldthwaite and Bogorad (9). Leaf tissue was ground in 0.4 M Tris-HCl buffer pH 8.0 containing 20 mM $MgCl_2$, 20 mM 2-mercaptoethanol and 1% PVP. Following the initial filtration and centrifugation steps, 1 ml samples of the crude supernatant were layered on 0.2 to 0.7 M linear sucrose gradients made up in 10 mM $MgSO_4$, 1 mM 2-mercaptoethanol and 0.02 M Tris-HCl buffer pH 8.0. After centrifugation for 30 h in an SW-27 rotor at 26000 rpm at 3°C, the gradients were eluted, monitored at 280 nm and fractions were collected for enzyme assay. All of the above operations were conducted at 2 to 4°C.

Enzyme Assays

RuBP-carboxylase and PEP-carboxylase activities were determined by measurement of the acid-stable radioactivity produced in the reaction between labelled bicarbonate and RuBP or PEP respectively. RuBP-carboxylase was assayed essentially as described by Chollet and Ogren (10), Fair et al (8) and Jensen and Bahr (11). The reaction mixture consisted of 60 mM Tris-HCl pH 8.0; 6 mM $MgCl_2$; 0.2 mM EDTA; 0.4 mM RuBP; 40 mM $NaHCO_3$ labelled with [^{14}C]$NaHCO_3$(0.25 μCi/μ mole). The enzyme was activated by preincubation for 15 min at 45°C in the presence of 10 mM $NaHCO_3$ and 40 mM $MgCl_2$. Ten to 20 μg of enzyme preparation was added to the reaction mixture to a final volume of 0.5 ml and the reaction was run for 1 or 2 min at 25°C.

PEP-carboxylase activity was estimated by the method of Chollet and Ogren (10). The reaction mixture consisted of 30 mM Tris-HCl pH 8.0; 3 mM $MgCl_2$; 0.1 mM EDTA; 1.5 mM DTT; 2 mM PEP; 5 mM sodium glu-

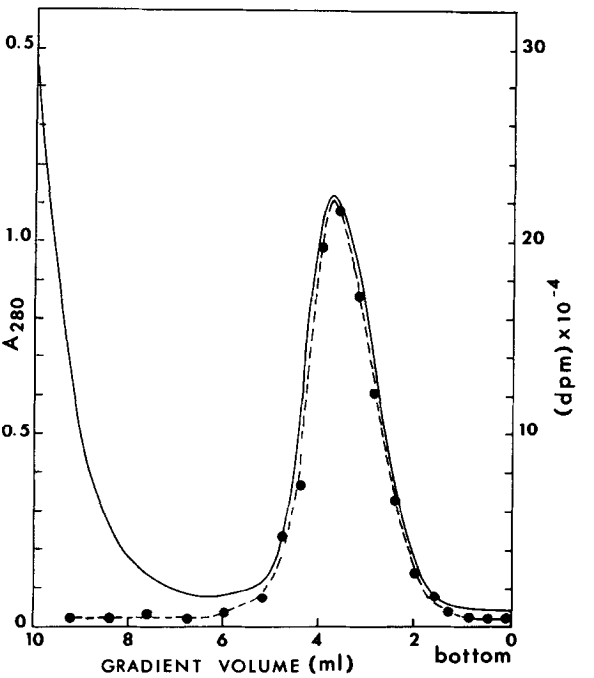

Figure 1. Isolation and activity of RuBP-carboxylase from barley leaf extracts. Samples of 1.0 ml of the crude extract were layered on a 0.2 to 0.7 M linear sucrose gradient. After centrifugation for 30 hr at 26,000 rpm at 3°C in an S.W.-27 rotor the gradients were eluted from the bottom, monitored at 280 nm and 0.75 ml fractions were collected. Aliquots of these fractions were assayed for carboxylase activity. Solid line is absorbance, hatched line is activity.

tamate and 5 mM $NaHCO_3$ labelled with [^{14}C]$NaHCO_3$(0.25 µCi/µ mole). The reaction volume was 0.5 ml and was run for 6 min at 30°C. For both enzymes the reaction was stopped by the addition of 0.5 ml of 6 N acetic acid and radioactivity was counted in a scintillation counter. Protein was estimated by Bradford's method (12) using BSA as the standard. All the results reported are based on at least 4 independent experiments which were in close agreement.

RESULTS

The elution profile of RuBP-carboxylase shown in Fig. 1 is characteristic of that obtained from subjecting extracts of the wild type and virescens seedlings to the one-step separation (9) and as shown the RuBP-carboxylase activity correlated directly with the elution profiles. However, the normal barley and its virescens mutant differed

TABLE 1: RuBP and PEP carboxylase activities in seedlings of Gateway barley(N) and its virescens mutant(M) grown in light or dark.

	RuBP carboxylase activity μM HCO_3/mg protein/hr		RuBP carboxylase activity μM HCO_3/g fr.wt./hr		PEP carboxylase activity μM HCO_3/g fr. wt./hr			
Age in Days	1 N	2 M	3 N	4 M	5 N	5/3+5 as %	6 M	6/4+6 as %
Light								
4	106 (3.08)*	64 (0.28)	327	18	29	8	41	70
6	81 (4.19)	64 (1.69)	339	108	---		---	
8	71 (4.65)	70 (1.70)	330	119	11	3	16	11
Dark								
8	40 (2.55)	41 (1.10)	102	45	22	17	19	30

*the values in brackets are mg enzyme protein/g fr. wt.

both in the amount and activity of RuBP-carboxylase. The largest difference between the lines was for seedlings grown 4 days in light, when the normal had 11 times more RuBP-carboxylase than the mutant (3.08 vs 0.28 mg protein/g fr.wt.) with 40% higher specific activity and 18 times higher RuBP-carboxylase activity per gram fresh weight than for the mutant (Table 1). A decline in the difference between the two lines occurred by 8 days in light resulting from an increase in the amount of enzyme in the mutant and the decrease in the specific activity of the enzyme of the normal.

As for RuBP-carboxylase activity, the sharpest difference between the PEP-carboxylase activity of the two lines was also with 4 days light. At this stage PEP-carboxylase represented only 8% of the total activity of the two carboxylases in the normal but accounted for 70% of the carboxylase activity in the mutant (Table 1). However, with further development of the mutant seedlings in light (8 days) it accounted for only 11% of the carboxylase activity compared to 3% for the normal. For seedlings grown in the dark for 8 days the proportion

of the activity in PEP-carboxylase was considerably higher in both lines, being 17% and 30% for the normal and mutant respectively.

DISCUSSION

This study has extended our characterization of the virescens mutant of Gateway barley and the general finding that it displays a delay in development similar to the slow greening of etiolated normal seedlings correlates well with our earlier studies in which we compared the two lines on the basis of their soluble leaf proteins and ultrastructural changes during development(1,2). Particularly the finding in this study that the greatest difference between the virescens mutant and the normal in the amount of RuBP-carboxylase was for 4-day-old light-grown seedlings corroborates the earlier study on the soluble leaf proteins (1) which showed that the mutant was deficient in fraction I protein at this stage. Not only did the mutant have less of this protein but the enzyme was less active (Table 1). The presence of RuBP-carboxylase in dark-grown barley leaves and increases in both the amount and specific activity of the enzyme from plants grown in light is in agreement with the earlier literature based on studies from a number of laboratories (10,13,14,15).

The least difference between the two lines in the quantity and activity of RuBP-carboxylase was found for the dark-grown seedlings when the PEP-carboxylase activity of both lines was nearly equal and relatively high (Table 1). In the mutant seedlings PEP-carboxylase activity was highest for the 4-day-old light-grown seedlings for which the amount of RuBP-carboxylase activity was lowest. The relatively high activity of PEP-carboxylase in these light-grown mutant seedlings indicates that the carboxylation of phosphoenolpyruvate via this carboxylase may be significant for CO_2 fixation in these seedlings. It may represent an adaptation to the condition when RuBP-carboxylase

activity is limited by the mutation. Although PEP-carboxylase is generally considered to be a major enzyme for the CO_2 fixation pathway in C_4 plants the above results support the view that PEP-carboxylase also plays a significant role in CO_2 fixation by C_3 plants in the etiolated and early greening stages (4-7).

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REFERENCES

1. Jhamb, S. and Zalik, S. (1973) Can. J. Bot. 51, 2147-2154.
2. Jhamb, S. and Zalik S. (1975) Can. J. Bot. 53, 2014-2025.
3. Stephansen, K. and Zalik, S. (1971) Can. J. Bot. 49, 49-51.
4. Hall, D.O., Huffaker, R.C., Shannon, L.M. and Wallace, A. (1959) Biochem. Biophys. Acta. 35, 540-542.
5. Hedley, C.L. and Rowland, A.O. (1975) Pl. Sci. Lett. 5, 119-126.
6. Hedley, C.L., Harvey, D.M. and Keely, R.J. (1975) Nature 258, 352-354.
7. Tolbert, N.E. and Gailey, F.B. (1955) Plant Physiol. 30, 491-499.
8. Fair, P., Tew, J. and Cresswell, C.F. (1973) Ann. Bot. 37, 831-844.
9. Goldthwaite, J.J. and Bogorad L. (1971) Anal. Biochem. 41, 57-66.
10. Chollet, R. and Ogren, W.L. (1972) Z. Pflanzenphysiol. 68, 45-54.
11. Jensen, G.R. and Bahr, T.G. (1977) Ann. Rev. Plant Physiol. 28, 379-400.
12. Bradford, M.M. (1976) Anal. Biochem. 72, 248-254.
13. Huffaker, R.C., Obendorf, R.L., Keller, C.J. and Kleinkopf, G.E. (1966) Plant Physiol. 41, 913-918.
14. Kleinkopf, G.E., Huffaker, R.C., and Matheson, A. (1970) Plant Physiol. 46, 416-418.
15. Margulies, M.M. (1964) Plant Physiol. 39, 579-585.