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ASPARTATE STIMULATION OF MALATE DECARBOXYLATION IN *ZEA MAYS* BUNDLE SHEATH CELLS : POSSIBLE ROLE IN REGULATION OF C_4 PHOTOSYNTHESIS

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SUMMARY: Aspartate stimulated by as much as three fold the rate of malate decarboxylation by *Zea mays* bundle sheath cells. Both the basal and aspartate stimulated rates of malate decarboxylation were light-dependent. Stimulation appeared to be due to aspartate as such, rather than depending on aspartate metabolism, and was due partly to a reduction in the malate concentration required for maximum decarboxylation and partly to an increased maximum velocity of decarboxylation. The extractable activities of NADP malic enzyme, glyceraldehyde phosphate dehydrogenase, and 3-phosphoglycerate kinase recoverable from cells were not increased by preincubating cells with aspartate, and aspartate did not affect the activity of these enzymes in cell-free extracts. It is suggested that aspartate may influence the transport of either malate into or pyruvate out of bundle sheath chloroplasts.

During C_4 photosynthesis in *Zea mays*, malate is transported from mesophyll to bundle sheath cells where it is decarboxylated in chloroplasts via NADP malic enzyme (1). Studies of $^{14}CO_2$ incorporation by *Zea mays* leaves under steady state conditions have shown that label is incorporated into both aspartate and malate, but that malate is the larger and more metabolically active C_4 acid pool at high light intensities (2). However, the pools of aspartate and malate involved in photosynthesis are close to equal at reduced light intensities (Hatch, unpublished), and the sizes of these pools also vary widely in *Sorghum bicolor* leaves following varying temperature pretreatments of plants (3). This evidence for the quantitative significance of aspartate in photosynthesis of *Zea mays* and related C_4 species prompted an investigation of the role of aspartate in C_4 acid decarboxylation by bundle sheath cells. In this paper we report a substantial effect of aspartate on malate decarboxylation by bundle sheath cells from *Zea mays*.

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TABLE I. Effect of Aspartate on Malate Decarboxylation to Pyruvate by Bundle Sheath Cells of *Zea mays*

Additions[a]	Pyruvate (μmol min^{-1} mg^{-1} of chlorophyll)			
	Light		Dark	
	- Aspartate	+ Aspartate	- Aspartate	+ Aspartate
Expt. 1				
None	0	0.06	-	-
Malate	0.69	0.93	0.06	0.07
Malate + HCO_3^- + R-5-P[b]	1.54	2.58	0.13	0.12
Malate + 3-PGA	1.46	3.39	0.37	0.40
HCO_3^- + R-5-P	0.21	0.28	0.04	0.04
3-PGA	0.26	0.26	0.41	0.43
Expt. 2				
Malate (3 mM) + 3-PGA	0.59	2.17	-	-
Malate (6 mM) + 3-PGA	0.83	2.89	-	-
3-PGA	0.30	0.33		

[a] Metabolite concentrations (unless otherwise indicated) were; 12.5 mM aspartate, 10 mM HCO_3^-, 5 mM ribose-5-phosphate, 12.5 mM malate, 5 mM 3-phosphoglyceric acid. All reactions also contained 0.5 mM AMP.

[b] Abbreviations: R-5-P, ribose-5-phosphate; 3-PGA, 3-phosphoglyceric acid.

METHODS

Procedures for the isolation of bundle sheath cells (after a short preliminary treatment with cellulase), the studies of [^{14}C]C_4 acid metabolism, and the enzymic determination of pyruvate production, have been described elsewhere (4). Modifications of the buffer medium used for studies of C_4 acid decarboxylation (pH increased to 8.2 and 5 mM K_2SO_4 included) contributed to the higher rates reported in this paper. Assay procedures have been described for NADP malic enzyme (5), glyceraldehyde phosphate dehydrogenase, and 3-phosphoglycerate kinase (6).

RESULTS AND DISCUSSION

During studies on the metabolism of aspartate by *Zea mays* bundle sheath cells we observed that aspartate substantially stimulated the rate of pyruvate production in the presence of malate (Table I). The aspartate

effect was variable when cells were provided with malate alone (no HCO_3^- plus ribose-5-phosphate or 3-phosphoglycerate), possibly reflecting the variable ability of cell preparations to generate ribulose-1,5-bisphosphate from endogenous sources. As shown previously (4), malate decarboxylation was substantially increased by adding 3-phosphoglycerate, or HCO_3^- plus ribose-5-phosphate which would increase the endogenous production of 3-phosphoglycerate. With both these systems the addition of aspartate substantially stimulated pyruvate production, and both the basal and aspartate-stimulated components of malate decarboxylation were dependent upon light under all conditions tested. The percentage increases due to aspartate were greatest in systems containing sub-optimal levels of malate (compare Expt. 2 versus Expt. 1 of Table I).

The inference from Table I that enhanced pyruvate production in the presence of aspartate was due to increased malate decarboxylation was confirmed by examining the decarboxylation of [4-^{14}C] malate and [4-^{14}C] aspartate (Table II). Decarboxylation of [4-^{14}C] malate, estimated by summing accumulated $^{14}CO_2$ and ^{14}C fixed into Calvin cycle intermediates and products, was approximately equal to pyruvate production determined enzymically (after subtracting the small quantities of pyruvate formed in controls containing only HCO_3^- plus ribose-5-phosphate or 3-phosphoglycerate). However, there was negligible decarboxylation of [4-^{14}C] aspartate with or without HCO_3^-, ribose-5-phosphate, or malate.

Under conditions where aspartate stimulated the conversion of malate to pyruvate there was substantial interconversion of malate and aspartate but no net conversion of aspartate to malate. As shown in Table II, the rates of conversion in each direction, measured in systems containing [4-^{14}C] malate/aspartate or [4-^{14}C] aspartate/malate, were similar. Increased pyruvate production in the presence of aspartate was not transitory since the rates for systems with and without aspartate remained constant for periods of 7.5, 15 and 22.5 min under all conditions used in Table I.

TABLE II. Decarboxylation of Malate and Aspartate by Bundle Sheath Cells of *Zea mays*

Additions[a]	Reaction rate (μmol min^{-1} mg^{-1} of chlorophyll)		
	$^{14}CO_2$ released ($^{14}CO_2$ + ^{14}C fixed into Calvin cycle)	Pyruvate formed	Aspartate-malate interconversion
Expt. 1			
[4-^{14}C] Mal + HCO_3^- + R-5-P[b]	1.55	1.60	
[4-^{14}C] Mal + HCO_3^- + R-5-P + Asp	2.39	2.50	0.63[c]
HCO_3^- + R-5-P	-	0.21	
HCO_3^- + R-5-P + Asp	-	0.28	
[4-^{14}C] Asp + HCO_3^- + R-5-P + Mal	0.12	2.69	0.68[d]
Expt. 2			
[4-^{14}C] Mal + 3-PGA	1.30	1.44	
[4-^{14}C] Mal + 3-PGA + Asp	2.65	3.18	0.50[c]
3-PGA	-	0.33	
3-PGA + Asp	-	0.36	
[4-^{14}C] Asp + 3-PGA + Mal	0.11	3.39	0.35[d]

[a] Metabolite concentrations as in Table I.

[b] Abbreviations: Mal, malate; Asp, aspartate; 3-PGA, 3-phosphoglyceric acid; R-5-P, ribose-5-phosphate.

[c] Measured by rate of labelling of aspartate from [4-^{14}C] malate.

[d] Measured by rate of labelling of malate and Calvin cycle intermediates and products from [4-^{14}C] aspartate.

The addition of aspartate resulted in a substantial reduction of the malate concentration required for maximum pyruvate production (Figure 1a); the apparent K_m for malate decreased from 10 mM to 2 mM (Figure 1b). Aspartate also increased the maximum rate of malate decarboxylation. For the experiment described in Fig. 1b, reciprocal plots indicated V_{max} values of 2.09 and 1.42 μmol min^{-1} mg^{-1} of chlorophyll in the presence and absence of aspartate, respectively.

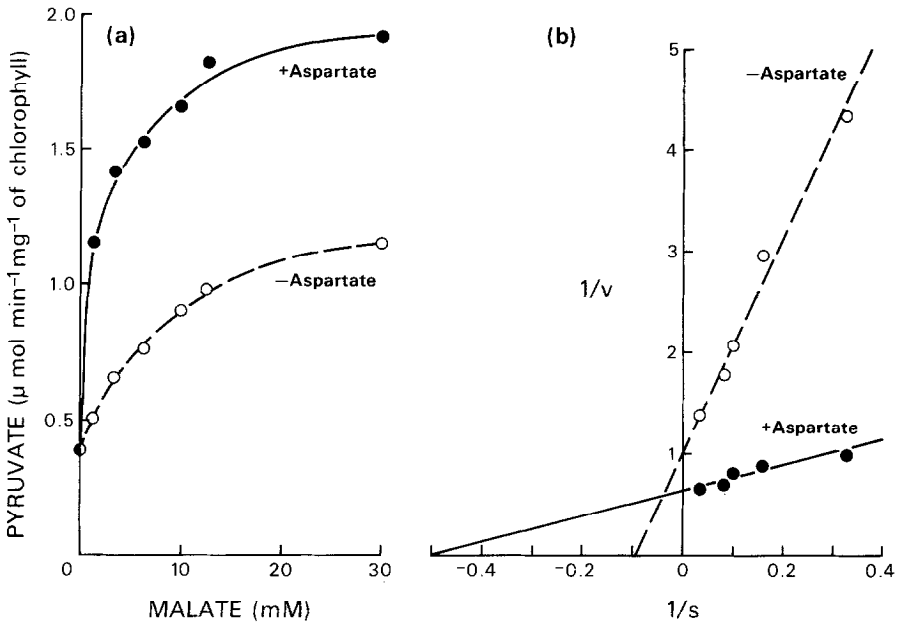

Figure 1. a: Effects of varying malate concentration (with and without aspartate) on the rate of pyruvate production by *Zea mays* bundle sheath cells. b: Reciprocal plot of data in a, after subtracting pyruvate produced in absence of malate. Bundle sheath cells were incubated with 3-phosphoglycerate (5 mM) plus varying concentrations of malate. The pyruvate produced was measured as described in the Methods.

Extractable NADP malic enzyme activity was not increased by incubating cells with aspartate. Activities of about 22 μmol min^{-1} mg^{-1} of chlorophyll were obtained for both treated and control cells. Neither did the addition of aspartate to assay mixtures for NADP malic enzyme have any effect on activity or the K_m for malate. To measure maximum NADP malic enzyme activity it is necessary to activate the freshly extracted enzyme in the presence of dithiothreitol at 25°C for 60-120 min (5). Aspartate did not affect the rate of activation or the final activity.

Activation of enzymes involved in the reductive phase of the photosynthetic carbon reduction cycle could indirectly increase the rate of malate decarboxylation by increasing the recycling of NADPH to $NADP^+$ (4,7). Neither NADP glyceraldehyde-3-phosphate dehydrogenase nor 3-phosphoglycerate kinase activity

was affected by preincubating cells with aspartate, or by adding aspartate to assay systems for the isolated enzymes.

Of several other compounds tested, only D-aspartate stimulated malate decarboxylation (76% stimulation compared with 142% for L-aspartate). Oxaloacetate, alanine, and potassium chloride, provided at the same concentrations were amongst the compounds that had no effect.

Isolated malic enzyme has a K_m for malate of 0.15 mM (8) compared with an apparent K_m of about 10 mM for malate decarboxylation by bundle sheath cells (Figure 1b). Presumably, diffusion of malate into either the cells or chloroplasts limits decarboxylation in the bundle sheath cell system. The reduction in the apparent K_m for malate in the presence of aspartate could be the result of facilitating malate transport into chloroplasts. Since pyruvate may inhibit the decarboxylation reaction (9), an increased rate of transport of pyruvate from the chloroplast could also result in higher rates of malate decarboxylation. We are currently attempting to isolate intact functional chloroplasts from *Zea mays* bundle sheath cells in order to test these possibilities. The precise physiological significance of this effect of aspartate on malate decarboxylation is uncertain. However, it is noteworthy that the concentration range over which aspartate is effective is very similar to the calculated concentration of aspartate in photosynthetic cells (1). Furthermore, the photosynthetically active pool of aspartate does vary with light intensity and other environmental variables (see Introduction). Since aspartate is synthesized in mesophyll cells, but would readily diffuse into bundle sheath cells, it may serve as a fine controller to integrate rates of carboxylation in mesophyll cells with decarboxylation in bundle sheath cells.

REFERENCES

1. Hatch, M.D., and Osmond, C.B. (1976) in Encyclopedia of Plant Physiology (New Series) Vol. 3, Transport in Plants III, (Eds C.R. Stocking & U. Heber), pp. 144-184, Springer-Verlag, Heidelberg.
2. Hatch, M.D. (1971) Biochem J. 125, 425-432.

3. Brooking, J.R., and Taylor, O.A. (1973) Plant Physiol. 52, 180-182.
4. Hatch, M.D., and Kagawa, T. (1976) Arch. Biochem. Biophys. 175, 39-53.
5. Hatch, M.D., and Mau, S. (1977) Arch. Biochem. Biophys. 179, 361-369.
6. Hatch, M.D., and Kagawa, T. (1973) Arch. Biochem. Biophys. 159, 842-853.
7. Farineau, J. (1975) Physiol. Plant. 33, 310-315.
8. Johnson, H.S., and Hatch, M.D. (1970) Biochem. J. 119, 273-280.
9. Rathnam, C.K.M., and Edwards, G.E. (1977) Arch. Biochem. Biophys. 182, 1-13.