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Short Communication

Amino Acid Stimulation of Carbon Dioxide Fixation in Cell-free Extracts of *Chlamydomonas reinhardtii*[1]

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R. F. Jones and J. H. Chen
Division of Biological Sciences, State University of New York at Stony Brook, Stony Brook, New York 11790

In *Chlamydomonas reinhardtii* the synthesis of glutamate and aspartate during photosynthesis has been shown to be closely associated with the Krebs cycle (2). The addition of P-enolpyruvate but not pyruvate was found to enhance greatly the incorporation of $^{14}CO_2$ in cell-free preparations of *C. reinhardtii* (3), suggesting that P-enolpyruvate carboxylase was important in CO_2 fixation in this alga in replenishing Krebs cycle intermediates. One of the functions of P-enolpyruvate carboxylase is to maintain an operative Krebs cycle by maintaining adequate levels of oxaloacetate for the continued generation of the cycle at times when aspartate and glutamate are being synthesized (5). Such an anaplerotic mechanism has been reported in bacteria where it was also found that acetyl-CoA stimulated P-enolpyruvate carboxylase activity but that aspartate inhibited the enzyme (7, 8). Little is known about the regulatory mechanisms of CO_2 fixation in algae, particularly in relation to the biosynthetic role of the Krebs cycle. Kates and Jones (4), however, reported that the activity of P-enolpyruvate carboxylase varied during the synchronized vegetative life cycle of the alga *C. reinhardtii*. The purpose of the present investigation, therefore, was to study the effect of acetyl-CoA, glutamate, aspartate, and their respective amides upon P-enolpyruvate carboxylase activity in cell-free extracts of *C. reinhardtii*.

Table I. *Requirement for CO_2 Fixation Reaction*

Reaction System	P-enolpyruvate System	Pyruvate System
	cpm/assay	
Complete	4384	144
− P-enolpyruvate or pyruvate	212	124
− NAD^+	4303	164
− ATP	3944	136
− GDP	3900	160
Complete	3588	160
− IDP	3740	144
− Acetyl-CoA	3288	136
− Mg^{2+}	756	156
− Mn^{2+}	3220	164
+ NADPH (4 mg/1.2 ml)	3360	

Cells of *C. reinhardtii* (strain 89+) were grown, and 100,000*g* supernatants from cell-free extracts were prepared as described previously (3), and dialyzed against several changes of 0.05 M phosphate buffer, pH 7.4, before enzyme assays. The CO_2 fixation reaction mixture contained, unless otherwise stated, the following constituents: (μmoles in 1.2 ml): ATP, 6; NAD^+, 6: GSH, 3; $MgSO_4 \cdot 7H_2O$, 10; acetyl-CoA, 1; $MnSO_4 \cdot 2H_2O$, 2; GDP, 2; IDP, 2; P-enolpyruvate, 40 (or pyruvate, 40); 100 μc $NaH^{14}CO_3$ (25 c/mole). All the reagents were dissolved in phosphate buffer, pH 7.4. An aliquot of 0.3 ml of dialyzed cell-free extract containing 2.5 to 3.5 mg of protein (6) was added to the reaction mixture minus substrates and preincubated for 5 min at 30 C. At the end of the preincubation period, P-enolpyruvate or pyruvate and $NaH^{14}CO_3$ were added simultaneously. The complete system was then incubated for 1 hr at 30 C, and the reaction was terminated by the addition of 2.5 ml of boiling acidic ethanol (80%, v/v). After centrifugation, an aliquot (0.2 ml) of the clear supernatant fraction was assayed for radioactivity with Bray's solution (1) and a Packard model 3320 scintillation spectrometer.

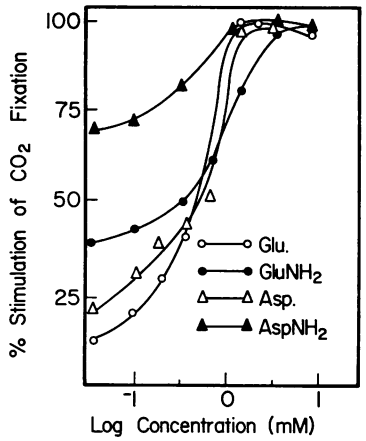

Fig. 1. Stimulation of CO_2 fixation by L-glutamate, L-aspartate and their corresponding amides.

The CO_2 fixation reaction depends upon the presence of P-enolpyruvate rather than pyruvate and is independent of ATP and NAD^+ (Table I). There is also a stringent requirement for Mg^{2+} but not for Mn^{2+}. The elimination of acetyl-CoA from the assay system did not affect the amount of CO_2 fixed. In fact, the presence of acetyl-CoA at concentrations between 10^{-6} M and 10^{-3} M did not result in any enhancement of fixation. Similarly, neither GDP nor IDP was found to be necessary in the P-enolpyruvate assay system, and the addition of NADPH was without effect.

Figure 1 illustrates the level of stimulation of CO_2 fixation that occurred with L-glutamate, L-glutamine, L-aspartate, and L-as-

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paragine. Although stimulation occurred at 5×10^{-2} mM, the maximum occurred at 1 mM and was maintained up to 10 mM. The analogues L-α-methylaspartate, D-aspartate, L-β-methylglutamate, and *p*-nitrobenzoyl-L-glutamate did not exert any stimulatory effect. Other amino acids tested but without effect were L-alanine, L-serine, L-methionine, L-phenylalanine, L-tryptophan, L-arginine, L-proline, and L-lysine.

In *C. reinhardtii* the anaplerotic fixation of CO_2 by P-enolpyruvate carboxylase appears, therefore, to be stimulated by distal products of the Krebs cycle, namely L-aspartate and L-glutamate, or their amides. This, together with the apparent nonrequirement for acetyl-CoA, suggests that the regulation of this enzyme differs from that found for bacteria (7, 8). The elucidation of this regulatory mechanism is currently under investigation in this laboratory.

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