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INHIBITION OF MAIZE LEAF PHOSPHOPYRUVATE CARBOXYLASE BY OXALOACETATE

J. LOWE AND C. R. SLACK*

Department of Biochemistry, University of Queensland, St. Lucia, Queensland 4067 (Australia) and David North Plant Research Centre, C.S.R. Research Pty. Limited, Brisbane (Australia)

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SUMMARY

Phosphopyruvate carboxylase was purified 60-fold from maize leaf lamina and the effect of various metabolites on the activity of the enzyme examined. Oxaloacetate was the only compound found that inhibited the enzyme at physiological concentrations; 50% inhibition occurring at 0.25 mM oxaloacetate. The significance of this inhibition in relation to regulation of the C_4-dicarboxylic acid pathway of photosynthesis is discussed.

In leaves of many tropical grasses[1,2] and some dicotyledenous species[3] phosphopyruvate carboxylase serves a photosynthetic function catalysing the first reaction of a pathway termed the C_4-dicarboxylic acid pathway of photosynthesis[4,5]. The level of the enzyme in leaves of species possessing this pathway is between 50- and 100-fold greater than in Calvin cycle species, it is associated with chloroplasts[2] and is probably located in, or on, the envelope of the mesophyll plastids[6,7]. Since phosphopyruvate carboxylases from the enterobacteraceae[8,9] and maize roots[10] have been found to be allosteric enzymes it was of interest to determine whether the activity of leaf enzyme, which serves a different metabolic function, is also subject to metabolite regulation.

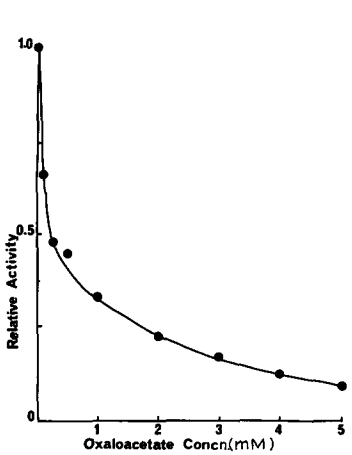
The enzyme used in the present study was purified 60-fold from maize leaves. Lamina (50 g) was homogenised with 250 ml of 100 mM Tris–HCl buffer (pH 8.3) containing 10 mM 2-mercaptoethanol, the homogenate filtered through muslin and the filtrate centrifuged at 20 000 $\times$ g for 10 min. Saturated $(NH_4)_2SO_4$ was added to the supernatant and the protein that precipitated between 38 and 50% saturation was suspended in 20 ml of 50 mM Tris–HCl buffer containing 5 mM dithiothreitol. Purification to this stage was performed at 3° and subsequent steps were carried out at room temperature. The supernatant was applied to a Sephadex G-200 column (column volume 400 ml), equilibrated with 50 mM Tris–HCl (pH 7.5) containing 2 mM

* Present adress: Plant Physiology Division, Palmerston North, New Zealand.

dithiothreitol and 5 mM $MgCl_2$. The first 40-ml fraction that emerged after the void volume was applied to a column (30 ml) of calcium phosphate gel (Hypatite C, Clarkson Chemical Co., Williamsport, Pa., U.S.A.), equilibrated with 50 mM Tris–HCl (pH 7.5), 2 mM dithiothreitol and 5 mM $MgCl_2$ and the column eluted with the above buffer mixture containing first 40 mM KCl, then 70 mM KCl. Phosphopyruvate carboxylase was eluted by 70 mM KCl and was stored as a precipitate in 60% $(NH_4)_2SO_4$.

The activity of the purified enzyme was equivalent to 78 μmoles of phosphopyruvate carboxylated per mg protein per min. As a precipitate in 60% $(NH_4)_2SO_4$ the enzyme was stable for at least 6 months, but rapidly lost activity when solutions of the enzyme were frozen. Assays were conducted by measuring the incorporation of $^{14}CO_2$ into aspartate in the presence of glutamate and aspartate aminotransferase[2] or into oxaloacetate without the addition of the glutamate and aminotransferase. Reaction times of less than 2 min were used in the latter assay and aliquots of acidified reaction mixtures were counted immediately to minimise the loss of fixed radioactivity due to non-enzymatic β-decarboxylation of oxaloacetate. Over this time period the reaction rates obtained with the two assay procedures were not significantly different.

The pH optimum of the enzyme was approximately 8.4 and at this pH the apparent K_m values for HCO_3^-, Mg^{2+} and phosphopyruvate were 0.4 mM, 3.3 mM and 0.6 mM respectively. These values agree closely with those recently reported for a crude preparation of maize leaf phosphopyruvate carboxylase[10].



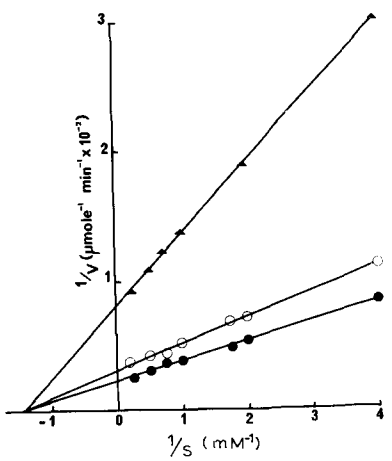


Fig. 1. Effect of oxaloacetate on activity of maize leaf phosphopyruvate carboxylase. Reaction mixtures (0.1 ml final volume) contained 50 mM Tris–HCl buffer (pH 8.3), 10 mM Mg Cl_2, 0.5 mM phosphopyruvate, 10 mM $NaH^{14}CO_3$, 5 mM dithiothreitol, enzyme and sodium oxaloacetate. Mixtures were incubated at 30° for 2 min, reactions stopped by additions of trichloracetic acid to a final concentration of 3% and the assay mixtures chilled to 0°. Aliquots were spotted onto paper, dried in an air stream and counted immediately using a 4 cm Geiger-Müller counter. The reaction rates in presence of oxaloacetate are expressed as fraction of the rate in absence of oxaloacetate.

Fig. 2. Lineweaver–Burk plots of the effect of phosphopyruvate on phosphopyruvate carboxylase activity in absence of oxaloacetate (●—●) and with 0.25 mM (○—○) and 1.0 mM oxaloacetate (▲—▲). Experimental details are described in Fig. 1.

Intermediates of the C_4-dicarboxylic acid pathway and compounds related to it were tested as possible modifiers of phosphopyruvate carboxylase activity. At concentrations of 5 mM, malate, aspartate, glutamate, fructose-1,6-diphosphate, fructose-6-phosphate, ribose-5-phosphate, ribulose-1,5-diphosphate, ATP, ADP, AMP, NAD^+ and NADH had no effect on the activity of the enzyme in the presence of either 0.5 or 5 mM phosphopyruvate. Oxaloacetate, however, inhibited the enzyme at physiological concentrations; 50% inhibition occurred at about 0.25 mM oxaloacetate (Fig. 1) and the inhibition by oxaloacetate was noncompetitive with respect to both phosphopyruvate (Fig. 2) and HCO_3^-. Pyruvate was found to be a less potent inhibitor of the enzyme; 50% inhibition occurring at 17 mM pyruvate in the presence of 5 mM phosphopyruvate. Inhibition by pyruvate, which is probably of little physiological significance in view of the high concentration required for appreciable inhibition, was competitive with respect to phosphopyruvate.

The reactions of the C_4-dicarboxylic acid pathway in maize and other panicoid grasses subsequent to the carboxylation of phosphopyruvate are believed to be the reduction of oxaloacetate by an NADP-specific malate dehydrogenase[12], the decarboxylation of malate by malate enzyme and the regeneration of phosphopyruvate from pyruvate by pyruvate kinase[13]. We have proposed[4,5] that this cyclic pathway functions to accumulate CO_2 into the parenchymal sheath chloroplasts where it is fixed *via* the Calvin cycle. The inhibition of maize leaf phosphopyruvate carboxylase by oxaloacetate could function *in vivo* to prevent an accumulation of oxaloacetate when the availability of phosphopyruvate for the carboxylation reaction exceeds that of NADPH for oxaloacetate reduction. It it noteworthy that aspartate which is a potent inhibitor of the phosphopyruvate carboxylase from Salmonella and malate which is an inhibitor of the maize root enzyme do not affect the activity of the leaf enzyme.

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