dodecyl sulphate was 36000, indicating a tetrameric structure.

T. aquaticus glyceraldehyde 3-phosphate dehydrogenase (the apo- as well as holo-enzyme) is stable for several hours at 90°C (the *B. stearrowthermophilus* enzyme is totally inactivated within 10 min at this temperature) but is rapidly inactivated above 100°C. Moreover, like the *B. stearrowthermophilus* enzyme (K. Suzuki & J. I. Harris, unpublished work), it retains its activity fully after exposure to 8M-urea and 1% sodium dodecyl sulphate. Some chemical, physico-chemical and kinetic parameters of the enzyme will be presented.

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Metabolic Regulation in the C₄ Dicarboxylic Acid Pathway of Photosynthesis

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We have suggested (Baldry *et al.*, 1971) that in the tropical grasses the C₄ dicarboxylic acid pathway of photosynthesis (Hatch & Slack, 1970) is located in the cytoplasm and acts as a mechanism for concentrating CO₂ at the site of reductive assimilation in the chloroplasts of the mesophyll cell layer. The initial incorporation of CO₂ into C₄ organic acids is catalysed by phosphopyruvate carboxylase (EC 4.1.1.31). Investigations of cytoplasmic phosphopyruvate carboxylase partially purified from leaves of the plant *Pennisetum purpureum*, which utilizes the C₄ dicarboxylic acid pathway, suggest that this enzyme may be regulated by photosynthetic activity.

Leaf tissue (100g) was homogenized in 200ml of chloroplast isolation medium [as described by Baldry *et al.* (1968), with the omission of thiols, which

were not necessary since this plant contains very low *o*-diphenol oxidase (EC 1.10.3.1) activity]. Subcellular particles were removed by centrifugation and cytoplasmic protein was precipitated with (NH₄)₂SO₄ (60% saturation). The dialysed freeze-dried precipitate was dissolved in 10mM-tris-HCl buffer, pH 7.0, applied to a column of DEAE-cellulose and eluted with a linear gradient of tris-HCl buffer, pH 7.0. Incorporation of ¹⁴C into oxaloacetate, trapped as the 2,4-dinitrophenylhydrazone, was measured in reaction mixtures containing phosphoenolpyruvate, Mg²⁺, H¹⁴CO₃⁻ and enzyme in 200 μl of 50mM-HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid) buffer, pH 8.0.

Variation of rate of reaction as a function of limiting concentrations of HCO₃⁻ or of Mg²⁺ approximated to first-order kinetics. However, sigmoid kinetics were observed when the rate of reaction was determined as a function of phosphoenolpyruvate concentration. Addition of intermediates of the photosynthetic carbon-reduction cycle (5 mM) stimulated (ribose 5-phosphate, fructose 1,6-diphosphate, fructose 6-phosphate, glucose 6-phosphate) or inhibited (phosphoglycerate) the reaction. The effects of glucose 6-phosphate were investigated further. Enzyme activity increased when the concentration of phosphoenolpyruvate exceeded that of glucose 6-phosphate, but was decreased when the concentration of glucose 6-phosphate exceeded that of phosphoenolpyruvate. The enzyme was also inhibited by oxaloacetate (50% at 1.5 mM) and malate (50% at 6.0 mM). In the presence of malate dehydrogenase (EC 1.1.1.37) NADH increased the rate of reaction, presumably owing to reduction of oxaloacetate to the less inhibitory malate. The increased enzyme activity observed when NADH was included in the reaction mixture was additive to that caused by glucose 6-phosphate. Activation by glucose 6-phosphate did not occur in the presence of thiols (β-mercaptoethanol, thioglycollate, dithiothreitol, GSH, at 0.1–10 mM) or 1.5M-urea.

These results suggest that the cytoplasmic phosphopyruvate carboxylase of *P. purpureum* is an allosteric enzyme regulated by intermediates of the photosynthetic carbon-reduction cycle. In this case the initial assimilation of CO₂ into oxaloacetate in plants that utilize the C₄ dicarboxylic acid pathway could be regulated by the photosynthetic activity of the mesophyll chloroplasts.

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