Effects of 2,4-dinitrophenol and anoxia on the inorganic-pyrophosphate content of the spadix of Arum maculatum and the root apices of Pisum sativum

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Abstract. This work was done to determine whether the inorganic-pyrophosphate (PPi) content of plant tissues changes when the rate of glycolysis is altered. Treatment of excised clubs of the spadix of Arum maculatum L. and root apices of Pisum sativum L. with 2,4-dinitrophenol increased the rates of respiration but had no detectable effects on PPi contents. When the two tissues were subjected to up to 60 min anoxia, no changes in PPi were detected. Anoxia was shown to lead to a fall in ATP and concomitant rises in ADP and AMP in pea roots. It is argued (i) that variation in the rate of glycolysis was not accompanied by detectable changes in PPi content, (ii) that this observation does not favour the view that pyrophosphate fructose 6-phosphate 1-phosphotransferase mediates appreciable entry into glycolysis, and (iii) that PPi content can be maintained when respiratory-chain phosphorylation is inhibited.

Key words: Anoxia – Arum – 2,4-Dinitrophenol – Pisum (pyrophosphate) Pyrophosphate, inorganic – Root apex (PPi) content – Spadix

Introduction

We know that pyrophosphate:fructose 6-phosphate 1-phosphotransferase [PFK(PPi); EC 2.7.1.90] is widely distributed with high activity in plants, that it catalyses a near-equilibrium reaction in vivo and is confined to the cytosol, yet we do not know the function of this enzyme (Weiner et al. 1987; see ap Rees 1988 for a review).

Suggestions that it is an important point of entry of substrate into cytosolic glycolysis continue to be made (Black et al. 1987). In general, the evidence for this view is not conclusive (see ap Rees and Dancer 1987 for a review). The best of such evidence are the demonstrations that illumination of guard-cell protoplasts is accompanied by a rise in fructose-2,6-bisphosphate and a fall in glucose 6-phosphate (Hedrich et al. 1985), and that fructose-2,6-bisphosphate rises when the metabolism of storage tissue is increased by slicing (Van Schaftingen and Hers 1983). However, these observations do not demonstrate a glycolytic role for PFK(PPi). First, hexose 6-phosphate is the substrate for the universally distributed classical glycolytic enzyme, 6-phosphofructokinase, as well as for PFK(PPi). Second, the changes in fructose-2,6-bisphosphate could be associated with increased production of inorganic pyrophosphate (PPi) by PFK(PPi), and the use of this PPi in the breakdown of sucrose via sucrose synthase and uridine 5'-diphosphoglucose pyrophosphorylase (ap Rees and Dancer 1987). We suggest that in determining whether PFK(PPi) mediates entry into glycolysis the key substrate to measure is PPi as this is not shared with 6-phosphofructokinase. Accordingly we have determined whether variation in the rate of glycolysis in the clubs of the spadix of Arum maculatum and in the root apices of Pisum sativum is accompanied by changes in PPi content. We used the uncoupler, 2,4-dinitrophenol, and anoxia to vary the rate of glycolysis. In some experiments we also measured hexose phosphates and adenine nucleotides.

Materials and methods

Materials. Enzymes were from Boehringer, Lewes, Sussex, UK, except that PFK(PPi) was from Sigma (London) Chemical Co., Poole, Dorset, UK. Substrates were from Sigma except that
ATP, NAD and NADP were from Boehringer. Peas (Pisum sativum L. cv. Kelvedon Wonder; Sanders Ltd., Cambridge, UK) were grown as described by Dancer and ap Rees (1988). The apical 2 cm of the roots of 5-d-old seedlings were excised and used within 10 min. Complete inflorescences of Arum maculatum L. were collected from local natural sites. Within an hour of collection, the clubs, the swollen ends of the appendices, were excised and used at once. The stages of development (α, β, γ) have been defined (ap Rees et al. 1976).

Measurement of gas exchange. Warburg's direct manometric method was used at 25°C. Replicate samples of 20 pea root apices (0.6 g fresh weight, FW) were suspended in 2.5 ml 0.02 M KH₂PO₄, pH 5.2, or 2.5 ml 25 µM 2,4-dinitrophenol in 0.02 M KH₂PO₄ adjusted to pH 5.2. For the experiments with Arum, replicate samples were made by cutting clubs into halves, or quarters, longitudinally. Each half or quarter club was stood in the centre well of a manometer flask in 0.1 ml 0.02 M KH₂PO₄, pH 5.2, or 0.1 ml 5 mM 2,4-dinitrophenol in 0.02 M KH₂PO₄, pH 5.2. The alkali was placed in the side-arm. Anoxia was achieved by gassing the flasks with nitrogen for 7–8 min. Control samples were run in each experiment. These samples were treated exactly as the experimental samples, except that measured amounts of the metabolites in question were added to the sample immediately after freeze-clamping. The amounts added were between one and two times the quantities found in the tissue sample. The percentages of the metabolites recovered were: Arum: PPI, 100; pea root: PPI, 92; fructose 6-phosphate, 83; glucose 6-phosphate, 102; ATP, 95; ADP, 96; AMP, 88.

Results

Effects of 2,4-dinitrophenol. We determined the effects of a range of concentrations of 2,4-dinitrophenol on the oxygen uptake of pea roots and Arum clubs and chose those that produced appreciable effects, i.e. a doubling of the oxygen uptake of the clubs and a 20% increase in that of pea roots (Table 1). In pea roots we showed that CO₂ production was increased to a greater extent (35%) than was oxygen uptake. The marked difference in the concentration of 2,4-dinitrophenol required to produce the above effects in the two tissues is ascribed to the different ways in which the uncoupler was supplied to the two tissues. No effect on respiration, no significant effect on PPI content could be detected (Table 1).

Effects of anoxia. Production of CO₂ by γ-stage clubs of Arum maculatum was 2.514 ± 865 µl h⁻¹ g⁻¹ FW. After 30 min anoxia this rate fell to 609 ± 23: values are means ± SE for three clubs. Extensive measurements of the PPI content of such clubs after 1, 15 and 60 min anoxia failed to reveal any effect of anoxia on PPI content (Table 2). When pea roots were made anoxic there was a fall in the rate of CO₂ production but no change in PPI content was detected (Table 3). Other metabolites did change: ATP fell and there were concomitant increases in ADP and AMP. Fructose-1,6-bisphosphate increased but a fall in hexose 6-phosphates was not demonstrated conclusively.