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SHORT COMMUNICATION

ACTIVITIES OF KEY ENZYMES OF CARBOHYDRATE OXIDATION IN DISKS OF CARROT STORAGE TISSUE

C. P. P. RICARDO* and T. AP REES

University of Cambridge, Botany School, Downing Street, Cambridge

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Abstract—In extracts of freshly cut disks (1 × 10 mm) of storage tissue of carrot (*Daucus carota* L.) the activities of hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and phosphofructokinase at 30° were 17, 29, 174 and 69 nmoles product formed/min/g fresh wt., respectively. The corresponding values for extracts of disks that had been incubated in aerated water for 24 hr were 67, 174, 261 and 39, respectively. These results are discussed in relation to the control of carbohydrate oxidation.

INTRODUCTION

IF THIN disks of carrot storage tissue are incubated under moist conditions for 12–24 hr their rate of respiration increases steadily to a value double that of freshly cut disks.[1,2] We refer to this incubation as ageing and to the increased respiration as induced respiration. In carrots the development of induced respiration is accompanied by, and is partially dependent upon, protein synthesis.[2,3] Thus respiratory enzymes could be synthesized during ageing. We have investigated this possibility by measuring the activities of a number of key respiratory enzymes in extracts of fresh and 24 hr-aged disks of carrot storage tissue. We chose hexokinase (E.C. 2.7.1.1) because the substrate for induced respiration may be hexose produced by the action of acid invertase on sucrose.[4] We chose glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44), and phosphofructokinase (E.C. 2.7.1.11) because these enzymes catalyse reactions that may limit the entry of hexose phosphate into respiratory pathways.

Appreciable inhibition or activation of the enzymes could occur during the preparation of extracts.[5] Thus differences in enzyme activities between extracts of fresh and aged disks could be due to differences in the recovery of the enzymes from the two types of disk. As a check against this possibility we compared enzyme activities in extracts of fresh and of aged disks with the activities in extracts of samples composed of 1:1 mixtures of fresh and aged disks. Substantial differences between the activity of the mixed sample of fresh and aged disks and the activity predicted from measurements made on separate samples of fresh and aged disks are taken as showing inadequate recovery.

RESULTS

The activities of the enzymes in extracts of fresh and 24 hr-aged disks are shown in Table 1. For 6-phosphogluconate dehydrogenase and phosphofructokinase, there was very close agreement between the predicted and the observed activities of the samples composed of

* Present address: Departamento de Fisiologia Vegetal, Estação Agronómica Nacional, Oeiras, Portugal.

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TABLE 1. ACTIVITIES OF KEY ENZYMES OF CARBOHYDRATE OXIDATION IN EXTRACTS OF FRESH AND 24-hr AGED DISKS OF CARROT STORAGE TISSUE

Enzyme	Activity (nmoles product formed/min/g initial fresh wt.) Fresh	Aged	Mixture of fresh and aged Predicted	Observed
Hexokinase	17	67	42	37
Glucose-6-phosphate dehydrogenase	29	174	101	87
6-Phosphogluconate dehydrogenase	174	261	218	232
Phosphofructokinase	69	39	54	55

a 1:1 mixture of fresh and aged disks. The activities of hexokinase and glucose-6-phosphate dehydrogenase were slightly less than the predicted values. For all enzymes except hexokinase, the relatively close agreement between the observed and predicted values was only obtained when Polyclar AT was included in the extraction medium. In the absence of Polyclar AT the activity of phosphofructokinase in extracts of fresh disks was reduced by 50 per cent, and the activities of the dehydrogenases in extracts of aged disks by 80 per cent. The pattern of changes in activity that occurred on ageing was obtained consistently in a minimum of five separate experiments for each enzyme. The absolute values of the enzyme activities and the precise extent of the changes varied with carrots taken at different times of the year. However, measurements made on different samples of the same batch of carrots at the same time of the year agreed closely. For example, in four separate experiments the activity of phosphofructokinase in extracts of fresh disks was 47, 42, 50 and 46 nmoles product formed/min/g fresh wt.

DISCUSSION

Our experiments with mixtures of disks strongly indicate that the differences in enzyme activities between extracts of fresh and aged disks were not primarily due to differences in recovery of the enzymes from the disks. Even with hexokinase and glucose-6-phosphate dehydrogenase, the disparity between the observed and predicted values for mixtures of disks was small compared with the differences between fresh and aged disks. Our estimates of glucose-6-phosphate dehydrogenase, particularly for aged disks, are much higher than those reported by Alberghina and Marré.[6] We attribute this difference to the methods that we used for the extraction and assay of the enzyme.

We suggest that the development of induced respiration in disks of carrot storage tissue is accompanied by a decrease in the maximum catalytic activity of phosphofructokinase, a marked increase in that of glucose-6-phosphate dehydrogenase and smaller but still appreciable increases in the activities of 6-phosphogluconate dehydrogenase and hexokinase. Increased activity of the dehydrogenases of the pentose phosphate pathway may be a general feature of induced respiration as there is evidence for such increases in disks of sweet potato[7] and red beet.[8]

We think that the above changes in activity, and the increase in acid invertase reported previously,[4] are important in the control of carbohydrate oxidation in carrot disks. Our evidence for this view is the very close correlation between the changes in enzyme activities

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and the manner in which the disks metabolize carbohydrate. The increase in acid invertase activity is paralleled by a decline in sucrose.[4] The rise in hexokinase coincides with the fall in sucrose and a marked increase in the ability of the tissue to metabolize exogenous glucose.[1,9] The rise in the activities of the two dehydrogenases and the fall in that of phosphofructokinase are accompanied by changes in the metabolism of [^{14}C] glucose that strongly suggest that the development of induced respiration involves an increase in the activity of the pentose phosphate pathway relative to that of glycolysis.[1] Ignorance of the fluxes through the reactions concerned makes it difficult to determine the precise relationship between the fluxes and the changes in enzyme activity. The maximum catalytic activities of the enzymes might limit metabolism during the development of induced respiration. Alternatively the changes that we have demonstrated may be secondary and represent the re-establishment of the ratio between maximum catalytic activity and flux that existed in fresh disks. Typical rates of CO_2 production at 30° for fresh and aged disks are 50 and 144 nmoles/g fresh wt./min, respectively. If all this CO_2 came from the complete and uninterrupted oxidation of glucose-6-phosphate entering glycolysis and the pentose phosphate pathway then glucose-6-phosphate would be oxidized at rates of 8·4 and 24 nmoles/g fresh wt./min in fresh and aged disks respectively. However, the rates may be double these because there is evidence that as much as 40 per cent of the hexose entering the respiratory pathways is retained in the tissue.[1,10] Comparison of these tentative estimates with the data in Table 1 indicates the following. Firstly, in the predominantly glycolytic fresh disks, glycolysis and the pentose phosphate pathway are probably not limited by the maximum catalytic activities of phosphofructokinase and the two dehydrogenases, respectively. This view is supported by the evidence that the activities of both pathways can be increased immediately in fresh disks.[10] Secondly, the decline in phosphofructokinase and the rise in glucose-6-phosphate dehydrogenase could contribute directly to the relative increase in the activity of the pentose phosphate pathway during the first 24 hr of ageing.

The above implies a regulatory role for phosphofructokinase during the first 24 hr of ageing. We are not convinced by arguments to the contrary by Adams and Rowan[11] for the following reasons. Firstly, Adams and Rowan show that the carrot extracts contained nothing that interfered with their assays of phosphate esters but do not show that there were no losses of these compounds during the preparation of the extracts. No figures are given for ATP. Secondly, we do not think that negative results are necessarily conclusive when the cross-over theorem is applied in complex tissues of compartmented cells to branched pathways operating through dissociable intermediates.[12,13] Finally it is difficult to see how a steady state would be obtained if glycolysis were regulated at the pyruvate kinase step but not at the phosphofructokinase step.

EXPERIMENTAL

Material. Mature carrots (*Daucus carota* L.) were bought locally and were used at once. The methods used to prepare and age the disks have been described.[4] Comparisons between fresh and aged disks were made only between replicate samples. Freshly cut disks for mixing with aged disks were prepared from carrots that were closely comparable to those used in the preparation of the aged disks.

Methods. Extracts were prepared in the following buffers: 0·05 M Tris (pH 8·2) for assay of phosphofructokinase, 0·05 M Tris (pH 7·8) for the two dehydrogenases, and 0·05 M Tricine (pH 8·0) for hexokinase.

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Four grams initial fresh weight of disks were homogenized with 1 g Polyclar AT (insoluble polyvinylpyrolidione) and 8·0 ml buffer. The homogenate was centrifuged at 35,000 *g* for 15 min and the supernatant was taken immediately for the enzyme assays. Extracts were prepared at 3° and assayed at 30°.

For hexokinase 0·5 ml extract was incubated for 3 min with 0·5 ml 0·05 M Tricine (pH 8·0) that contained: 250 μmoles glucose, 5 μmoles ATP, and 10 μmoles $MgCl_2$. The reaction was stopped by the addition of 1 ml 6% perchloric acid and the reaction mixture was then neutralized with 1 N KOH and centrifuged for 10 min at 35,000 *g* at 4°. Portions (1·0–2·0 ml) of the resulting supernatant were assayed for glucose-6-phosphate by incubation with 0·7 μmole NADP, 2 μg glucose-6-phosphate dehydrogenase and sufficient 0·05 M tricine buffer (pH 8·0) to bring the total volume to 3·0 ml.

Dehydrogenase activity was determined according to Glock and McLean.[14] The assay mixture contained in a volume of 3·0 ml: 0·5–1·0 ml extract, 9·0 μmoles $MgCl_2$, 90 μmoles tris (pH 7·6 for glucose-6-phosphate dehydrogenase, pH 8·4 for 6-phosphogluconate dehydrogenase), 0·3 μmole NADP and 6·0 μmoles glucose-6-phosphate or 6-phosphogluconate. Glucose-6-phosphate dehydrogenase activity is the difference between activity measured with 6·0 μmoles 6-phosphogluconate and that measured in the presence of 6·0 μmoles 6-phosphogluconate plus 6·0 μmoles glucose-6-phosphate. Corrections were made for the very low rates of NADP reduction that were observed in the absence of glucose-6-phosphate and 6-phosphogluconate.

Phosphofructokinase was assayed by the method of Atkinson and Walton.[15] Extract (0·5 ml) was incubated for 3 min with 0·5 ml 0·005 M Tris-HCl (pH 8·2) that contained 1 μmole ATP, 1 μmole $MgCl_2$, and 4 μmoles fructose-6-phosphate. The reaction was then stopped by the addition of 1 ml cold 5% trichloroacetic acid and the precipitate removed by centrifugation. A 1·5 ml portion of the neutralized supernatant was assayed for fructose-1,6-diphosphate in a total volume of 3·0 ml that contained 10 μmoles Tris (pH 7·4), 0·4 μmole NADH, and aldolase, triose phosphate isomerase and α-glycerophosphate dehydrogenase in excess.

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