Regulation of Glycolytic Metabolism in Fresh-cut Carrots under Low Oxygen Atmosphere

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Abstract. Carrot (Daucus carota L.) shreds were stored under a continuous flow of air or 0.5% and 2% O2 (balance N2) for 9 days at 5 and 15°C. The resulting changes in respiration, levels of glycolytic intermediates, and activities of ATP : phosphofructokinase (ATP-PFK), and PPI : phosphofructokinase (PPI-PFK) were monitored. Carrots under low O2 atmosphere exhibited an increase in RQ due to a greater reduction in O2 consumption than in CO2 production, and the increase in RQ was greater at 0.5% than at 2% O2 at both temperatures. Fructose 1,6-bisphosphate (F1,6P) accumulated with decreased O2 atmosphere and was 2-fold greater at 0.5% than at 2% O2 atmosphere at both temperatures. The levels of other glycolytic intermediates were not significantly influenced by low O2. The increase in PPI-PFK activity occurred at the same time as F1,6P accumulation. A similar relationship was not found with ATP-PFK. These results suggest that PPI-PFK may be involved in regulation of glycolysis under low O2 atmosphere.

Much research has been conducted to evaluate the effects of controlled and modified atmosphere on fruits and vegetables (Hardenburg et al., 1990; Isenberg, 1979; Smock, 1979). However, the mode of action of O2 and CO2 on fresh produce is not fully understood (Kader, 1986; Wang, 1990; Weichmann, 1986).

Since very low O2 and very high CO2 atmospheres caused accumulation of acetaldehyde and ethanol in tissues of harvested plants (Kennedy et al., 1992; Thomas, 1929), studies have been undertaken to understand the mechanism for regulation of fermentation in harvested plant parts in response to low O2 and high CO2 (Kanellis et al., 1991; Ke et al., 1994; Leshuk and Saltveit, 1991; Nichols and Patterson, 1987; Milanick and Fouse, 1989).

Kerbel et al. (1988) studied the effect of high CO2 on glycolysis and reported a significant reduction in the activities of ATP : phosphofructokinase (ATP-PFK) and PPI : phosphofructokinase (PPI-PFK) and in the content of fructose 1,6-bisphosphate (F1,6P) in pear fruit stored under high CO2 atmosphere; a discernible increase in the content of fructose 6-phosphate (F6P) was also observed. To our knowledge, no information is available on the effects of low O2 concentration on glycolytic intermediates and related enzymes in harvested plant tissues.

The objective of this research was to determine the effects of low O2 on glycolysis. Thus, respiration rate, levels of glycolytic intermediates, and certain glycolytic enzymes were determined in carrot root shreds stored under low O2 atmosphere.

Materials and Methods

Plant materials and treatments. Carrot roots (Daucus carota) were purchased from a local wholesale distributor in Jessup, Md. Uniform roots were washed, peeled, trimmed of their root tips and stem plates, and shredded with a food processor (DLC-10; Cuisinart, East Windsor, N.J.). Shreds (about 50 mm in length, 5 mm in width, and 4 mm thick) were placed in plastic trays (100 g fresh weight per container), and each tray was stored in a 3.8-liter glass jar at 5 and 15°C. A stream of air, or 0.5% or 2% O2 (balance N2) was metered through the jar at a rate of 10 ml·min−1 at 5°C and 15 ml·min−1 at 15°C, which was sufficient to keep CO2 accumulation below 0.3%. Distilled water (100 ml) was placed at the bottom of the jar to maintain a high relative humidity, and the tray was elevated above the water. Oxygen consumption and CO2 production of the shreds were measured four times every day with a computer controlled electrochemical O2 analyzer (S-3A1; Ametec, Pittsburg, Pa.) and CO2 analyzer (CD-3A; Ametec).

For quantification of glycolytic intermediates and determination of enzyme activities, one jar for each treatment was removed from storage at various intervals. Then, the carrot shreds were immediately frozen in liquid N2 and stored at −80°C until extraction.

Quantification of glycolytic intermediates. All intermediates were analyzed by standard enzymatic methods adapted from Bergmeyer (1983). Frozen carrot shreds (about 5 g fresh weight equivalent) were transferred to liquid N2 in a mortar and ground to a fine powder using a pestle. The powder was mixed with 10 ml of 10% (w/v) trichloroacetic acid solution for 1 h at 0°C with occasional shaking. The mixture was then centrifuged at 30,000×g for 30 min at 0°C. The supernatant was partitioned 4 times against an equal volume of ether; the lower layer was used for quantification of intermediates.

Quantification was carried out spectrophotometrically by monitoring the oxidation/reduction of NADH/NADPH at 340 nm for 10 to 15 min at 30°C in 1 ml of triethanolamine buffer (TEA, pH 7.6) containing the following reagents: 0.5 ml sample, 200 mM TEA, 0.2 mM NADP, 5 mM MgCl2, and 0.2 units of glucose 6-phosphate dehydrogenase for glucose 6-phosphate (G6P); the above mixture plus 0.8 units of phosphoglucose isomerase for F6P; 0.5 ml sample, 200 mM TEA, 20 mM EDTA, 0.1 mM NADH, and 0.2 units of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for glyceraldehyde 3-phosphate (GAP); the above mixture for GAP plus 0.9 units of triosephosphate isomerase (TIM) for dihydroxyacetone phosphate (DHAP); the above mixture for DHAP plus 0.5 units of aldolase for F1,6P; 0.5 ml sample, 100 mM TEA, 1 mM EDTA, 0.2 mM NADH and 8 units of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for glyceraldehyde 1,3-bisphosphate (1,3PGA); 0.5 ml sample, 100 mM TEA, 1 mM EDTA, 0.1 mM NADH, 8 mM NaSO4,
Results and Discussion

Oxygen uptake and CO₂ production of carrot shreds were less under low O₂ than in air atmosphere, and the reduction of O₂ uptake was greater than the reduction in CO₂ production (data not shown). This resulted in a larger RQ for samples in low O₂ than in air. The RQ was also greater at the lower O₂ atmosphere (Fig. 1), which has been noted with other fruits and vegetables (Leshuk and Saltveit, 1991; Solomos, 1983; Weichmann, 1987). During storage, the RQ of samples in air remained unchanged, whereas those in low O₂ increased until day 4, followed by a decrease until day 7 at both 5 and 15°C. Glycolysis operates under both aerobic and anaerobic conditions, but when O₂ is deprived even for short periods of time, many plants accelerate glycolysis and the glycolytic pathway replaces the Krebs cycle as the main source of energy (Kennedy et al., 1992; Mocquot et al., 1981).

The amount of each glycolytic intermediate in low O₂ was compared with that in air using a crossover plot of the data for days 1, 3, and 7 at 15°C (Figs. 2 and 3). Data on the X-axis indicate there were no differences between samples in low O₂ and air atmospheres. The most significant change was the accumulation of F1,6P, with the accumulation being greater at 15°C. F6P, F1,6P, GAP, DHAP, 1,3PGA, 3PGA, 2PGA, and PYR, respectively, as calculated from 5 repetitions with pure glycolytic intermediates in the extracts.

Determination of enzyme activities. Tissue powder (5 g fresh weight equivalents) was prepared as described above and mixed with 10 ml solution containing 100 mM Tris-HCl (pH 7.8), 2 mM EDTA, 1 mM MgCl₂, 5 mM DTT and 2.5 g insoluble PVP at 0°C for 1 h with occasional shaking. The mixture was centrifuged at 30,000×g for 25 min and the supernatant used immediately for measurement of enzyme activities.

Pyrophosphate:phosphofructokinase (PPi:PFK) was assayed spectrophotometrically by monitoring the oxidation of NADH (0.16 mM) in the following: 1-ml reaction mixture according to Smyth et al. (1984a); 100 mM HEPES-NaOH (pH 7.8), 4 mM magnesium acetate, 2 mM MgCl₂, 1.2 units of aldolase, 14 units of TIM, 1.8 units of GDH, 0.05 ml of extract, and 1 mM PPi to initiate the reaction. Twenty µl of 100 µM fructose 2,6-phosphate (F2,6P) was added after a linear rate of production was obtained. The same mixture was used for determination of ATP: phosphofructokinase (ATP:PFK), except that PPi was replaced by 1 mM ATP, and F2,6P was omitted.

One unit is the amount of enzyme that catalyzed the conversion of 1 µmol of substrate per min under our conditions. The recovery of enzyme activity through the quantification process was 95% ± 3% and 97% ± 5% for PPi:PFK, and ATP:PFK, respectively, according to 5 repeated assays with pure enzymes in the extracts. Protein was determined according to Bradford (1976) with bovine gamma globin as a standard.

Three entirely separate experiments were conducted, and determinations and assays were replicated at least twice in each experiment.

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than at 2% O₂. The greater accumulation of F1,6P at 15C was probably due to the higher rate of metabolism at 15C than at 5C.

The activities of PPI-PFK and ATP-PFK in carrots under low O₂ and air atmospheres are shown in Figs. 5 and 6. Activity of PPI-PFK increased sharply until day 3 and then decreased in samples under low O₂, whereas it remained unchanged in samples stored under air. By day 3, PPI-PFK activity increased 3.6- and 2.2-fold in samples under 0.5% O₂ and 2% O₂ at 15C, respectively, and 1.9- and 1.4-fold in samples under 0.5% O₂ and 2% O₂ at 5C, respectively. The time, amount, and pattern of change in the levels of PPI-PFK activity were similar to changes in F1,6P and RQ. The effect of mechanical stress on PPI-PFK activity in carrots caused by processing can be ruled out under our experimental conditions because there were no changes in carrots under air (Figs. 5 and 6).

ATP-PFK activity did not change during storage, and the rate was similar at different O₂ atmospheres and temperatures (Figs. 5 and 6). The lack of change of ATP-PFK activity under low O₂ has been reported previously (Black et al., 1987; Mertens et al., 1990) and support the conclusion that ATP-PFK activity is not affected by low O₂. Under high CO₂ (10%) storage of pears, Kerbel et al. (1988) reported a significant reduction in activities of both ATP-PFK and PPI-PFK. Apparently, low O₂ and high CO₂ do not have a similar effect on these enzymes.

ATP-PFK catalyzes the formation of F1,6P and ADP from F6P and ATP. PPI-PFK catalyzes the reaction in the same direction, but with the formation of F1,6P and Pi from F6P and PPI. Since it also catalyzes the reaction in the reverse direction, PPI-PFK can theoretically catalyze both glycolytic and gluconeogenic carbon flow (Black et al., 1987; Stitt, 1990). Several lines of evidence suggest that F2,6P is an activator of PPI-PFK in the glycolytic direction (Bennett et al., 1987; Mertens et al., 1990; Smyth et. al., 1984b; Tobias et al., 1992). The amount of F2,6P increases substantially under anaerobic conditions when glycolysis becomes very active (Mertens et al., 1987) and also in the presence of an uncoupler (Hatzfeld et al., 1989). In the present paper, F2,6P content was not determined. The effect of low O₂ on F2,6P content in carrots, which is essential to determine the active direction of PPI-PFK in situ, is under investigation.

In summary, low O₂ atmospheres increased the RQ, F1,6P level and PPI-PFK activity but not ATP-PFK activity. These findings indicate that PPI-PFK may contribute to the regulation of glycolysis under low O₂ atmospheres, and that could be a controlling point in the glycolytic pathway affected by low O₂.