Metabolism of maltose and sucrose by microspores isolated from barley (Hordeum vulgare L.)

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Abstract. The aim of this work was to discover why barley (Hordeum vulgare L.) microspores die when cultured on media containing 40 mM sucrose but undergo embryogenesis on 40 mM maltose. Freshly isolated microspores were cultured for 6–24 h on media containing either [U-14C]maltose or [U-14C]sucrose at 40 mM, and the detailed distribution of 14C was determined. The amounts of glycolytic intermediates, ATP, ADP and AMP, in microspores were also measured. Cultures on sucrose differed from those on maltose in that the initial rate of metabolism was faster but declined rapidly, less 14C was recovered in polymers and more in alanine, there was extensive leakage of assimilated carbon, significant accumulation of ethanol and a lower adenylate energy charge. It is argued that microspores cultured on 40 mM sucrose die because they metabolize the sugar rapidly, become hypoxic and, as a result, accumulate large quantities of ethanol within the cells. Metabolism of maltose is slower and there is sufficient oxygen available to allow cells to survive in culture. Consequently some of the cultured cells undergo embryogenesis.

Key words: Embryogenesis – Hordeum – Maltose Microspores – Sucrose

Introduction

There are a number of reports that maltose is greatly superior to sucrose in supporting the regeneration of plants from cultured cells. These include embryogenesis from anthers of barley (Finnie et al. 1989), potato (Batty and Dunwell 1989), wheat (Last and Brettel 1990) and Petunia (Requin 1983), embryogenesis from callus of Medicago sativa (Strickland et al. 1987), and regeneration of shoots from protoplasts of Brassica napus and Brassica oleracea (Loudon et al. 1989). This phenomenon was studied in greater detail by Scott and Lyne (1994a, b) in cultures of isolated barley microspores. This work showed that different sources of carbohydrate have markedly different effects on microspore viability in the first few days of culture. Barley microspores died within 3 d of culture in the presence of sucrose or glucose. Cell death was more rapid in the presence of the latter sugars than in the complete absence of any carbohydrate source. Culture on maltose allowed some of the barley cells to undergo embryogenesis. These observations suggest that glucose and sucrose act, directly or indirectly, to cause cell death. This is unexpected for two reasons. First, sucrose, not maltose, is the form in which carbon and energy are delivered to most of the non-photosynthetic cells of higher plants. Second, the only two enzymes known to metabolize maltose in plants are α-glucosidase and maltose phosphorylase. The former yields glucose, and the latter produces glucose and glucose 1-phosphate. Subsequent metabolism of glucose will give a mixture of hexose monophosphates that includes glucose 1-phosphate. Thus it is difficult to see why the microspores thrive on maltose but die on glucose and sucrose. Finnie et al. (1989) suggested that the rate at which sucrose was metabolized was the cause of the above observations but no data are available to allow this hypothesis to be tested.

The purpose of our work was to discover why barley microspores die when cultured on media containing sucrose or glucose, but some cells undergo embryogenesis when cultured on maltose. Accordingly, we have investigated this phenomenon by studying the uptake and metabolism of [U-14C]maltose and [U-14C]sucrose by microspores isolated from barley.

Materials and methods

Materials. All sugars and the components of the culture media were of the highest grade available from BDH Chemicals, Poole, Dorset, UK. Isotopes were from Amersham International, Bucks., UK. Histopaque was from Sigma Chemicals, Co., Poole, Dorset, UK. Seeds of winter barley (Hordeum vulgare L. cv. Igrï) were germinated and grown at 25°C for a week, vernalized at 4°C for 8 weeks, and then grown at 12°C exactly as described by Hunter (1985).
Microspore culture. The culture medium was that described by Foroughi-Wehr et al. (1976) except that it also contained 5 mM glutamine and 4.4 mM benzyladenine. Microspores were isolated as described by Scott and Lynce (1994a,b). If more than 60% of any preparation was found to be not viable the suspension was purified further by layering it onto 8 ml Histopaque and centrifuging 60-g for 5 min. The microspores that remained on the surface of the Histopaque were collected, resuspended in 5 ml culture medium, centrifuged at 60-g for 5 min and the sediment was collected and suspended in culture medium at a density of 700000 cells ml-1. The viability of the microspore preparations was assessed as described by Scott and Lynce (1994a). The percentage of microspores, that were viable, in the preparations that we used was 51.7 ± 1.2 (mean ± SE, n = 52). Once prepared, the microspores were cultured in the chambers of a Lab-Tek slide. Each slide had eight 0.5-ml sterile chambers to each of which we added 75 ml of microspore suspension and 425 ml of culture medium containing the appropriate concentrations of the required sugars. The cultures were kept atopic and were incubated in the dark at 25°C on an orbital shaker at 76 rev min-1.

Metabolism of 14C-labelled substrates. Labelled substrates were included in the culture medium at the specific activities given in the text. The media were sterilized by filtration. The slides were incubated at 25°C on an orbital shaker at 76 rev min-1.

Enzyme assays. For α-glucosidase (EC 3.2.1.20) samples of freshly harvested microspores (3 x 106) were homogenized in 200 μl 50 mM glycyglycine (pH 7.5) with a ground-glass homogenizer. The homogenate was centrifuged at 10 000 g for 10 min. The supernatant was removed and the sediment was washed twice with two further washes in 200-μl portions of 50 mM glycylglycine (pH 7.5). All the supernatants were combined and desalted by passage through a 10-ml column of Sephadex PD-10 G-25M. The washed sediment was resuspended in 500 μl of 50 mM sodium acetate buffer (pH 5.6). Portions (150 μl) of the resuspended sediment, and of the desalted supernatants were assayed for α-glucosidase as described by Kruger and ap Rees (1983) at 25°C in a 1.0-ml reaction mixture that contained 15 μM maltose or 50 mM sodium acetate buffer (pH 5.6).

Results

Assimilation of labelled sugars. We determined the extent to which freshly harvested microspores assimilated 14C from culture media labelled with [14C]maltose or [14C]sucrose (Table 1). There was assimilation from each sugar and, at every concentration tested, it was much greater from [14C]sucrose than from [14C]maltose. We investigated whether this was so for longer incubations in 40 mM sugar, the concentration in which a relatively high rate of embryogenesis occurs, and a high specific activity of 14C could be achieved. The data in Table 2 show that assimilation from [14C]maltose was roughly constant for the first 10 h and then declined. Assimilation from [14C]sucrose declined throughout the experiment and after 10 h there was a net loss of label from the microspores. For the period in which there was net assimilation from [14C]sucrose, it was greater than from [14C]maltose. Comparable experiments were carried out with [14C]glucose, the results (not shown) were similar...