Short Communications

The Distribution of Glucose Phosphate Isomerase Isoenzymes in Sweet Potato and its Tissue Culture

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The reaction in which glucose 6-phosphate is reversibly converted into fructose 6-phosphate could conceivably play a part in the regulation of metabolism of glucose 6-phosphate, the key intermediate of glycolysis.

Sasaki *et al.* (1972) have reported that glucose phosphate isomerase (D-glucose 6-phosphate ketol-isomerase, EC 5.3.1.9) from sweet-potato root tuber exists in three chromatographically distinguishable isoenzyme forms. However, the factors governing the distribution of these isoenzymes are not known. Analysis of changes in the enzymic pattern in the native plant and its tissue culture may allow some insight into the physiological role of different multiple enzymic forms. Accordingly the glucose phosphate isomerase isoenzymes from sweet potato and its tissue culture and their distribution were investigated.

The root tubers of *Ipomoea batatas* var. Kokei no. 1 were purchased from a local market and stored at 20°C. Callus was obtained by the procedure reported by Okazawa *et al.* (1967) for potato tuber callus. The slices (5 mm × 0.5 mm), which were prepared under sterile conditions with a cork-borer, were transferred on to 30 ml of agar medium (modified White's medium) containing 14 μ M-2,4-dichlorophenoxyacetic acid in a 100 ml Erlenmeyer flask, as described by Okazawa *et al.* (1967). Usually two slices were used per flask and they were incubated in darkness at 23°C for 4 weeks. The liquid suspension cultures were derived from a few pieces of the callus formed and the cultures were propagated by transferring approx. 0.5 g of cells every 3 weeks to fresh medium. All experiments were carried out with cells that had been transferred at least five times.

The standard liquid medium contained Gamborg's (1966) PRL-4 medium modified by the inclusion of 0.2% of casamino acids instead of N-Z Amine Type and the omission of growth hormone. The cells were grown in 500 ml Erlenmeyer flasks each containing 100 ml of the medium with an initial pH of 5.8, and the cultures were grown in the dark at 24–25°C. The flasks were shaken at 80 rev./min on a rotary shaker. The growing cells were used for enzyme extraction at 3 weeks.

The cells in the suspension were harvested by filtering under sterile conditions, and approx. 2 g of these cells was transferred and spread on 30 ml of agar medium in a 100 ml Erlenmeyer flask. They were incubated in the dark at 24–25°C. The standard agar medium is similar to the basal medium for callus formation and contained 2% sucrose and no growth hormone. The enzyme was extracted from agar-cultured cells at 3 weeks.

In our previous work glucose phosphate isomerase was extracted from the tissue of sweet potato by homogenization and from the cultured cells by brief ultrasonic treatment. In our present work, to keep the conditions of cell rupture as constant as possible, the extraction of enzyme from both sweet potato and cultured cells was done under standard conditions involving ultrasonic treatment (1 kV for 2 min) after homogenizing (for 1 min in a Universal homogenizer) about 2.5 g wet wt. of cells in 15 ml of 0.1 M-potassium phosphate buffer, pH 7.0, containing 7.3 mM-2-mercaptoethanol at 4°C. Under these conditions no differences in enzyme pattern were observed between the extract obtained by homogenization and ultrasonic treatment and the extract obtained by either homogenization or ultrasonic treatment alone. The crude extracts were adjusted to pH 7.0 with 0.1 M-KOH and the insoluble material was removed by centrifugation. The glucose phosphate isomerase activity was collected in the fraction precipitated by 20–45% saturation with $(\text{NH}_4)_2\text{SO}_4$, as described by Sasaki *et al.* (1972).

Separation of the glucose phosphate isomerase isoenzymes was done on a DEAE-cellulose column. Glucose phosphate isomerase activity was assayed spectrophotometrically as described by Sasaki *et al.* (1972) (see Fig. 1).

Protein was determined spectrophotometrically by measuring E_{280} . Fructose 6-phosphate (disodium salt), NADP⁺ and glucose 6-phosphate dehydrogenase were purchased from Boehringer und Soehne, Mannheim, Germany. DEAE-cellulose (0.8 mequiv./g) was obtained from Brown, Berlin, N.H., U.S.A.

The multiple forms of glucose phosphate isomerase from sweet potato, liquid-cultured cells and agar-

Fig. 1. Elution of glucose phosphate isomerase isoenzymes of sweet potato and its tissue culture from DEAE-cellulose

The column (1cm×20cm) was equilibrated with 0.02M-potassium phosphate buffer, pH 7.0, containing 7.3mM-2-mercaptoethanol. The sample was then applied and the glucose phosphate isomerase isoenzymes were eluted stepwise. The column was eluted with 50ml of the same buffer, then with 50ml of the same buffer containing 0.1M-NaCl and then with 50ml of the buffer containing 0.2M-NaCl. Fractions (5ml) were collected and subjected to enzyme assay. The reaction mixture contained, in a final volume of 1.0ml: 0.1M-glycine-NaOH buffer, pH 9.0, 0.5ml; $MgCl_2$, 2μmol; fructose 6-phosphate, 1μmol; $NADP^+$, 0.2μmol; glucose 6-phosphate dehydrogenase, 5μg; enzyme source, 50μl. The measurements were made in a recording spectrophotometer for 5 min at 20°C. —, Enzyme activity ($\Delta E_{340}/5\text{ min}$); ----, protein (E_{280}). (a) Sweet potato (12.5mg of enzyme protein); (b) agar-cultured cells (11.4mg of enzyme protein); (c) liquid-cultured cells (17.0mg of enzyme protein).

cultured cells were compared. As shown in Fig. 1, glucose phosphate isomerase of sweet-potato root tuber gave three isoenzymes, I, II and III, as reported by Sasaki *et al.* (1972), but in the liquid-cultured cells the peaks corresponding to glucose phosphate isomerase isoenzymes II and III were absent and almost all of the isomerase activity was recovered in the peak

corresponding to isoenzyme I. However, the enzyme from agar-cultured cells separated into glucose phosphate isomerase isoenzymes I and III, and isoenzyme II was not detected. In each tissue the total activity recovered from the column was about 95% of that applied, and further elution with 1.0M-NaCl in phosphate buffer revealed no other peak with

isomerase activity. The protein profiles of sweet potato, liquid-cultured cells and agar-cultured cells obtained by chromatography on DEAE-cellulose were thus significantly different (Fig. 1). We find it remarkable that isoenzymic composition and protein profiles among sweet potato and cultured cells are so very different. Our results suggest that the control of synthesis, and therefore of the use, of glucose phosphate isomerase isoenzymes is governed by the conditions in each type of cell, and further that these

different isoenzymes have a significant, although as yet poorly understood, role in the regulation of glucose 6-phosphate and fructose 6-phosphate metabolism in the cell.

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