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ACTIVITY OF PHOSPHORYLASE IN *SOLANUM TUBEROSUM* DURING LOW TEMPERATURE STORAGE

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; tuber; phosphorylase; starch–sugar interconversion.

Abstract—Changes in the activity of phosphorylase were measured during storage of potatoes at +2° when the sugar content rises rapidly and subsequently at +10° when the accumulated sugar is converted mainly to starch. The observed changes were relatively small and could not be related to any of the components of the phosphorylase system, which was shown to be complex.

INTRODUCTION

Phosphorylase (α-1,4 glucan orthophosphate glucosyl-transferase E.C. 2.4.1.1) probably catalyses the first step in the cold-induced sweetening of potatoes [1]. This is the breakdown of starch in the presence of orthophosphate to glucose-1-phosphate. A purified enzyme with well characterized properties indicating that it is a single protein has been prepared from potato [2], but the initial crude enzyme extract contains a number of phosphorylase isoenzymes because electrophoresis in polyacrylamide gels separates five zones [3] with starch synthesising activity. The polysaccharide synthesised in the different zones does not give the same colour with iodine, which suggests that a branching enzyme is associated with some of the isoenzymes. Since the whole phosphorylase complex appears to be very readily extracted by aqueous solvents, a crude aqueous extract was used in the present study to ascertain whether there was any connection between either the overall activity or an individual isoenzyme and the progress of the starch–sugar interconversion [1].

RESULTS AND DISCUSSION

Changes in phosphorylase activity during storage

The potatoes (King Edward) were stored for varying periods at +2°. Some which had been at +2° for 28 days were transferred to +10°. Activity was measured in extracts from both fresh potatoes and from frozen potato powder. In preliminary experiments the activity was measured at pH values from 4·85 to 7·5. The maximum apparent activity occurred at a pH between 5·5 and 6 but since a purified enzyme [2] which represents the major component of the complex is known to have maximum activity at 6·5 in citrate buffer, it appeared that either another enzyme was interfering or that the isoenzymes of phosphorylase had different pH maxima. The pH curve under these conditions had a broad maximum, the difference in activity between 5·8 and 6·3 being less than 10%. In the standard assay procedure the pH was adjusted to 6·1 as a compromise to limit any interference which might be due to phospho-glucomutase (this enzyme is inactive at this pH). The rate of reaction with time was linear up to 20 min reaction time. Using bulk frozen potato powder the coefficient of variation (16 samples) was 8·7% and for fresh potatoes (16 separate batches of potatoes) 29%. The recovery of purified potato phosphorylase [2] (in amount about equal to the endogenous phosphorylase activity) added to frozen potato powder and extracted in the standard method was better than 95% (four sets of duplicate extractions). Some of the extracts were passed down a G25 Sephadex column to remove low molecular weight metabolites and others after this treatment were allowed to stand for 20–30 days at 0°. The results are shown in Fig. 1.

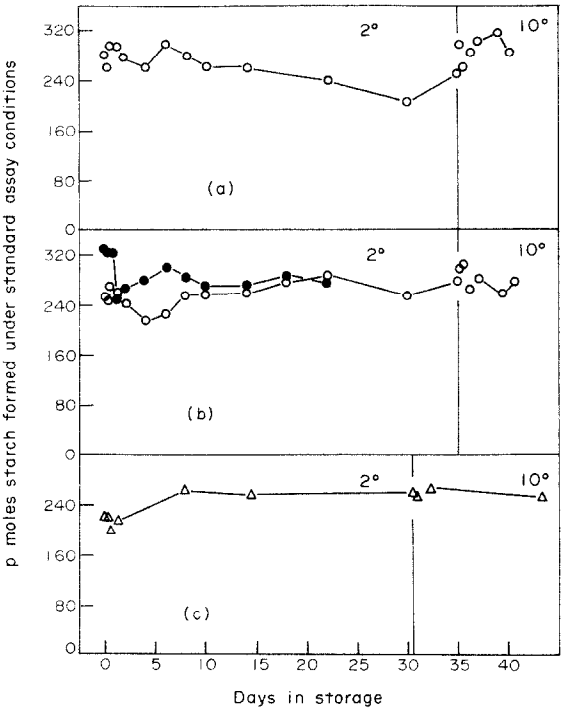

Fig. 1. Changes in phosphorylase activity during storage of potatoes at +2° and +10°. (a) Fresh material, crude extract, activity measured within 30 min —○—; (b) fresh material, crude extract freed from substances of low MW by passing down column of Sephadex G25 (coarse) previously equilibrated with 0·001 M MES buffer, pH 6, 0·1 M NaCl —○—; purified extract allowed to stand 20–30 days at 0° —●—; (c) frozen powder, crude extract, activity measured within 30 min —△—; vertical lines indicate times at which potatoes were transferred.

In general, whether observations were made on fresh potato tubers (Fig. 1a) or on bulked frozen powder, changes in storage temperature did not cause significant changes in total phosphorylase activity. Measurement of the overall phosphorylase activity did not differentiate between the various isoenzymes nor did it indicate any changes in the debranching enzyme. However, electrophoretograms showed no obvious changes in the pattern of isoenzymes and it is interesting that three varieties of potato, Désirée, Bintje and King Edward, all showed the same pattern. The removal of low molecular weight solutes (Fig. 1b) did not immediately affect the activity but, on allowing these extracts to stand, the activity increased appreciably. The presence of ATP (5·5 mM) inhibited part of the phosphorylase activity. In extracts from potatoes stored at +2° for 2–10 days the mean relative inhibition at a confidence level of 99% (for 12 observations) was 7·4% ± 2·0%. The same extracts were those which showed the greatest increase in activity on standing (Fig. 1b). The reasons for this are not known but the changes in activity were not associated with particulate matter (the soln was centrifuged for 4 hr at 200000 *g*). The obvious complexity of this group of enzymes prompted a more detailed examination to determine whether any had any special significance in relation to the interconversion of starch and sugar.

Fractionation of crude extracts

A crude extract was fractionated on a column of Sepharose 6B in the presence of 0·15 M NaCl. In the absence of NaCl a large part of the phosphorylase was eluted in the void volume. The reason for this was not clear but the phenomenon may be connected with the presence on the agarose gel of a number of charged groups. The shape of the curve (Fig. 2) indicated the presence of at least three components but perhaps even more important was the observation that during the assay the polysaccharide synthesized in fractions under different parts of the curve stained a different colour with iodine.

The same general picture was obtained when a crude extract was fractionated on a DEAE Sephadex column. The enzyme was partially purified by precipitation with ammonium sulphate to

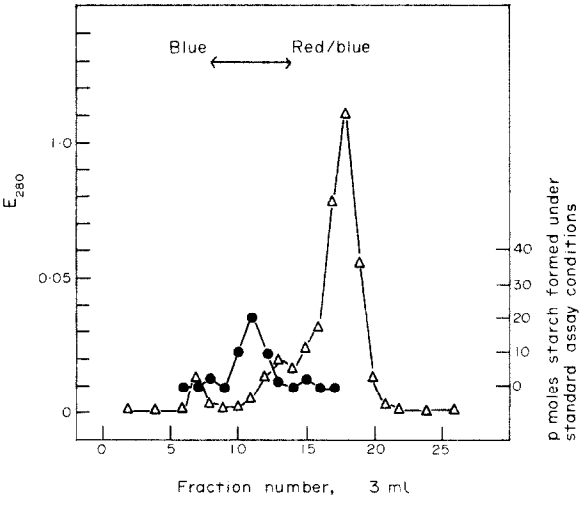

Fig. 2. Chromatography of crude potato extract on a column of Sepharose 6B previously equilibrated with 0·001 M MES buffer, pH 6, 0·15 M NaCl. Protein as absorption at 280 nm —△—; phosphorylase activity —●—; reaction colour (with iodine) of polysaccharide synthesized in assay test indicated appropriately.

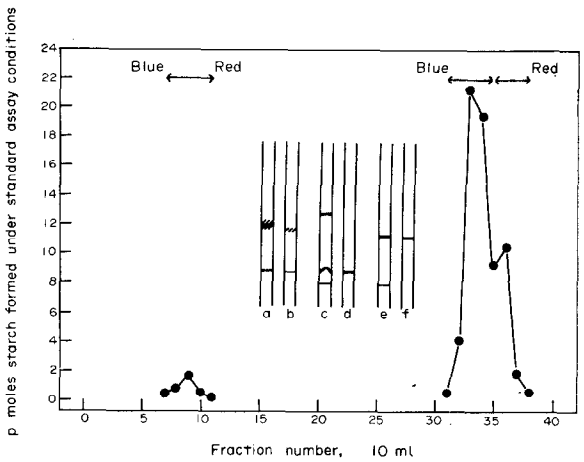

Fig. 3. Chromatography of crude potato extract after ammonium sulphate fractionation on a column of DEAE Sephadex A25 previously equilibrated with 0·1 M Tris–HCl buffer, pH 9 (+ 1°). Peaks eluted with a linear NaCl gradient (0–0·5 M) Phosphorylase activity —●—. Reaction colour of polysaccharide synthesized in assay test (with iodine) indicated as in Fig. 2. Gel electrophoresis patterns of aliquot fractions a and b contained 0·1% amylopectin + 0·002 M EDTA, c and d 0·1% soluble starch, e and f 0·1% glycogen. a, c and e were of Fraction 33, b, d and f of Fraction 37. Solid lines represent bands which stained blue with iodine and cross hatched areas bands which stained red.

remove phenolic material which otherwise caused the enzyme to be absorbed at the top of the column. The curve (Fig. 3) showed the presence of at least three components and again, as with gel filtration, the polysaccharide synthesized during the assay procedure stained a different colour with iodine depending on the part of the curve. Electrophoretograms of selected fractions confirmed the complexity of the phosphorylase enzyme and also the presence of a branching enzyme. The first component of the curve gave only a single band on the electrophoretograms and this has not been included in Fig. 3. Modifying the composition of the gel affected the separation of the various isoenzymes. When glycogen was incorporated only one main band was present, with soluble starch two were present (Fraction 33) and with amylopectin and EDTA three main bands were present, but two of them stained red with iodine. Of the various factors studied, the two most significant were the type of polysaccharide incorporated in the gel and the addition of EDTA to the buffer. The various isoenzymes showed different adsorption properties towards polysaccharides and by incorporating the appropriate polysaccharide in the gel it was possible to change the pattern of separation. The presence of phosphate also appeared to affect the separation by causing an apparent dissociation of a complex.

The evidence from the fractionation procedures indicated that the phosphorylase group of enzymes was complex. While the overall activity of phosphorylase itself appeared to be largely unaffected by a change in temperature of storage, which suggested that control of the starch–sugar interconversion was not localized with this enzyme, the evidence could be misleading. Other factors may prevent the enzyme from attacking the starch grain, e.g. the enzyme *in vivo* may be attached to a membrane and only released on a suitable stimulus even though aqueous extraction removes it completely. Even if phosphorylase is not prevented from attacking the starch grain, it is likely that the nature of the multi-branched polysaccharide and its position in the starch grain will largely restrict any attack. Attack will only proceed in the presence of a debranching enzyme. Most assay systems consider only the role of phosphorylase as a synthetic enzyme in which the exterior chains of the polysaccharide are lengthened and there is no convenient assay in which the attack on the starch is measured. There is little information about changes in the debranching enzymes.

EXPERIMENTAL

All column and fractionation procedures were carried out in a cold room at + 1°.

Preparation of phosphorylase extracts. The washed potatoes (King Edward; 10 tubers in 2 lots of 5, 700 g) were sliced and ground in a Moulinex juice extractor together with an equal quantity of aq. 0·02% EtSH. An additional amount of EtSH, 0·02% by weight, was added to the final extract to prevent phenol oxidase action. A representative sample (50 ml) was centrifuged (25 min 50000 *g*) for the assay of phosphorylase activity [4].

Gel electrophoresis. This was performed with 8, 7 and 5% acrylamide usually in the presence of a polysaccharide.

Gel filtration. A crude extract (1 ml) was fractionated on a column (29 × 1·7 cm dia.) of Sepharose 6B which had been equilibrated against either 1 mM MES buffer, pH 6, or 0·15 M NaCl and the MES buffer. Fractions of 3 ml were collected at a flow rate of 10 ml/hr.

DEAE-Sephadex column chromatography. The crude extract (126 ml from 50 g potato) was fractionated by the addition of $(NH_4)_2SO_4$ before applying it to the DEAE-Sephadex column [5]. The protein precipitating between 30 and 50% satn contained most of the activity. The $(NH_4)_2SO_4$ was removed from the soln (the ppt. was dissolved in 10 ml 0·02% aq. mercaptoethanol) by passing down a column (27 × 1·5 cm dia.) of G25 Sephadex gel which had been previously equilibrated against 0·1 M Tris–HCl buffer, pH 8·3. The phosphorylase was eluted in the void vol.

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