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# CONVERSION OF POTATO PHOSPHORYLASE ISOZYMES

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**Key Word Index**—*Solanum tuberosum*; Solanaceae; potato phosphorylase; isozymes; proteases; PAA gel electrophoresis.

**Abstract**—The conversion of a slow moving potato phosphorylase isozyme to a fast one, in sprouting tubers, either on freezing the whole tubers or on storage of their crude extracts, is due to limited proteolysis. High protease inhibitor concentration seems to be the primary factor preventing this conversion in freshly harvested tubers under similar conditions. Though MW determinations on both isozymes show the removal of a peptide during conversion, it is also likely that the enzyme may take up a different conformation due to the removal of this peptide.

## INTRODUCTION

The presence of multiple forms of potato phosphorylase has been demonstrated by various investigators [1–8]. The isozyme pattern obtained by polyacrylamide electrophoresis depends upon the physiological state of the tubers [3,9]. Among the isozymes, one which migrates about half way in the PAA gel is most prominent; this enzyme is converted into a faster moving isozyme on freezing the sprouting potato tubers [10].

The present investigation was carried out in order to obtain some insight into the factor(s) governing this conversion and its probable physiological significance. The isozymes are numbered, according to Ref. [3] (see Fig. 1).

## RESULTS

*Conversion experiments.* When the crude extracts of sprouting tubers are stored at 4°, the slower moving phosphorylase isozyme (isozyme 6) is converted into a faster moving isozyme [10]. After 24 hr incubation the conversion was not complete and besides the slower and faster (isozyme 6, 6A) zones, an intermediate zone was also observed. However, on longer storage (72 hr) only

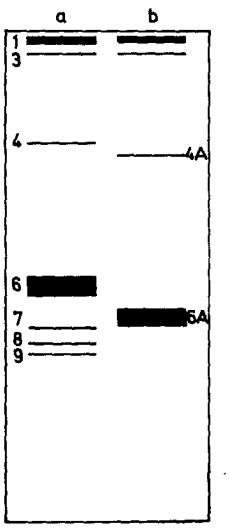


Fig. 1

Fig. 1. Phosphorylase isozymes before (a) and after (b) freezing of potato tubers. Electrophoresis was conducted in 6% Cyanogum® in Tris–HCl pH 8·9 and Tris–glycine pH 8·3 as the chamber buffer. Isozymes are numbered according to [3].

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the faster moving zone was present. The conversion was independent of the pH of the extracts and took place in the pH range 5–9, using universal buffer, [11]. This conversion did not take place in crude extracts from freshly harvested young tubers. A similar conversion could also be brought about by freezing the whole (sprouting) tubers (Fig. 1), whereas freezing did not have any effect on freshly harvested tubers. Isozyme 4 is also converted to a faster moving enzyme on freezing the sprouting tubers. However, no further studies were carried out on the conversion of this isozyme.

*Action of enzymes.* In order to understand this conversion, the action of various exogenous enzymes was investigated. Extracts of sprouting tubers in borate buffer pH 8·2, (0·04 M) were incubated with α-amylase, lysozyme, and proteases such as trypsin, chymotrypsin and papain, for 4 hr at 4°. Enzymes were used in concentrations of 2 and 10 mg/ml of crude extract. The phosphorylase pattern did not change after incubation with amylase and lysozyme, while complete conversion of the slower moving to the faster moving isozyme took place in presence of papain (2 and 10 mg/ml) and also with 10 mg trypsin and chymotrypsin, respectively. Trypsin at a lower concentration could bring about only a partial conversion. The complete conversion by trypsin (10 mg/ml) was accompanied by a considerable loss in activity of the converted enzyme. As papain was most effective, subsequent conversion experiments were carried out only with papain.

Experiments with the crude extracts of freshly harvested tubers showed that only high concentrations of papain (10 mg/ml) could bring about the conversion, and that too, only a partial conversion. Papain in both of the concentrations could convert the semipurified (Sephadex G-200 fractions) isozyme 6 into 6A. Once converted, the faster moving isozyme was stable in presence of papain for a longer time (72 hr), whereas the activity disappeared in presence of trypsin and chymotrypsin.

*MW.* Gel filtration on Sephadex G-200, with catalase and phycoerythrin as markers, revealed a MW of 320000 for the slow zone and 260000 for the fast zone. Electrophoresis of both the isozymes, in gels of varying amounts of acrylamide ence in MW's of both the zones. However, MW determination by porosity gradient polyacrylamide gel (6–24%) electrophoresis, using Tris–borate buffer pH 8·3 (400 V, 66 hr) with rabbit muscle phosphorylase b and bovine serum albumin as markers, revealed a MW of 200000 and 162000 for the slower and faster moving isozymes respectively [13]. Sucrose gradient centrifugation experiments [14] with catalase as a marker showed a sedimentation velocity of 8·4 S for both isozymes.

## DISCUSSION

The conversion of the slower moving phosphorylase (isozyme 6) into the fast moving one (isozyme 6A) takes place in sprouting tubers after freezing or storing their extracts for about 48 hr at 4°. The same conversion, traced by gel electrophoresis [1], can also be affected in a short time by the action of added proteases. Therefore, it is likely that the conversion observed without added proteases is caused by the action of endogenous proteolytic enzymes. The absence of the fast moving phosphorylase in freshly prepared extracts of sprouting tubers indicates that phosphorylase and the endogenous protease(s) do not come in contact *in vivo*, probably due to the compartmentalization of the cell. Freezing or homogenization disrupts the integrity of the cell, with subsequent release of the endogenous protease(s). The difference in the behaviour of sprouting and fresh tubers, could be due either to the difference in protease activity or to a high inhibitor concentration.

The inability of the exogenously added papain to bring about the conversion, indicates high inhibitor concentration to be the most likely factor, preventing the conversion in fresh tubers. Hence, we assume that the observed phenomenon does not have an obvious physiological significance in carbohydrate metabolism. On the other hand, the two enzymes have quite different kinetic properties (K.N. Shivaram, unpublished results). Since Fekete [15] has shown that phosphorylases do not only catalyze the degradation of starch but also the synthesis, this phenomenon of a distinct shortening of the enzyme chain with full activity retained (in case of papain and endogenous pro-

Further, the ability of papain to convert the semi-purified isozyme 6 to 6A shows that its action is direct, and not indirect, i.e. through activation of potato proteases by hydrolyzing the inhibitors.

Though the difference in the MW's of both the isozymes obtained by gel filtration, polyacrylamide gel electrophoresis and porosity gradients, indicate the removal of a rather big peptide, it is also possible that the enzyme may be taking up another conformation, due to removal of a comparatively small peptide. The latter explanation is supported by the identical behaviour of both isozymes on sucrose gradients and also by the high resistance of the faster moving isozyme to papain.

## EXPERIMENTAL

Potato varieties, Grata and Bintje, stored at 4°, were used.

*Preparation of the extract.* Tubers were peeled, sliced and homogenized in a Waring blendor, with equal vol. of appropriate buffer. In order to prevent browning 2 ml of a sulfite soln, containing 5% $Na_2SO_3$ and 3·75% $Na_2S_2O_5$ in $H_2O$, was added to every 100 ml of juice. Juice was then centrifuged at 12000 *g* for 15 min at 0° and supernatant was used as the crude extract. The proteolytic enzymes used were purchased commercially. Gel filtration on Sephadex G-200 columns were carried out according to Andrews [16]. Centrifugation on sucrose gradients was performed according to Martin and Ames [14]. Gel electrophoresis was performed on cooled slabs of 3 mm thickness [1,17]. Buffer systems used were Tris–borate (0·04 M, pH 8·2), both for the gel and the chambers; Tris–HCl (0·375 M, pH 8·9), for the gels and Tris–glycine (0·05 M, pH 8·3) for the chamber. Gel electrophoresis was performed in tubes (0·6 × 10 cm) as previously described [3,12]. The porosity gradient in gel slabs was done according to the modified method of Margolis and Kenrick [13]. The phosphorylase activity was demonstrated by overnight incubation of the gels at room temp. in citrate buffer (0·1 M, pH 5·1) containing 0·1% glucose-1-phosphate, and staining the synthesized polyglucans with aq. soln of dil iodine [1].

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