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Amylase activity in potato tubers

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Summary

Amylase activity in extracts of sprouted tubers was optimised at final concentrations of soluble starch in the incubation medium of 0.6 – 2.0 mg cm^{-3}. Optimum pH was 6. The exclusion of calcium ions from extraction and incubation media did not result in reduced enzyme activity. This, together with a shift in the absorption maximum of the starch-iodine complex almost identical to that observed with pure β-amylase, indicates the predominance of β-amylase in the extracts. Over a 15-min incubation period the linearity of the response was dependent upon the volume of tuber extract included in the assay medium. Gel filtration of extracts did not influence this response.

Introduction

Although the mechanism of starch breakdown in germinating seeds has received much attention in recent years, little is known about the sequence of events operating in the potato tuber (Preiss & Levi, 1980; Halmer & Bewley, 1982). More information on the pathways of degradation is required if we are to examine possible limitations to sprout growth set by the maximum catalytic activities of enzymes controlling reserve breakdown. Since starch degradation appears to provide the primary source of carbon for reducing sugar accumulation (Isherwood, 1973) the expression of amylolytic and phosphorolytic enzyme activity is also important in determining the quality of tubers for the crisping and chipping industries (Sowokinos, 1973 and references therein). In this paper we outline some of the properties of amylase from sprouting tubers.

Materials and methods

Tubers of *Solanum tuberosum* L. cv. Maris Piper were planted in compost, prepared as described by Thompson & Taylor (1979), and allowed to sprout for 21 days at 15°C in the dark. Thick, transverse sections were taken from the mid-region of the tuber and the periderm discarded. An amount of 5 g fresh weight of tuber was extracted at 2°C in a pestle and mortar with 1 g polyvinyl polypyrrolidone and 10 cm^3 citrate-phosphate buffer (0.1 mmol dm^{-3} (pH 6) containing 20 mmol dm^{-3} $CaCl_2$. After centrifugation at 3000 *g* for 5 min, samples of the supernatant, made up to 1 cm^3 with extraction medium, were incubated with soluble potato starch (0.6 mg cm^{-3} final concentration) for 15 min at 30°C. The reaction was stopped with I_2/KI (Chrispeels & Varner, 1967) and the decrease in absorbance at 620 nm recorded. Some extracts were desalted on a 20 cm × 1 cm column of Bio-gel P-6PG (Bio-Rad, UK). The contributions of α- and β-

amylases towards total amylase activity were assessed; (a) by comparing the shift in the absorbance spectrum of the starch-iodine complex with tuber enzyme extracts with the shift observed with pure α- and β-amylases, and (b) by determining the reduction in total amylase activity following the chelation of calcium ions with ethyleneglycolbis (β-aminoethyl ether) N,N,N^1, N^1,-tetraacetic acid (EGTA).

Results and discussion

Two of the main criteria that have to be met in the development of assays with relatively crude enzyme extracts are; (1) that activity is proportional to the quantity of extract used, and (2) that activity is linear over the assay period chosen. Fig. 1a shows that total amylase activity was almost linear for 15 min with the lowest volume of extract, but that linearity was disturbed by an increase in enzyme volume. The effect is unlikely to be due to low molecular weight inhibitors as gel-filtration failed to improve the response to increased amounts of extract (Fig. 1b). Time course analysis should therefore be carried out routinely as a check against any change in the linearity of the response.

Tuber amylase activity has a pH optimum of 6 (Fig. 2a) and activity appears to be optimal at substrate concentrations between 0.6 and 2.0 mg starch per cm^3 (Fig. 2b). However, we were unable to demonstrate an obligate requirement for calcium ions in the extraction and incubation medium (Fig. 2c). Since plant α-amylases are reported to be calcium-requiring metalloenzymes (Preiss & Levi, 1980), this would indicate that β-amylase predominated in the samples used in the present study. An analysis of the absorption spectrum of the starch-iodine complex before and after incubation with tuber extracts showed a shift in maximum absorbance from 575 nm to 551 nm (Table

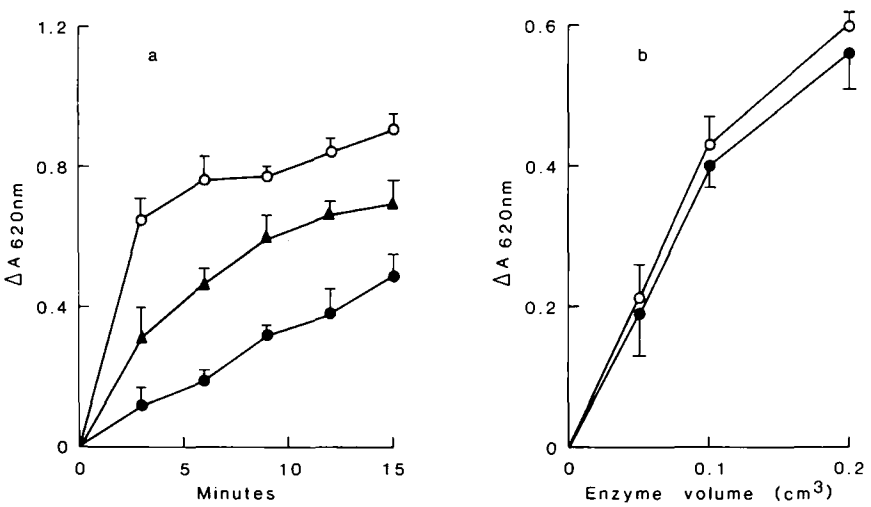

Fig. 1. (a) The influence of extract volume on the time course of amylase activity. ● - 0.1 cm^3; ▲ - 0.25 cm^3; ○ - 0.5 cm^3. (b) The effect of desalting by gel filtration on the time course of amylase activity with different volumes of extract. ○ - desalted; ● - not desalted. Bars indicate ± standard error of the mean.

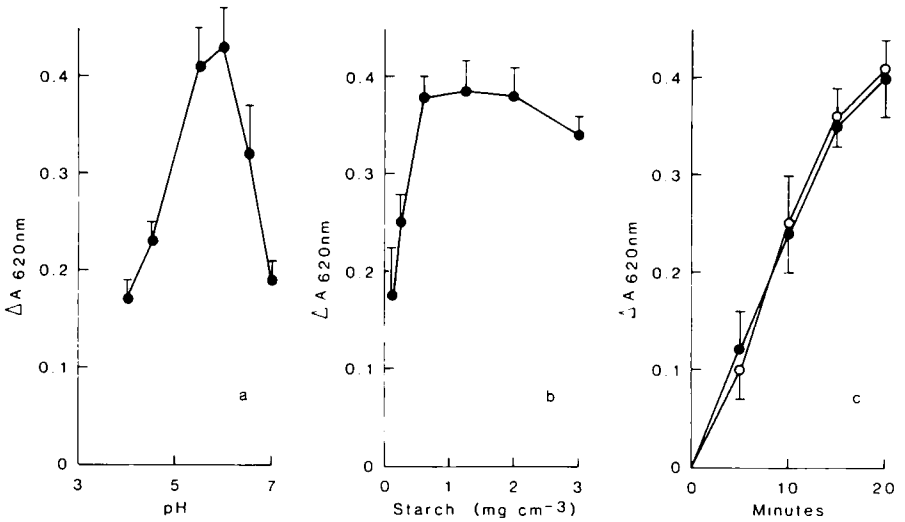

Fig. 2. The influence of (a) pH, (b) soluble starch concentration and (c) calcium ions on amylase activity. (○ - assay medium containing 20 mmol dm^{-3} $CaCl_2$: ● - $CaCl_2$ omitted; 10 mmol dm^{-3} EGTA included in extraction and assay medium). Extract Volume 0.1 cm^3: incubation period 15 min.

1). This was close to the shift observed with pure β-amylase (Sigma, type 1-B) whereas the shift in maximum absorbance with pure α-amylase (Sigma, type X11-A) was much smaller. These differences again point to the predominance of β-amylase in the tuber extracts.

Other methods reported to be specific for α-amylase also failed to detect activity (Davies & Ross, 1986). The possibility, with the use of phosphate buffer, that starch breakdown occurred through the action of starch phosphorylase rather than amylase was tested by replacing citrate-phosphate buffer with sodium acetate buffer and by desalting the extracts. The results showed no major contribution of starch phosphorylase to starch breakdown in the enzyme assay employed (data not presented).

The authors of two papers (Nowak, 1977; Sowokinos et al., 1985) who used methods similar to the one outlined here, refer to measuring α-amylase in potato tubers but in the light of our findings the validity of this assumption must be questioned. The

Table 1. Absorption maxima (nm) of starch iodine complex before (T0) and after 30 min incubation (T30) with tuber extract or either purified α- or β-amylase. Units are those defined by the manufacturer (Sigma).

	T0	T30
Extract	575	551
β-amylase (0.1 unit)	576	552
α-amylase (0.1 unit)	576	573

potential importance of differentiating between α- and β-amylases lies in the suggestion that α-amylase is responsible for the initiation of starch granule degradation (Dunn, 1974) and in the observation that β-amylase activity is high in unsprouted tubers, i.e. prior to the onset of rapid starch depletion, while α-amylase activity is undectectable (Davies & Ross, 1986).

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