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# THE EFFECT OF TEMPERATURE ON ADENOSINE DIPHOSPHATE GLUCOSE PYROPHOSPHORYLASE FROM *SOLANUM TUBEROSUM*

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**Key Word Index**—*Solanum tuberosum*; Solanaceae; potato; adenosine diphosphate glucose pyrophosphorylase; temperature; sweetening.

**Abstract**—A partially purified extract of adenosine diphosphate glucose pyrophosphorylase has been prepared from *Solanum tuberosum*. The effect of temperature on the initial rate of reaction has been determined in the presence and absence of activator. The results are discussed in relation to the sweetening of potatoes at 2°.

## INTRODUCTION

Potatoes stored at temperatures below 10° rapidly sweeten as a result of the conversion of starch to sugar and the lower the temperature of storage, the greater the accumulation of sugar (at 2° the total sugar reaches 2%). Since it is probable that even at 10° and above the starch and sugar are continuously being interconverted [1], the accumulation of sugar at low temperature could be caused by restriction of starch synthesis as a result of cold induced changes in the properties of the ADP-glucose pyrophosphorylase. It was of interest therefore to study the effect of temperature on a partially purified preparation of the above enzyme from potatoes between 0° and 10°.

## RESULTS

The crude extract, after centrifugation but before dialysis, had a sp. act. of 1·8 mmol of ADP-glucose formed/min/mg protein. Dialysis did not significantly change this figure though both the total activity and the amount of protein fell to one-third of their initial values. After heat treatment, centrifugation and dialysis, the sp. act. was 36, and after $(NH_4)_2SO_4$ precipitation and dialysis, 100. The most notable feature of the potato enzyme was the fact that the heat treatment did not cause any loss of activity but actually caused a several-fold increase in overall activity. The activity of the partially purified enzyme was enhanced both at 10° and 30° by 3-phosphoglycerate (10-fold at either temperature) and by phosphoenolpyruvate (4- and 3-fold respectively). Fructose-6-phosphate and glucose-6-phosphate had a negligible effect. These results suggest that the ADP-glucose pyrophosphorylase from potato tuber has similar properties to the leaf enzyme [2], viz. activation by 3-phosphoglycerate and stability to heat treatment at 65° in the presence of 0·02 M phosphate buffer, pH 7.

A study of the activity of the enzyme at different temperatures in the presence and absence of 3-phosphoglycerate, presented as Arrhenius plots, is given in Fig. 1. These clearly show that, within the limits of the assay method, an approximately linear slope is obtained in all cases. The error in the method [3] is greatest at 0° and, though observations at this temperature are included in Fig. 1, they have been given less weight when the line of best fit was drawn.

The potato is a chilling-resistant plant tissue [4] and it is significant that Arrhenius plots of the respiration rates of mitochondria isolated from potatoes showed a linear decrease over the entire temperature range from 25° to 1·5°, whereas similar plots of the respiration rates of mitochondria isolated from chilling-sensitive plant tissue (tomato and cucumber fruit and sweet potato roots) showed a linear decrease from 25° to *ca* 10°, at which point there was a marked deviation with increased slope as temperatures were reduced to 1·5°.

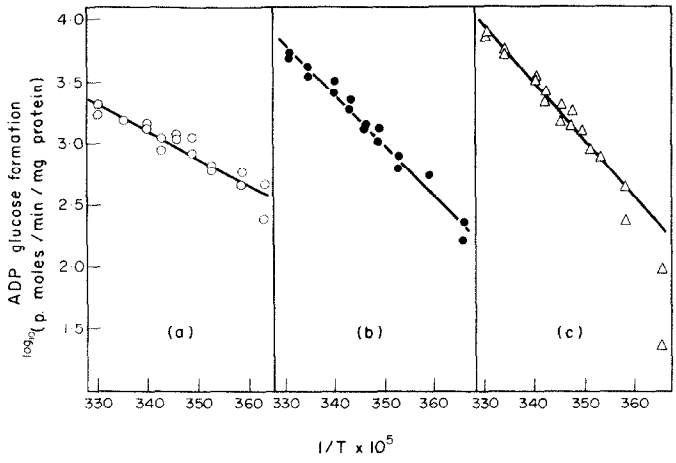


Fig. 1. Arrhenius plots of the formation of ADP-glucose by ADP-glucose pyrophosphorylase in the presence and absence of an activator (3-phosphoglycerate). (a) No 3-phosphoglycerate —○—: (b) 2 mM —●—: (c) 20 mM —△—.

The behaviour of ADP-glucose pyrophosphorylase from potato is thus in line with the temperature properties of the oxidative activity of the mitochondria. It seems likely therefore that the explanation for the conversion of starch to sucrose during low temperature storage does not lie with a change in the properties of ADP-glucose pyrophosphorylase with temperature.

## EXPERIMENTAL

*Preparation of enzyme.* The enzyme was prepared from potato tubers (cv. King Edward) by blending the diced tubers with half their weight of a buffer containing 0·05 M Tris-HCl, pH 7·5, 2 mM EDTA, 10% glycerol and 10 mM cysteine and then purifying the enzyme as described previously [2], except that the solutions contained mercaptoethanol in place of reduced glutathione. The extract, subdivided into quantities suitable for the subsequent enzymic experiments, was kept at −80°.

*Assay of enzyme.* For the measurement of the stimulation of the enzyme by various activators, the usual method [3] was modified. The reaction mixture (total vol. 0·2 ml) contained, in μmol, glycyl-glycine buffer, pH 7·5, 5·3; $MgCl_2$, 2; NaF, 1·7; ATP, 0·13; together with bovine serum albumin 1 mg, enzyme 0·01 ml and glucose 1-[$^{14}$C]phosphate (0·1 μmol, sp. act. 8 × $10^5$ cpm/μmol). The concentration of the activators present in the reaction mixtures was 1 mM.

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## REFERENCES

1. Isherwood, F. A. (1973) *Phytochemistry* **12**, 2579.
2. Sanwal, G. G., Greenberg, E., Hardie, J., Cameron, E. C. and Preiss, J. (1968) *Plant Physiol.* **43**, 417.
3. Shen, L. and Preiss, J. (1964) *Biochem. Biophys. Res. Commun.* **17**, 424.
4. Lyons, J. M. and Raison, J. K. (1970) *Plant Physiol.* **45**, 386.