Phytochemistry, 1975, Vol. 14, pp. 351–352. Pergamon Press. Printed in England.

THE EFFECT OF WOUNDING ON GLUCOSE-6-PHOSPHATE DEHYDROGENASE OF *SOLANUM TUBEROSUM* TUBER TISSUE

SJ. J. GERBRANDY and J. D. VERLEUR

Biologisch Laboratorium der Vrije Universiteit, De Boelelaan 1087, Amsterdam, The Netherlands

(*Received* 28 *April* 1974)

Key Word Index—*Solanum tuberosum*; Solanaceae; potato; glucose-6-phosphate dehydrogenase; isoenzymes; MW; wound reaction.

Abstract—Two forms of glucose-6-phosphate dehydrogenase were separated by disc electrophoresis of potato tuber extracts. The slower moving enzyme has a MW of 260000 the faster one of 130000. Wounding of potato tubers enhances the relative activity of the slower moving enzyme. Addition of $NADP^+$ to the cathode buffer during electrophoresis has the same effect as wounding, whereas addition of glucose-6-phosphate has an opposite effect. The role of the wound induced increase of the pyridine nucleotide level in the interconversion of the two forms of glucose-6-phosphate dehydrogenase is discussed.

INTRODUCTION

Wounding of potato tubers increases respiration and the activity of the pentose phosphate pathway and the citric acid cycle [1]. In studies of the pentose phosphate pathway, attention has been paid to the increased activity of glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) and 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44) and the regulating function of the pyridine nucleotide cofactors concerned in these enzyme reactions [2–4]. This report deals with the glucose-6-phosphate dehydrogenase isoenzymes of potato tubers, in order to interpret on the molecular level the wound induced rise in enzyme activity.

RESULTS AND DISCUSSION

Two forms of glucose-6-phosphate dehydrogenase were separated from crude extract of potato tuber tissue by polyacrylamide gel electrophoresis. Wounding of the tubers induces an increase of the activity of the slower moving enzyme (R_f 0·25) and a decrease of the activity of the faster moving enzyme (R_f 0·44) (Figs. 1a, b). Identical differences in enzyme pattern were obtained with extracts which had been purified by Sephadex G200 chromatography before electrophoresis. Consequently, it is unlikely that the change in pattern observed after wounding was caused by a difference in composition of the extracts.

This change in enzyme pattern could be caused by a preferential synthesis of one form or by a transformation of one form into the other. The glucose-6-phosphate dehydrogenases from *Neurospora* [5] and from *Ipomoea* [6] are reported to

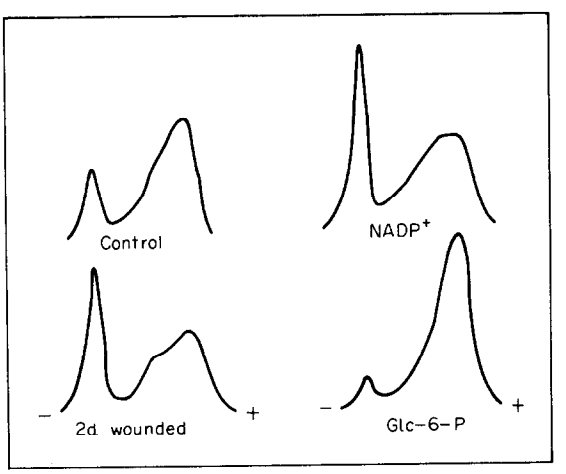

Fig. 1. Densitograms of glucose-6-phosphate dehydrogenase. The forms were separated by polyacrylamide gel electrophoresis: (a) of crude extract from intact potato tubers; (b) of crude extract from tissue two days after wounding; (c) of crude extract from intact potato tubers with 0·02 mM $NADP^+$; or (d) 1·5 mM glucose-6-phosphate, in the cathode buffer.

produce high MW forms after addition of $NADP^+$. The effect of cofactors and substrate on the enzyme pattern of crude potato extract was studied by electrophoresis with either $NADP^+$ (0·02 mM) or NADPH (0·02 mM) or glucose-6-phosphate (1·5 mM) added to the cathode buffer. As is indicated in Fig. 1(c), $NADP^+$ enhances the relative activity of the slower moving enzyme at the expense of the faster one. NADPH exerts the same effect as $NADP^+$ whereas glucose-6-phosphate shows the opposite effect (Fig. 1d).

The MWs of the two enzyme forms were estimated according to Hedrick and Smith [7] by electrophoresis of crude extract in gels with acrylamide concentrations of 6, 7, 8 and 9%. The slopes of the lines obtained by plotting the R_f values logarithmically against the acrylamide concentrations are a measure of the MW. With 6-phosphogluconate dehydrogenase (MW 100000) [8] and phycoerythrin (MW 262000) [9] as reference proteins, values of 260000 and 130000 were obtained. As is the case with glucose-6-phosphate dehydrogenases from other sources with comparable MWs [10], the slower moving potato enzyme might represent a tetramer and the faster one a dimer. The effects of $NADP^+$, NADPH and glucose-6-phosphate on the enzyme pattern suggest an equilibrium between dimer and tetramer that is influenced by these compounds.

Consequently the change of the glucose-6-phosphate dehydrogenase pattern after wounding of potatoes is interpreted as a shift of the equilibrium to the tetramer. An increased level of pyridine nucleotides in potato tuber tissue after wounding, has been reported by Brinkman *et al.* [4]. So the effect of wounding on the glucose-6-phosphate dehydrogenase pattern might be mediated by the increased level of the pyridine nucleotides.

EXPERIMENTAL

Plant materials. Potato tubers cv Bintje were stored since harvest at 8° in the dark.

Preparation of crude extract. Potato tuber tissue was cut into small pieces and homogenized in a blender for 1 min with 2× the volume of homogenizing medium (0·05 M Tris–HCl, pH 7·5; 4 mM Na_2SO_3; 3 mM Na_2SO_3). The homogenate was pressed through perlon gauze and centrifuged at 10000 *g* for 15 min at 4°. The supernatant was used as the crude extract.

Disc electrophoresis. Discontinuous polyacrylamide gel electrophoresis was performed as described by Maurer [11] (system No. 2) in gels containing 7% acrylamide. Tris–glycine (pH 8·3) served as electrode buffer.

Assay of glucose-6-phosphate dehydrogenase. The enzyme was assayed after electrophoresis by incubation of the gels for 10 min at room temp. in the dark in 0·05 M triethanolamine buffer (7·5) to which per 10 ml had been added 4 mg nitroblue tetrazolium, 40 mg glucose-6-phosphate, 6 mg $NADP^+$, 12 mg $MgCl_2$ and 0·2 mg phenazine metasulphate. After incubation, the gels were fixed in 7% HOAc. The colour intensity of the reduced tetrazolium salt was measured at 700 nm.

Wounding. Potato tubers were divided into halves. Half was cut into *ca* 10 mm thick pieces, the other half remained intact and served as a control. Both were incubated at 25° in humid air. Crude extracts were prepared from the tissue in the centre within the ring of vascular bundles. In the control halves a zone of 5 mm from the cut surface was rejected.

REFERENCES

1. Kahl, G. (1973) *Bot. Rev.* **39,** 274.
2. Verleur, J. D. (1969) *Acta Bot. Neerl.* **18,** 353.
3. Lange, H., Kahl, G. and Rozenstock, G. (1972) *Physiol. Plant.* **23,** 60.
4. Brinkman, F. G., Plas, L. H. W. van der and Verleur, J. D. (1973) *Z. Pflanzenphysiol.* **68,** 364.
5. Scott, W. A. and Tatum, E. L. (1971) *J. biol. Chem.* **246,** 6347.
6. Muto, S. and Uritani, S. V. (1971) *Plant Cell Physiol.* **12,** 803.
7. Hedrick, J. L. and Smith, A. J. (1968). *Arch. Biochem. Biophys.* **126,** 155.
8. Gerbrandy, Sj. J. (1973) Dissertation. Free University, Amsterdam.
9. Velde, H. H. van der (1972) *Biochim. Biophys. Acta* **303,** 246.
10. Bonsignore, A. and De Flora, A. (1972) *Current Topics in Cellular Regulation* (Horecker, B. L. and Stadtman, E. R., eds.), Vol. 6, pp. 21–61, Academic Press, New York.
11. Maurer, H. R. (1968) *Disk Electrophorese*, Walter de Gruyter, Berlin.