Phytochemistry, 1972, Vol. 11, pp. 2981 to 2983. Pergamon Press. Printed in England.

ISOENZYMES OF PHOSPHOGLUCOISOMERASE IN SWEET POTATO

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(*Received* 14 *March* 1972. *Accepted* 1 *May* 1972)

Key Word Index—*Ipomoea batatas;* Convolvulaceae; isoenzymes; phosphoglucoisomerase.

Abstract—Isoenzymes (I, II and III) of phosphoglucoisomerase in sweet potato were demonstrated by DEAE-cellulose chromatography. Some properties of the two main types (I and III) were examined. Phosphoglucoisomerase III was very heat-sensitive compared with phosphoglucoisomerase I, and the K_m of phosphoglucoisomerase I was smaller than that of phosphoglucoisomerase III with fructose-6-phosphate as substrate.

INTRODUCTION

PHOSPHOGLUCOISOMERASE (D-glucose-6-phosphate ketolisomerase, E.C. 5.3.1.9) which catalyses the isomerization between glucose-6-phosphate and fructose-6-phosphate has been isolated in crystalline form from animal skeletal muscle[1] and brewer's yeast.[2] Furthermore, it has been reported that the crystalline phosphoglucoisomerase isolated from brewer's and baker's yeast has been resolved into three isoenzyme fractions.[3] In higher plants, however, the presence of isoenzymes of this enzyme has not been reported, and the crystallization of this enzyme has not been achieved. The experiments reported here show the presence of three types of phosphoglucoisomerase in sweet potato. Separaton of the isoenzymes was effected on a DEAE-cellulose column, and some properties of the two main types were examined.

RESULTS AND DISCUSSION

The phosphoglucoisomerase was precipitated between 20 and 45% saturaton with ammonium sulfate and the specific activity of the enzyme increased about 2-fold with 90% recovery. No activity was lost after storage at 4° for 10 days.

Three peaks of enzyme activity were found when this preparation was chromatographed on a DEAE-cellulose column. As shown in Fig. 1, the phosphoglucoisomerase I was eluted with 0·02 M phosphate buffer (pH 7·0) at first, the phosphoglucoisomerase II was eluted with 0·1 M sodium chloride and the phosphoglucoisomerase III in 0·02 M sodium chloride. The activities of these three peaks (phosphoglucoisomerase I, II and III) were approximately 55, 5 and 40%, respectively The total recovery from the DEAE-cellulose column was

[1] E. A. NOLTMANN, *J. Biol. Chem.* **239,** 1545 (1964).
[2] Y. NAKAGAWA and E. A. NOLTMANN, *J. Biol. Chem.* **240,** 1877 (1965).
[3] Y. NAKAGAWA and E. A. NOLTMANN, *J. Biol. Chem.* **242,** 4782 (1967).

about 96% and no other peak of phosphoglucoisomerase was eluted on increasing the NaCl concentration to 1·0 M.

In brewer's yeast the presence of three isoenzymes (PGI *A*, *B* and *C*) with DEAE-cellulose chromatography has been reported,[3] separated by elution with a linear gradient from 0·02 to 0·07 M phosphate buffer, pH 6·0.

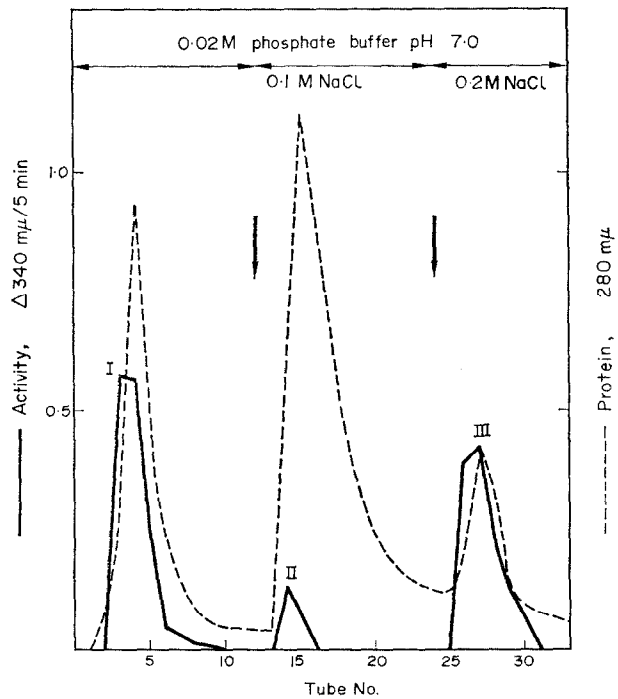

FIG. 1. ELUTION OF PHOSPHOGLUCOISOMERASE FROM DEAE-CELLULOSE.

Phosphoglucoisomerase I and III were both optimately active at pH 9·0. Half maximal activity was found at pH 6·5 and 10·3 for both enzymes. The rate of heat inactivation of phosphoglucoisomerase I and III were studied at 55°. Phosphoglucoisomerase III was much more heat-sensitive than phosphoglucoisomerase I, and 75% of the activity was lost after 5 min and over 90% was lost by 20 min treatment under the conditions employed. Phosphoglucoisomerase I activity did not change significantly with the heat treatment. The effect of fructose-6-phosphate concentration on the rate of reaction for phosphoglucoisomerase I and III was tested. These data were plotted according to the method of Lineweaver and Burk and the Michaelis constants for sweet potato phosphoglucoisomerase I and III were calculated to be 1·1 and 5·3 $\times$ 10^{-4} respectively.

EXPERIMENTAL

Chemicals. Fructose-6-phosphate disodium salt, NADP and glucose-6-phosphate dehydrogenase were purchased from Boehringer. DEAE-cellulose (0·87 meq/g) was obtained from Brown. All other chemicals were the analytical grade from commercial sources.

Preparation of enzyme. Sweet potatoes, Var. Kokei No. 1, purchased locally and stored at 20°, were washed, peeled and passed through a juicer (Fuju Electric Co.). All of the purification steps were carried out at

0–4° unless indicated otherwise. Mercaptoethanol (7·3 mmol/100 ml) was added to each homogenate, which was filtered through cheese cloth and the crude extract adjusted to pH 7·0 with N KOH. The insoluble material was removed by centrifugation for 20 min at 15 000 rpm and the supernatant was dialysed against 1 l. of 0·02 M phosphate buffer (pH 7·0) containing $7·3 \times 10^{-3}$ M mercaptoethanol.

Ammonium sulfate fractionation. Solid $(NH_4)_2SO_4$ was added slowly with stirring to 50 ml of the supernatant fraction. The precipitate that formed at 20–45 % saturation was dissolved in 20 ml of 0·02 M phosphate buffer (pH 7·0) containing $7·3 \times 10^{-3}$ M mercaptoethanol. The concentrated enzyme was then dialysed 3 times against the same buffer (2 hr each time).

DEAE-cellulose column chromatography. DEAE-cellulose column chromatography was based on that described by Noltomann.[3] The column (1 × 20 cm) was equilibrated with 0·02 M phosphate buffer (pH 7·0) containing $7·3 \times 10^{-3}$ M mercaptoethanol. The sample was then applied and the phosphoglucoisomerase were eluted stepwise. The column was eluted with 60 ml of the same buffer and then with 60 ml of the buffer containing 0·1 M NaCl and following with 60 ml of the buffer containing 0·2 M NaCl. 5 ml of each fraction was collected and subjected to enzyme assay.

Enzyme assay. Acivity was measured by the change in absorbance at 430 nm produced by NADPH, with fructose-6-phosphate as substrate and glucose-6-phosphate dehydrogenase as the indicator enzyme.[4] The reaction mixture contained in a final vol. of 1·0 ml; 0·1 M glycine–NaOH buffer (pH 9·0), 0·765 ml; $MgCl_2$, 2 μmol; fructose-6-phosphate, 1 μmol; NADP, 0·2 μmol; glucose-6-phosphate dehydrogenase, 5 μg; and enzyme source, 10 μl. The measurements were made in a recording spectrophotometer for 5 min and temperature was maintained at 20°.

pH optimum. The buffers used were 0·1 M glycine–NaOH in the pH range 8·8–10·7 and 0·1 M Tris-maleate in the pH range 6·0–9·0.

Kinetic studies. The Michaelis constant (K_m) studies were performed at 20° with the preparation purified by DEAE-cellulose column. Heat inactivation studies on phosphoglucoisomerase I and III were effected at 55° in glycine–NaOH buffer (pH 9·0). Activity was estimated after cooling rapidly.

Protein. Protein was determined spectrophotometrically by absorbance of 280 nm.

[4] E. A. NOLTMANN, in *Methods in Enzymology* (edited by W. A. WOOD), Vol. IX, p. 557, Academic Press, New York (1966).