EVIDENCE OF THE EXISTENCE OF TWO FORMS OF SUCROSE SYNTHETASE

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Received 17 June 1971

1. Introduction

Sucrose is a key metabolite in the control of carbohydrate metabolism in Jerusalem artichoke tubers [1, 2]. As sucrose synthetase (UDP-glucose: D-fructose 2-glucosyltransferase, EC 2.4.1.13) catalyzes the synthesis and cleavage of sucrose [3], a study was undertaken to determine its role as regulatory enzyme. In the course of this investigation it was found that sucrose synthetase could be separated in two forms. This paper reports the evidence supporting these findings.

2. Materials and methods

Unless stated otherwise, all operations were done in the cold. Tubers were stored at 2–4° in damp sand until used.

Jerusalem artichoke (*Helianthus tuberosus*) tubers were homogenized with twice their volume in 0.1 M tris buffer pH 7.6, containing 5 mM mercaptoethanol and 0.01 M KCN, in a Waring blender. The homogenate was filtered through four layers of cheese-cloth and the filtrate was centrifuged at 10,000 *g* for 10 min. To the supernatant, solid ammonium sulphate was added to 20% of saturation. The precipitate that formed was removed by centrifugation and discarded. The ammonium sulphate concentration in the supernatant solution was increased to 70% of saturation and the precipitate was collected by centrifugation, at 20,000 *g* for 20 min. The precipitate was suspended in the minimum volume of 0.02 M tris buffer pH 8.0 containing 0.1 mM mercaptoethanol and 0.1 mM EDTA. The suspension was dialyzed overnight against the same buffer with two changes. The dialyzate was reprecipitated between 0 to 25 and 25 to 45% ammonium sulphate saturation, and the precipitates were collected and treated as before. The enzyme activity was mainly in the 25 to 45% fraction.

Sucrose synthetase (forward reaction) was assayed, incubating in a total volume of 0.1 ml, 1 μmole of fructose, 0.2 μmole of UDP-glucose, 2 μmole of tris buffer pH 8.0 and enzyme. Incubation was carried out at 30° for 30 min. The reaction was stopped by adding 0.2 ml of 1 N NaOH and heating in the water bath at 100° for 10 min. The sucrose formed was determined by the thiobarbituric acid method [4] in a total volume of 1.2 ml.

If the UDP formed was determined, the reaction was stopped by boiling 1 min and the nucleoside diphosphate estimated according to Avigad [5]. Sucrose synthetase (reverse reaction) was assayed incubating in a total volume of 0.05 ml, 20 μmole of sucrose, 0.5 μmole of UDP, 1 μmole of NaF, 2.5 μmole of tris buffer pH 7.3 and enzyme. Incubation was carried out for 30 min at 30°. After stopping the reaction by boiling 1 min, fructose formed was determined in suitable aliquots by the Park and Johnson method [6].

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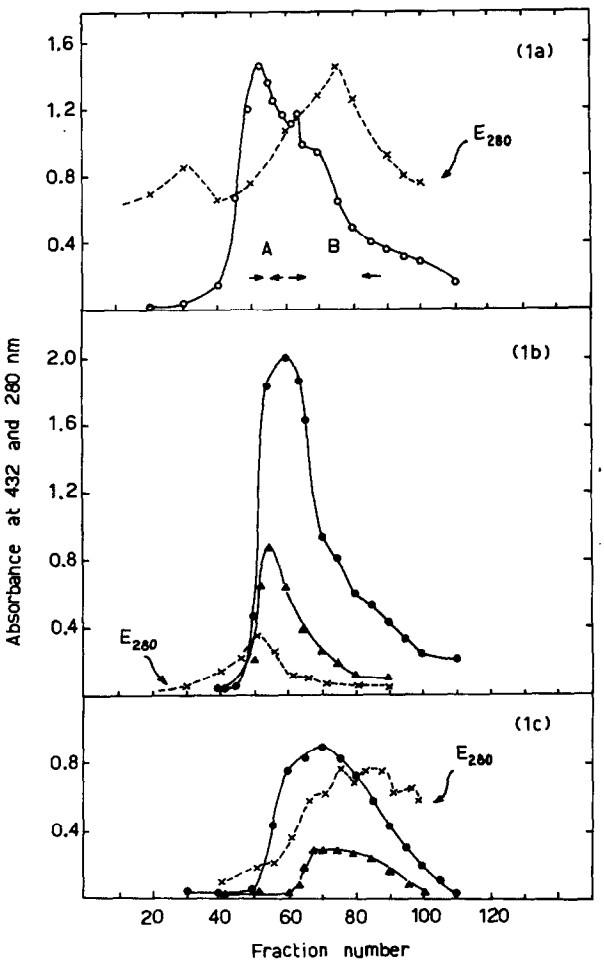


Fig. 1. Chromatography of ammonium sulphate-fractionated sucrose synthetase on DEAE-cellulose (column 2.0 × 18 cm) previously equilibrated in 0.1 mM mercaptoethanol, 0.1 mM EDTA, 0.02 tris buffer pH 8.0. Flow rate: 0.1–0.5 ml per min. Elution: 220 ml total volume of 0.05 to 0.5 M NaCl linear gradient in the equilibration buffer. Fractions: 1 ml. Fraction aliquots were analyzed for sucrose synthetase (○). Fractions under areas A and B were pooled, dialyzed, concentrated by vacuum dialysis and rechromatographed (1b is area A and 1c is area B). ●, First rechromatography; ▲, second rechromatography; ×, protein (E_{280}).

3. Results and discussion

Chromatography of the ammonium sulphate purified sucrose synthetase from Jerusalem artichoke tubers on DEAE-cellulose columns produced the elution profile presented on fig. 1a. An identical profile was obtained when sucrose synthetase activity was estimated as the UDP formed or when the reverse reaction was measured. This elution pattern seems to indicate the presence of two peaks of sucrose synthetase activity. Fractions at the peaks were analyzed also for sucrose phosphate synthetase (UDP-glucose: D-fructose 6-phosphate 2-glucosyltransferase, EC 2.4.1.14), but no activity could be detected. Improvement of the separation could be obtained neither by modifying the gradient or eluting buffers, nor by chromatography on a different supporting material like AE and TEAE-cellulose. On the other hand, the pattern obtained was reproducible and independent of the extraction method used for preparing the enzyme from the tuber.

If the pattern obtained in the DEAE-cellulose chromatography was due to artefacts arising from systematic, reproducible discontinuities in the pH or salt gradient, rechromatography of the pooled areas of apparently different enzyme activity would result in changes in the distribution of the rechromatographed material. That this is not the case can be seen clearly in fig. 1. When each area of activity isolated by pooling the indicated fractions (see fig. 1a) was submitted to rechromatography on DEAE-cellulose, both peaks emerged in the effluent at their original position. Even if the enzyme fractions lost activity, a second rechromatography failed again to modify the initial elution profile (see figs. 1b and 1c).

As column chromatography did not seem to be the most convenient method for separation, the ammonium sulphate purified sucrose synthetase was submitted to isoelectrofocusing. The profile obtained showed at least two peaks with enzymatic activity and pH values of 5.5 and 5.9 (fig. 2). Furthermore, as a control that the results obtained by the two methods were in agreement, the sucrose synthetase was first chromatographed on DEAE-cellulose as shown in fig. 1. Fractions under the ascending (A) and descending (B) parts of the elution curve (see fig. 1a) were pooled, concentrated, dialyzed and submitted to electrofocusing. The results presented in fig. 3 indicate that the enzymatic activities under different parts of the DEAE-chromatography elution curve correspond to the forms with pH values of 5.6 and 6.1 in close agreement with those obtained for the ammonium sulphate enzyme.

The results presented here seem to indicate that

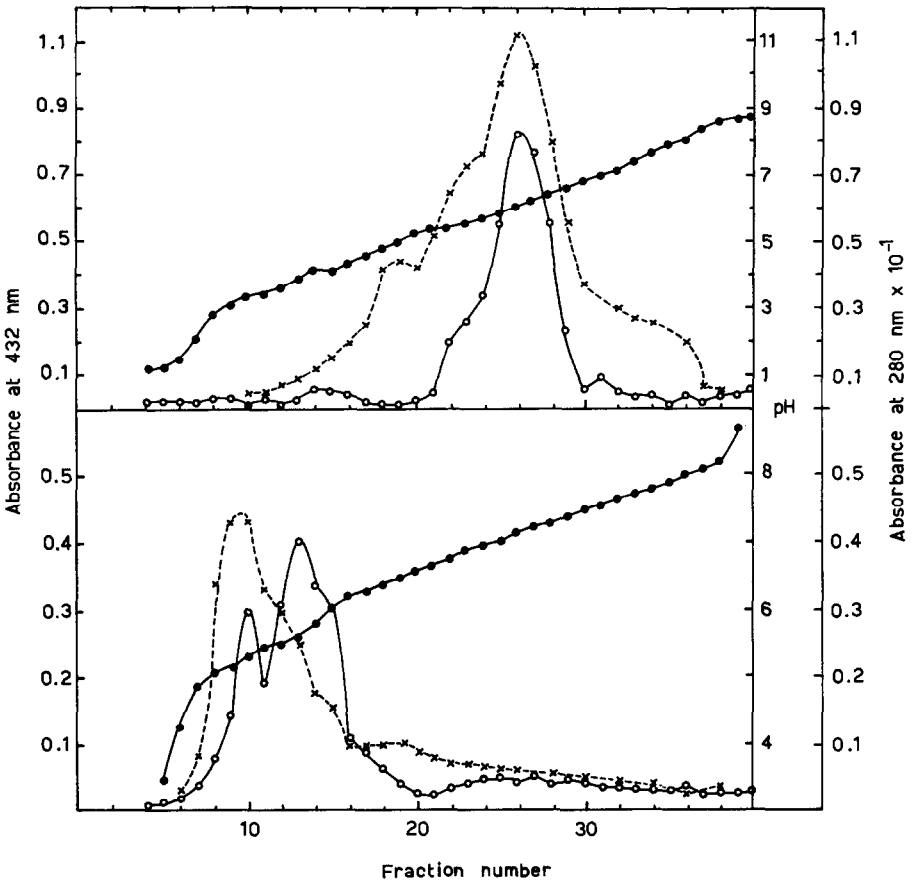


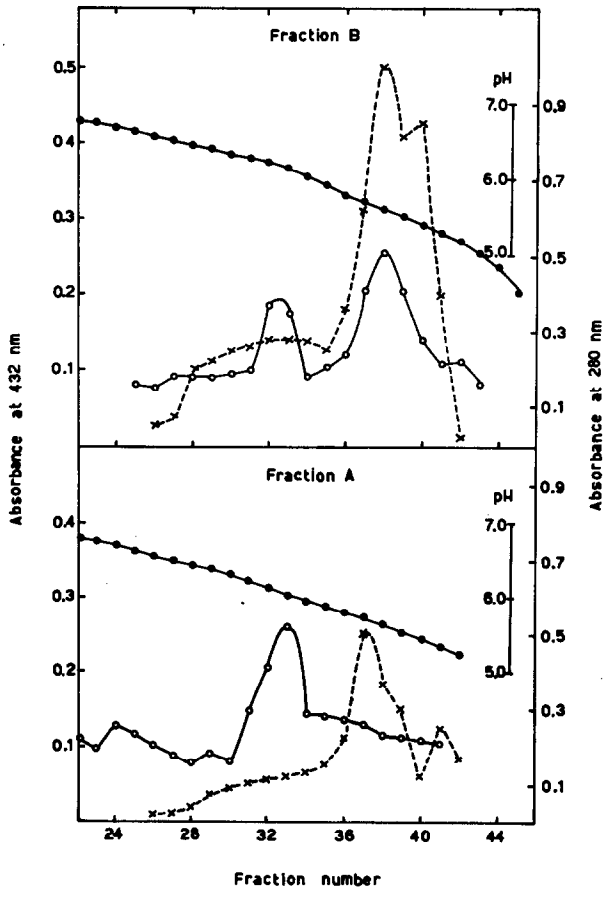
Fig. 2. Isoelectrofocusing of ammonium sulphate-fractionated sucrose synthetase (5–10 mg protein) in LKB 110 ml column. Electrophoresis in 1% ampholite (pH 3–10 and 5–8) for 40 hr, 500 V. Anode: 80% glycerol, 0.09 M H_3PO_4 at column bottom. Cathode: 0.25 N NaOH. Sucrose gradient was substituted by a glycerol gradient. Fractions collected after electrophoresis: 2 ml. ○, enzyme activity; ●, pH; ×, absorbance at 280 nm.

there are two forms of sucrose synthetase in Jerusalem artichoke tuber extracts. Preliminary kinetic data seem to indicate that the two forms could be functionally different. The affinity of form B for sucrose is markedly low (K_m = 200 mM, pH 7.2) compared with that of form A (K_m = 56 mM, pH 7.2) which is more in agreement with the value reported previously by Avigad [5] (K_m = 33 mM, pH 8.3).

Several authors [8, 9] have suggested that sucrose cleavage and synthesis could be attributed to different enzymes. The data presented here are relevant to the problem even if they do not solve it. The metabolic significance of the results reported is under study.

Acknowledgements

This work was supported in part by a Research Grant from the Ford Foundation and by the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina). The authors wish to thank Dr. J.M. Dellacha, Biochemistry Department, Buenos Aires University, for the use of a LKB isoelectrofocusing apparatus, and they are indebted



to Mrs. Beatriz Bettinelli for her able technical assistance.

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Fig. 3. Isoelectrofocusing of fractions A and B from DEAE-cellulose chromatography (fig. 1). Protein (0.1–0.5 mg) was applied to Gordon's microscale isoelectrofocusing apparatus [7]. Electrophoresis in 1% ampholite (pH 5–8) for 15 hr, 400 V. Anode and cathode as in fig. 2. Fractions collected after electrophoresis: 0.25 ml. ○, enzyme activity; ●, pH, ×, absorbance at 280 nm.