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Enlargement of amylopectin by ADP-D-glucose:α-1,4-glucan α-4-glucosyltransferase of spinach

During the purification of ADP-D-glucose:α 1,4-glucan α-4-glucosyltransferase of spinach it was found that the enzyme catalyzes the extensive elongation of outer chains of a primer, potato amylopectin.

The partially purified enzyme with a specific activity[1] of 12 units/mg protein was passed through a column consisting of cellulose impregnated with potato amylopectin. The elution pattern of the enzymatic activity was illustrated in Fig. 1. The pooled eluate usually had a specific activity of 300–500 units/mg protein and was essentially free from amylase(s) (EC 3.2.1.1 or EC 3.2.1.2). By use of this preparation an attempt was made to enlarge potato amylopectin with ADP-D-glucose as glucose donor.

The time course of reaction in the presence of varying concentrations of the primer was shown in Fig. 2. Only with a low concentration of the primer was formation of a flocculent precipitate observed, which indicated the formation of a polymeric product distinguishable from the primer, amylopectin. After collection and washing by centrifugation the precipitate was examined for its properties. As shown in Table I, the amount of the precipitate completely corresponded to that expected from the amount of liberated ADP. Because crystalline sweet potato β-amylase (EC 3.2.1.2) degraded the polymeric product to nearly the theoretical limit (Table II), it was concluded that the newly synthesized portions of the product consisted almost exclusively of linear chains of α-1,4-glucose units. The absorption characteristics of its iodine complex were rather similar to those of potato amylose (Fig. 3). Assuming a relation between DP and the intensity of colour of the iodine complex of linear α-1,4-glucan[6], it was expected that the newly synthesized linear chain portions of the

Abbreviation: DP, degree of polymerization.

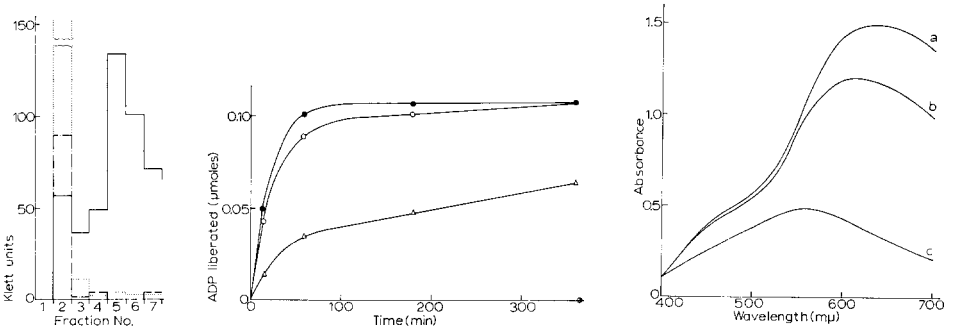

Fig. 1. Elution pattern of the spinach transferase on a cellulose column impregnated with amylopectin. A solution of the partially purified transferase (80 units, 3 ml) was put onto the top of a column (1 cm × 10 cm) of cellulose impregnated with potato amylopectin, which had been equilibrated with 0.005 M phosphate–0.001 M EDTA(pH 7.5), and eluted with the same buffer at a flow rate of 0.2 ml/min at 4°. Fractions (8 ml) were collected and assayed for transferase and amylase(s). Transferase was assayed as described previously[1]. Amylase(s) was estimated by a decrease in colour intensity (at 680 mμ) of the iodine complex of potato amylose, which had been incubated, at a concentration of 0.2%, with each fraction at 30°, pH 7. Protein was determined according to the method of LOWRY *et al.*[2]. A Klett value of 100 represents 1.6 units/ml for the transferase, a decrease of 50% in colour intensity/h per ml for amylase(s), and 63 μg/ml for protein, respectively. ———, transferase; —·—, amylase; · · · · ·, protein.

Fig. 2. Time course of reaction with varing concentrations of primer. The reaction mixture contained 0.54 μmole of ADP-Glc, 75 μmoles of glycine(pH 8.4), 1.75 μmoles of EDTA, 0.24 unit of the enzyme and the indicated amounts of potato amylopectin in a total volume of 0.25 ml. Incubation temperature was 30°. At intervals a 0.05-ml aliquot was removed for determination of liberated ADP. Amount of amylopectin: ●—●, 152 μg; ○—○, 15.2 μg; △—△, 1.52 μg; ◑, without amylopectin.

Fig. 3. Absorption curve of the polymeric product. Polymers were stained with iodine–potassium iodide under the conditions described by BOURNE *et al.*[5]. a, potato amylose; b, polymeric product obtained under the conditions described in Table I; c, potato amylopectin(primer).

polymeric product would have a DP of 135–195, the figure being comparable to that calculated from the extent of enlargement of the primer molecule, using a figure of 24 for the DP of the unit chain of the latter (about 170).

TABLE I

BALANCE BETWEEN LIBERATED ADP AND POLYMER PRODUCED

Reaction mixture contained 2 μmoles ADP-Glc, 300 μmoles glycine (pH 8.4), 7 μmoles EDTA, 30.4 μg (as glucose) potato amylopectin and 1.2 units of the enzyme in a total volume of 1.0 ml. After incubation for 6 h at 30°, liberated ADP in a 0.05-ml aliquot of the reaction mixture was measured. One ml of methanol was added to the rest of the reaction mixture and polymer was collected by centrifugation and washed 3 times with 50% methanol. Its amount was determined by the phenol–sulfuric acid method[3].

	μmoles as glucose
Polymer recovered*	1.35
Liberated ADP*	1.19
Primer amylopectin	0.16
Liberated ADP *plus* primer amylopectin	1.35

* Allowance was made for the aliquot portions removed for analysis.

TABLE II

β-AMYLOLYSIS OF THE POLYMERIC PRODUCT

The polymer obtained under the same conditions as described in Table I was hydrolyzed with 27 μg of sweet potato β-amylase in 0.02 M acetate (pH 4.8, 2 ml), at 37° for 24 h. Maltose was estimated by the Somogyi–Nelson method[4]. A theoretical limit was calculated by considering the β-amylolysis limit of the primer (52%) and its enlargement by the transferase (7.6 times).

	μmoles as maltose
Polymer hydrolyzed	0.29
Theoretical limit	0.27
Maltose liberated	0.24

The above results indicate that, as is the case with animal glycogen synthetase[7] (EC 2.4.1.11), ADP-D-glucose:α-1,4-glucan α-4-glucosyltransferase of spinach catalyzes formation of long chains of α-1,4-glucose units in the presence of the appropriate primer.

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