CHARACTERIZATION AND PARTIAL PURIFICATION OF A BRANCHING ENZYME FROM ESCHERICHIA COLI

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Under conditions of growth limitation by either nitrogen or sulfur, Escherichia coli and Aerobacter aerogenes accumulate, in the presence of excess glucose, large intracellular amounts of a highly branched glycogen closely similar to animal and yeast glycogens (Segel et al., 1965 ; Sigal et al., 1964).

The (1 → 4)α-D-glycosidic linkages of this bacterial glycogen are formed in the presence of a primer by an ADP-glucose-transglucosylase which has only a very low activity with UDP-glucose as glucosyl donor (Greenberg and Preiss, 1964). This finding is in agreement with the previous observation (Sigal et al., 1964) that normal or even larger amounts of glycogen are accumulated by UDPG-pyrophosphorylase negative mutants of E. coli.

The present paper reports the isolation from E. coli and partial purification of a branching enzyme, or amylo-1,4 → 1,6-transglucosidase (systematic name : α-1,4 glucan : α-1,4 glucan, 6-glycosyltransferase, EC 2.4.1.18). Found in plants, animal tissues and yeast, this enzyme had been little studied in bacteria, its presence being only reported in crude dialyzed extracts of Arthrobacter globiformis (Zevenhuizen, 1964). The branching enzyme from E. coli is active with both amylose and amylopectin, and has been separated from an amylolytic enzyme producing reducing sugars from the same substrates.

Analytical methods : Branching activity was assayed and estimated from the change in iodine coloration, as described by Zevenhuizen (1964). Reducing sugars (as apparent maltose) were determined by the method of Park and Johnson (1949), with a ferric reagent slightly modified in

order to contain 1.5 g of $FeCl_3$ and 20 g of polivinyl-pyrollidone per liter of 1.0 N H_2SO_4. These oligosaccharides have been separated by paper (Whatman n° 1) chromatography (36 hr) with n-butanol : acetic acid : H_2O (4 : 1 : 5, v/v).

Proteins were estimated in crude extracts by the biuret method of Gornall et al. (1949) and in Sephadex fractions by spectrophotometric measurement at 260 and 280 mμ (Warburg and Christian, 1941).

Preparation and activity of extracts : E. coli K 12 (wild type) was cultivated aerobically at + 32° C in a nitrogen deficient medium containing 3 g glucose and 240 mg NH_4Cl per liter (Segel et al., 1965). The cells were harvested 2 hr after the end of the log phase, centrifuged, resuspended in 3 vol. of Tris-HCl buffer (0.05 M, pH 7) and disrupted for 10 min. in a Raytheon oscillator. Cell debris were removed by centrifugation (1 hr., 27 000 x g) and the clear supernatant dialyzed overnight against 0.01 M Tris-HCl buffer (pH 7) containing 10^{-4}M reduced glutathione.

Precipitation of nucleoproteins by protamine sulfate increased the $OD_{280\ m\mu}$ / $OD_{260\ m\mu}$ ratio from 0.57 to 1.0. The supernatant was dialyzed for 3 hr. against the same buffer as above. The protein fraction precipitated between 30 and 60 % ammonium sulfate saturation was redissolved in 0.1 M Tris-HCl buffer (pH 7) containing 10^{-4}M reduced glutathione and dialyzed overnight against the same buffer. All operations were carried on at + 4° C.

The activities of these crude and semi-purified extracts were assayed at + 32° C and pH 7 (Tris-citrate buffer 0.1 M) in the presence of amylose or amylopectin (Calbiochem), the reaction being followed on small aliquots taken at time intervals. These preparations contain both the branching enzyme and an amylase, as shown by the liberation of reducing sugars. No evidence was found for the presence of a phosphorylase, as judged from constantly negative results of assays by the method of Illingworth et al. (1953)

Purification on Sephadex : Ammonium sulfate treated preparations (7 ml), containing 280 - 350 mg of total proteins, were further purified (Fig. 1) by passing through a column of Sephadex G-100 as indicated in the legend of Fig. 1. This resulted in separation of the branching activity, which is eluted first with a bulk of inactive proteins, from the amylolytic activity, which is located in a second, well separated peak.

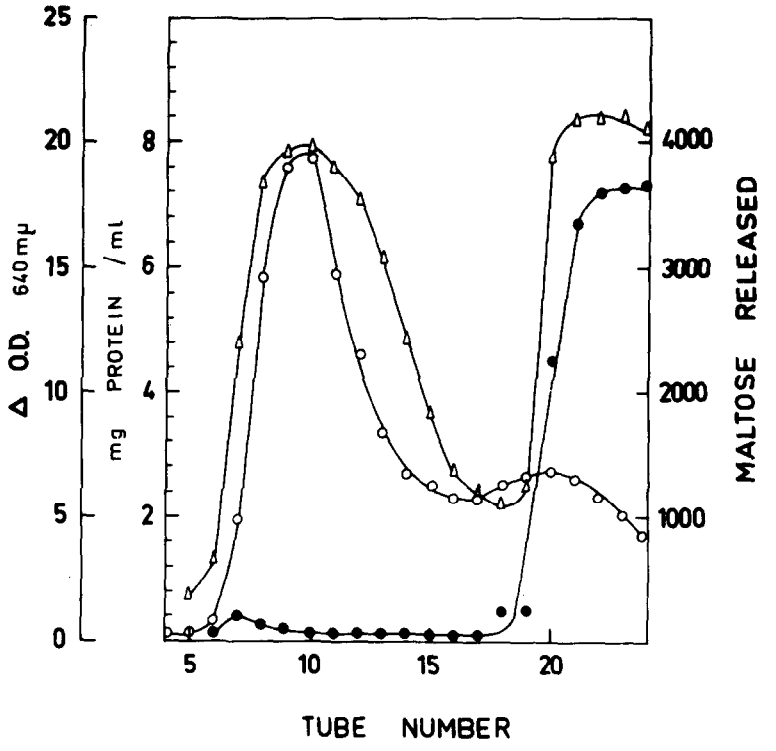

Fig. 1. Gel filtration on a Sephadex G-100 column (2 x 90 cm) equilibrated and eluted with the same Tris-HCl buffer (0.1 M, pH 7). Total protein load : 280 mg. Flow rate : 9 ml/hr., in 3 ml fractions. Curve -O-O-O- : mg proteins/ml of fraction. Curve -Δ-Δ-Δ- :ΔOD (640 mμ) of reaction mixture. Curve -●-●-●- maltose released (μg/ml of reaction mixture).

As verified by paper chromatography, maltose and other reducing oligo-saccharides are exclusively formed by the fractions corresponding to the second elution peak.

The fractions of the first peak, having branching activity, were collected, concentrated by 90 S ammonium sulfate precipitation and redissolved in distilled water. These preparations were incubated overnight at + 32° C with amylose or amylopectin in 0.1 M Tris-citrate buffer (pH 7) and the polysaccharides formed were isolated as described by Zevenhuizen (1964). The formation of branch points on amylose and the introduction of new 1→6 glycosidic linkages on amylopectin has been demonstrated by usual methods (Segel et al., 1965). The results (Table I) show that the polyglucose formed in vitro by the purified extracts is identical to the bacterial glycogen isolated from the

TABLE I

PROPERTIES OF POLYGLUCOSES FORMED FROM AMYLOSE AND AMYLOPECTIN

The polysaccharides were formed under the following conditions : enzyme preparation : 160 mg of total protein, amylose or amylopectin : 150 mg, Tris-citrate buffer (pH 7) : 2.5 µmoles, total volume : 25 ml. After overnight incubation at + 32° C in a closed vessel, containing toluene vapors for protection against bacterial contamination, the polyglucose formed was extracted as described by Zevenhuizen (1964).

Properties	Polysaccharide formed from		Glycogen of *E. coli*	Amylopectin	Amylose
	Amylose	Amylopectin			
α-amylase limit (%)	55	63	59-71	85-92	
β-amylase limit (%)	46	46	47-56	50-60	
$\overline{CL}$ α-amylase method	8.5	9.5			2 700
periodate oxidation method	8	10	10-13	18-24	
ECL β-amylase method	6	7	8-9	9-16	
ICL	1	2	1-4	6-8	
λ max of iodine complex (mµ)	460	470	420-455	540	

$\overline{CL}$: average chain length ; ECL and ICL : exterior and interior chain lengths. (For analytical methods see : Segel *et al.*, 1965).

whole cells, in regard to the percentage of 1→6 linkages and to the average interior and exterior chain lengths. The kinetics of branching activity has been followed as shown on Fig. 2.

The properties of this *E. coli* enzyme are quite similar to those of the branching enzymes from animals (Krisman, 1962) and yeast (Gunja *et al.*, 1960).

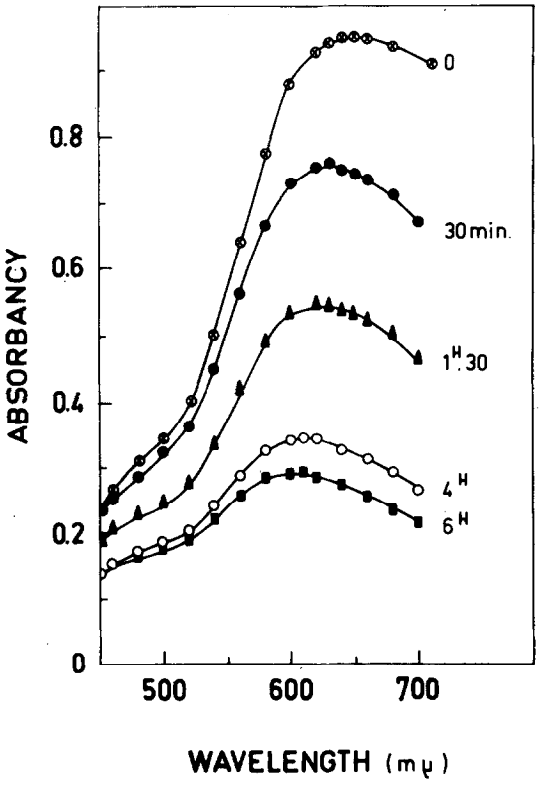

Fig. 2. Action of the branching enzyme on amylose.
Reaction mixture : 20 mg amylose, 100 μmoles Tris-citrate buffer (pH 7), enzyme preparation : 64 mg of protein, final volume : 5 ml.
The progression of branching is marked by the diminution of absorbance at 640 mμ.

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