LAMINARIBIOSE PHOSPHORYLASE FROM EUGLENA GRACILIS

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Several examples of reversible phosphorolytic cleavage of disaccharides are known (Doudoroff, 1961). Sucrose phosphorylase and cellobiose phosphorylase catalyze phosphorolysis to α-glucose-1-P, while with maltose phosphorylase β-glucose-1-P is obtained. Evidence for the presence of a similar enzyme in the protist E. gracilis is presented in this note. This enzyme, tentatively called laminaribiose phosphorylase (laminaribiose:orthophosphate glucosyl transferase), catalyzes the following reaction:

$$\text{Laminaribiose } \left[3\text{-O-}\beta\text{-D-glucopyranosyl D-glucose}\right] + P_i \rightleftharpoons \alpha\text{-D-glucose-1-P} + \text{glucose} \quad (1)$$

E. gracilis strain z was cultivated, disrupted and centrifuged as described for the preparation of paramylon synthetase (Goldemberg and Maréchal, 1963). The 100,000xg supernatant was used as source of enzyme. It was purified by the following steps: precipitation with solid ammonium sulfate (30-70% saturation), and dialysis; precipitation with protamine sulfate; fractional adsorption on calcium phosphate gel

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and elution with 0.005 M sodium pyrophosphate, pH 8.7, and finally dialysis against 0.01 M citrate buffer-0.001 M EDTA, pH 6.5. A 16-18 fold purification was obtained, with a yield of 35-38%.

Activity of the enzyme was tested starting with α-glucose-1-P and glucose, as indicated in Table I. Under these conditions the reaction proceeds linearly for 20 min.

TABLE I

Acceptor Specificity

The incubation mixture contains: 0.066 M citrate buffer, pH 6.5; 0.005 M EDTA; 0.017 M α-D-glucose-1-P; 0.083 M acceptor, and 0.01 ml. of purified enzyme, in a final volume of 0.06 ml. After 10 min. incubation at 37°, and heating in a boiling water bath, P_i was determined according to Fiske and SubbaRow (1925).

Acceptor	P_i (μmoles)
-	0
Glucose	0.33
Cellobiose	0.10
Laminaribiose	0.05
2-Deoxyglucose	0.05
Maltose	0.05
D-Glucosamine D- or L-Xylose Sucrose Fructose D-Mannose D-Galactose Laminarin (0.2 mg.) Paramylon treated with KOH (0.2 mg.)	<0.02

Other esters such as β-glucose-1-P, glucose-6-P, galactose-6-P and fructose-1-P did not substitute for α-glucose-1-P as donor.

Table I shows the results obtained with several acceptors; it can be seen that glucose is the best one. The activity increases with the concentration of glucose up to 0.08 M.

The products formed in the reaction were detected by paper chromatography (Fig. 1). It can be seen that a spot of the same R_G as laminaribiose is formed. It was isolated by chromatography on a charcoal-Celite column (Barry and McCormick, 1962). Its identity was confirmed by the electrophoretic mobility in 0.05 M borate, pH 9.2, at 600 volts for 2 hrs. When reducing power was determined according to Somogyi (1945)-Nelson (1944) using maltose as standard, and total sugar with the phenol-sulfuric method (Dubois, Gilles, Hamilton, Rebers and Smith, 1956), a ratio of 1:1.95 was obtained. An increase in reducing power was observed after incubation with β-glucosidase. After oxidation with lead tetraacetate according to Charlson and Perlin (1956), arabinose could be detected by paper chromatography. This test is considered to be characteristic for 1,3 linkages.

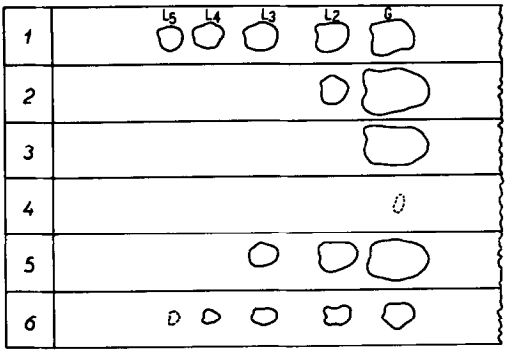

Fig. 1. Products formed in the reaction. Complete system as described in Table I, but in double amount; 10 min. incubation. After heating in a boiling water bath, and passage through Amberlite MB-3 resin, the samples were chromatographed on Whatman 4 paper for 16 hrs. in butanol-pyridine-water (6:4:3). 1: Standards. G, glucose, L_2, laminaribiose; L_3, laminaritriose, L_4, laminaritetraose; L_5, laminaripentaose. 2: Complete system. 3: Glucose-1-P omitted. 4: Glucose omitted. 5: Complete system, 16 hrs. incubation under toluol vapors. 6: The same as 5, but using 0.0083 M glucose.

With longer incubation period (16 hrs.), higher homologues of laminaribiose appeared (Fig. 1). These had the same mobility as the products obtained by hydrolysis of paramylon. The higher homologues did not seem to arise by the action of a transglucosylase because no oligosaccharide formation could be shown when laminaribiose and glucose (without glucose-1-P) were incubated under the same conditions. If the concentration of glucose was lowered to 0.008 M, less laminaribiose and more of the higher oligosaccharides were formed.

Reaction (1) in the direction from left to right could be detected by measuring the increase of labile P after incubation with laminaribiose and P_i.

Further studies on the enzyme are being carried out.

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REFERENCES

Barry, V. C. and McCormick, J. E., in "Methods in Carbohydrate Chemistry" (Whistler, R. L. and Wolfrom, M. L., eds.) Vol. I, p. 328. Academic Press, Inc., New York, (1962).

Charlson, A. J. and Perlin, A. S., Canad. J. Chem. 34, 1200 (1956)

Doudoroff, M., in "The Enzymes" (Boyer, P. D., Lardy, H. and Myrbäck, K., eds.), Vol. 5, p. 229. Academic Press, Inc., New York, (1961).

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F., Anal. Chem. 28, 350 (1956).

Fiske, C. H. and SubbaRow, Y., J. Biol. Chem. 66, 375 (1925).

Goldemberg, S. H. and Maréchal, L. R., Biochim. Biophys. Acta 71, 743 (1963).

Nelson, N., J. Biol. Chem. 153, 375 (1944).

Somogyi, M., J. Biol. Chem. 160, 61 (1945).