Preliminary Notes

PN 1258

Biosynthesis of paramylon in *Euglena gracilis*

Paramylon, the reserve carbohydrate of *Euglena gracilis* and other Euglenoids, is a glucose polymer with β-1,3 linkages[1]. Evidence for the synthesis of paramylon with an enzyme from *E. gracilis*, and with UDPG as glucosyl donor, is presented in this note. FEINGOLD *et al.*[2] have described a similar reaction which leads to the formation of a β-1,3-glucan with enzymes from mung-bean seedlings and other plants.

E. gracilis strain z was cultivated in a peptone–yeast extract–glucose medium, at 26–28° in the dark for 6–8 days. The etiolated cells were harvested by centrifugation and washed with water. They were suspended in 2.2% $(NH_4)_2HPO_4$ and passed through a French pressure cell. The extract was centrifuged at 2000 × *g* at 4° for 5 min and the supernatant was spun at 105000 × *g* for 1 h. The precipitate was thoroughly homogenized in water at 4° in a glass homogenizer ("particulate fraction"). The enzyme could be extracted from the particles by a procedure similar to that described by JÄRNEFELT *et al.*[3]. For this purpose sodium deoxycholate solution (1 mg/mg of protein), $MgCl_2$ (1 mM final concentration) and KCl (1.5 M final concentration) were added, the mixture was shaken in the cold and then centrifuged for 1 h at 105000 × *g*. The clear supernatant ("solubilized enzyme") was dialyzed against 0.01 M phosphate buffer (pH 7.4)–0.001 M EDTA in the cold for 40 h.

Activity of the solubilized enzyme was measured with the incubation system indicated under Fig. 1, either by counting the radioactivity incorporated into the precipitate, or by the UDP liberated in the reaction[4]. It was not necessary to add any acceptor. With these preparations, 5–20% transformation of the substrate could be obtained in a 30 min incubation.

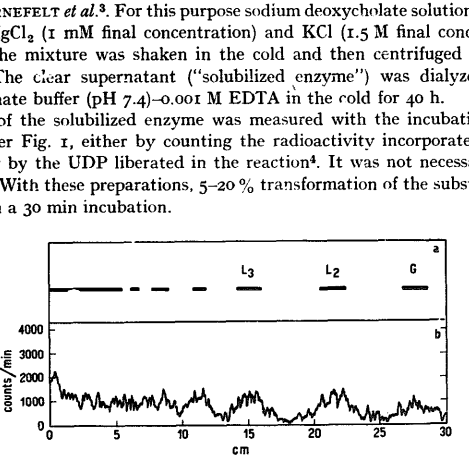

Fig. 1. Products obtained from the enzymically formed material by partial digestion with HCl. The incubation system contained: 7.5 μmoles of glycylglycine buffer (pH 7.5), 0.1 μmole of $MgSO_4$, 0.25 μmole of UDP-[^{14}C]glucose (88750 counts/min) and solubilized enzyme in a final volume of 0.05 ml. After 30 min at 23°, 0.4 mg of carrier paramylon (see footnote on p. *III*) and 0.5 ml of 66% ethanol were added. The precipitate was centrifuged and washed 3 times with 66% ethanol. It was transferred to a vial and hydrolyzed with concentrated HCl saturated with HCl gas at −18° according to FEINGOLD *et al.*[2]. The hydrolyzate was chromatographed on Whatman No. 4 paper with butanol–pyridine–water for 16 h and automatically scanned. a, hydrolyzed laminarin; b, hydrolyzed radioactive product. G. glucose; L_2, laminaribiose; L_3, laminaritriose.

In order to identify the reaction product, the radioactive material was hydrolyzed with HCl as indicated in Fig. 1. It can be seen that the location of the radioactive spots corresponds with that of the oligosaccharides formed from similarly hydrolyzed laminarin, another β-1,3-glucan, and also with that of authentic glucose, laminaribiose and laminaritriose.

The radioactive product was also degraded enzymically using laminarase, a β-1,3-glucan hydrolase obtained from *E. gracilis*[5]. Radioactivity determinations showed that 51% of the original counts were recovered in the supernatant after laminarase action. When chromatographed, these counts were located in the zone corresponding to glucose. Under the conditions of the experiment, laminarase hydrolyzed 40% of the original paramylon* and only 2% of starch, 1% of glycogen and 1% of soluble cellodextrin.

The enzyme seems to quite specific for UDP-α-glucose. Thus adenosine diphosphate glucose was 25% and deoxyadenosine diphosphate glucose 15% as effective as UDP-α-glucose, while with UDP-β-glucose no activity could be detected.

Further studies on the enzyme are being carried out.

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1 A. E. CLARKE AND B. A. STONE, *Biochim. Biophys. Acta*, 44 (1960) 161.
2 D. S. FEINGOLD, E. F. NEUFELD AND W. Z. HASSID, *J. Biol. Chem.*, 233 (1958) 783.
3 J. JÄRNEFELT R. E. BASFORD, H. D. TISDALE AND D. E. GREEN, *Biochim. Biophys. Acta*, 29 (1958) 123.
4 L. F. LELOIR AND S. H. GOLDEMBERG, *J. Biol. Chem.*, 235 (1960) 919.
5 J. FELLIG, *Science*, 131 (1960) 832.

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* Paramylon granules obtained according to CLARKE AND STONE[1] were solubilized with 1 N KOH (about 1 ml/100 mg) at room temperature, and afterwards dialyzed against water for 24 h. The gelatinous suspension obtained in this way was kept at 4°.

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