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Note

Preparation of ^{14}C-labelled (1→3)-β-D-gluco-oligosaccharides by transglycosylation

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End-labelled oligosaccharides of known d.p. have value in studies of the mechanism of the action of glucanases. The endo-(1→3)-β-D-glucanase L-IV from the marine bivalve *Spisula sachalinensis*[1] has significant transglycosylation activity and we now report the preparation of the (1→3)-β-D-gluco-oligosaccharides ^{14}C-labelled at the reducing end by the action of L-IV on laminarin and D-[1-^{14}C]-glucose.

TABLE I

DISTRIBUTION OF ^{14}C ON THE PAPER CHROMATOGRAM (% OF THE TOTAL RADIOACTIVITY)

Products[a]	*Reaction time*					
	0	*3 min*	*9 min*	*18 min*	*30 min*	*7 h*
G	99.12	97.64	94.83	91.21	88.07	73.22
G_2	0.16	1.07	2.53	4.15	5.38	15.31
G_3	0.15	0.43	0.72	1.32	1.88	6.43
G_4	0.15	0.19	0.49	0.89	1.19	2.71
G_5	0.13	0.11	0.25	0.42	0.67	1.04
G_6	0.09	0.12	0.14	0.29	0.44	0.52
G_7	0.07		0.36	0.55	0.36	0.27
$\geq G_8$	0.05	0.15			0.62	0.30
Start	0.07	0.29	0.68	1.17	1.39	0.19
Degree of hydrolysis of glucan (%)	0	1	3	5	9	≥30

[a]G, ^{14}C-glucose; G_2, ^{14}C-laminaribiose; *etc.*

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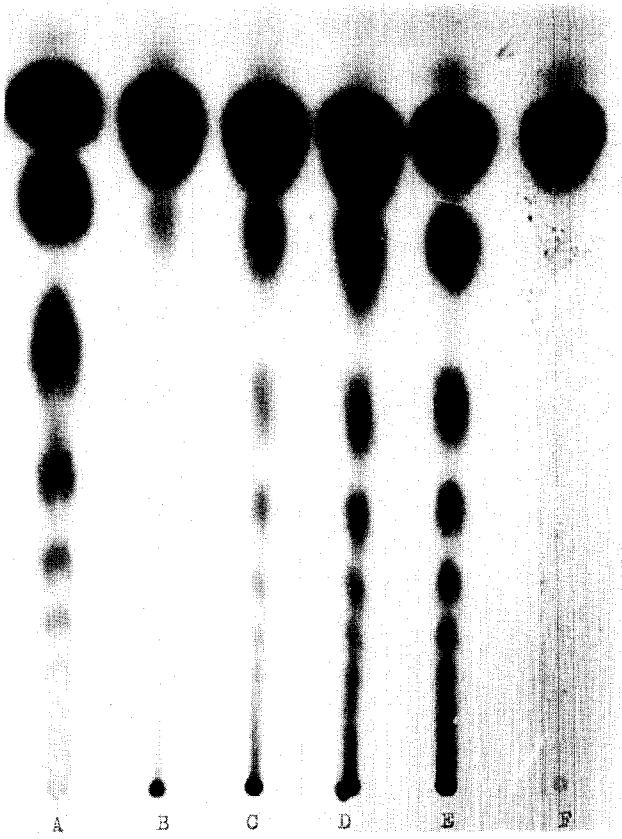

Fig. 1. Radioautograph of the paper chromatogram (Whatman No. 3 paper, 6:4:3 1-butanol–pyridine–water, 3 ascents) of the products of transglycosylation formed from D-[1-^{14}C]glucopyranose (0.2 mg/mL) and (1→3)-β-D-glucan (4 mg/mL). Times of reaction: F, 0; B, 3 min; C, 9 min; D, 18 min; E, 30 min; A, 7 h.

The paper chromatogram of the radioactive products of transglycosylation is shown in Fig. 1, and the relative radioactivities of the labelled sugars are presented in Table I.

Each of the radioactive products extracted from the paper chromatogram was degraded by exo-(1→3)-β-D-glucanase L-II from *Eulota maakii* into glucose and laminaribiose, *i.e.*, they were (1→3)-β-D-gluco-oligosaccharides. Of the ^{14}C-labelled laminaribiose produced by L-IV, ≤6% had the non-reducing unit labelled. No oligosaccharides were formed when L-IV was incubated with D-glucose (5 mg/mL).

Since ~25% of ^{14}C-D-glucose was incorporated into the oligosaccharides (see Table I), the above method may be used for large-scale preparations.

EXPERIMENTAL

Homogeneous exo-(1→3)-β-D-glucanase L-II (EC 3.2.1.58) from *Eulota maakii* and L-IV (EC 3.2.1.6) were prepared as described[2,3]. Smith-degraded linear (1→3)-β-D-glucan (d.p. ~25) was produced from laminarin in our laboratory[4]. D-[1-^{14}C]Glucopyranose (1.9 MBq/μmol) was purchased from V/O "Isotop" (U.S.S.R.).

Enzymic reaction. — The reaction mixture contained (1→3)-β-D-glucan (1–4 mg/mL) dissolved in mM Na acetate buffer (pH 5–5.4) with mM NaCl and L-IV (0.06–0.08 U/mL*). The mixture was kept at 25°. The concentration of D-[1-^{14}C]glucose varied from 0.2 to 0.9 mg/mL.

Aliquots taken at intervals were heated for 5 min at 100° and then subjected to p.c. (see Fig. 1). The location of the labelled sugars was ascertained by autoradiography using PM-1 or PT-1 X-ray Film (U.S.S.R.) and exposure for 24–72 h. The appropriate zones were excised and their radioactivity was determined.

The detection of non-reducing end-labelled laminaribiose in the products was performed by borohydride reduction followed by acid hydrolysis and separation of the resulting glucose and glucitol[5]. The mixture of radioactive glucitol and glucose was fractionated also by electrophoresis on Whatman No. 3 paper, using a 0.1M Na_2MoO_4 buffer (pH 5.0) at 20 V/cm for 90 min.

All other methods have been described[4].

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*One unit is the amount of enzyme which catalyses the formation of 1 μmol of reducing sugar (as glucose)/min under the conditions described.