Enzymic Synthesis of *O*- β -D-Glucopyranosyl-(1 $\rightarrow$ 6)-D-galactose

By K. W. KNOX

Institute of Dental Research, United Dental Hospital, Sydney, New South Wales, Australia

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1. The enzymic synthesis of *O*- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-D-galactose has been described and evidence for the structure presented. 2. It has been shown that the transglycosylase of *A. niger* provides a convenient means of synthesizing (1 $\rightarrow$ 6)-linked disaccharides.

Crook & Stone (1957) studied the transglucosylase activity of an enzyme preparation from *Aspergillus niger* and identified a number of oligosaccharides, including *O*- β -D-glucosyl-(1 $\rightarrow$ 6)-D-glucose, among the products of action of the preparation on cellobiose. When galactose was also present, an extra component was detected, and it was suggested that this was glucosyl-(1 $\rightarrow$ 6)-galactose. Evidence has been obtained for the presence of *O*- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-D-galactose amongst the products of acid hydrolysis of the cell-wall polysaccharide from *Lactobacillus casei* (Knox & Hall, 1965), and the ability of *Aspergillus niger* to synthesize this disaccharide has therefore been examined.

METHODS AND RESULTS

The commercial enzyme preparation from *A. niger* E19 (Rohm and Haas Co., Philadelphia, Pa., U.S.A.) had been further purified as described by Krishna Murti & Stone (1961). To 40 ml. of 0.01 M-acetate buffer, pH 5.0, containing 3 g. of cellobiose and 2 g. of D-galactose, was added 10 ml. of the same buffer containing 50 mg. of freeze-dried enzyme. The mixture was incubated at 37° for 1 hr. (previously shown to be the optimum time for production of the disaccharide), and then heated at 100° for 5 min.

The solution was applied as a band on washed sheets of Whatman no. 3MM paper and chromatograms were developed for 40 hr. in butan-1-ol-pyridine-water (6:4:3, by vol.) (solvent A). The position of the oligosaccharides was located by developing guide strips with silver nitrate (Trevelyan, Procter & Harrison, 1950). The bands corresponding to glucosyl-(1 $\rightarrow$ 6)-galactose were cut out and eluted with water. Examination of the eluates by paper chromatography in solvent A showed the presence of a slower-moving minor component, presumably a trisaccharide. The eluates were pooled and applied to a column containing 5 g. of charcoal and 5 g. of Celite 535; the disaccharide was

eluted with aq. 3% (v/v) ethanol and the trisaccharide by 15% ethanol (Whistler & Durso, 1950). To follow the rate of elution, carbohydrate was estimated by the primary cysteine-sulphuric acid reaction (Dische, 1953). From the carbohydrate content of the pooled 3% ethanol eluate it was calculated that the yield of disaccharide was 40 mg.; this represents a recovery of 1% of the galactose present in the incubation mixture.

Only one component was detected on chromatography of the disaccharide in solvent A ($R_{0.10}$ 0.46) and on electrophoresis in 0.05 M-sodium tetraborate (M_R 0.76) (Frahn & Mills, 1959). Acid hydrolysis followed by chromatography in solvent A showed the presence of glucose and galactose. Quantitative determination of the component sugars with D-glucose oxidase (Huggett & Nixon, 1957) and D-galactose oxidase (Avigad, Amaral, Asensio & Horecker, 1962) gave a ratio 1.00 mole of D-glucose:0.96 mole of D-galactose. D-Galactose oxidase had no detectable action on the disaccharide.

Evidence for the identity of the terminal reducing sugar was obtained by reduction with sodium borohydride followed by acid hydrolysis; chromatography in butan-2-one-acetic acid-boric acid (aq. saturated solution) (9:1:1, by vol.) (Rees & Reynolds, 1958) showed the presence of glucose and galactitol. It is further concluded that the disaccharide is a β -glucoside as it is hydrolysed by almond-emulsin β -glucosidase, and has a low positive rotation $[\alpha]_D^{20} + 15 \pm 1^\circ$ (c 2 in water).

On oxidation of the disaccharide with sodium metaperiodate (final concentration 8 mM) for 1 hr. at room temperature, the yield of formaldehyde (Kabat, 1961) was 0.1 mole/mole of disaccharide; the amount of periodate consumed after 2.5 hr. was 4.7 moles/mole of disaccharide. In a comparable experiment in which the disaccharide had been reduced with sodium borohydride the yield of formaldehyde was 0.9 mole/mole. This yield is consistent with the presence of a sugar substituted on either C₍₂₎ or C₍₆₎ by a pyranose unit. However,

the possibility of a (1→2)-linkage in the disaccharide was excluded by the results of periodate consumption, and by the formation of a red formazan (Wallenfels, 1950). The high electrophoretic mobility in tetraborate and relatively low chromatographic mobility are consistent with a (1→6)-linkage.

These results therefore indicate that the disaccharide is an *O*-β-D-glucopyranosyl-(1→6)-D-galactose. Freudenberg, Wolf, Knopp & Zaheer (1928) have described the chemical synthesis of this product and reported $[\alpha]_D + 14^\circ$.

The transglycosylase of *A. niger* has also been used for obtaining products chromatographically identical with *O*-β-D-galactosyl-(1→6)-*N*-acetyl-D-glucosamine (from lactose and *N*-acetyl-D-glucosamine), and *O*-α-D-galactosyl-(1→6)-*N*-acetyl-D-glucosamine (from melibiose and *N*-acetyl-D-glucosamine).

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