β-1,2-GLUCOSYL TRANSFER BY MEMBRANE PREPARATIONS FROM *ACETOBACTER XYLINUM*

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1. Introduction

Acetobacter xylinum is a Gram-negative bacterium well-known for its production of crystalline cellulose. It was demonstrated as early as 1958 [1], and subsequently confirmed [2–6], that membrane preparations from this organism catalyze the transfer of D-glucose from UDP-glucose to cellulose. In the original study by Glaser [1] cellulose was determined as the alkali-insoluble glucan fraction, the alkali-soluble material being discarded. Subsequent studies showed that there were also unidentified alkali-soluble transfer products [4–7] which have been discussed as intermediate polymers of cellulose biosynthesis [5,7].

It is now shown that the major polymeric product formed in vitro from UDP-glucose is alkali-soluble and that glucose is transferred predominantly in a β-1,2- rather than a β-1,4-linkage. A brief abstract of some of these results has appeared [8].

2. Experimental

2.1. *Materials and general procedures*

The bacterial strains and materials used and the general procedures were as in [9,10]. The solvent systems and buffers used for separation were as follows (in parts per vol.): (A) isobutyric acid/1 M ammonia, 5:3; (B) ethyl acetate/pyridine/acetic acid/water, 5:5:1:3; (C) ethyl acetate/pyridine/water, 2:1:2 (upper phase); (D) propanol-1/ethyl acetate/water, 7:1:2; (E) 0.1 M sodium molybdate (pH 5.0) [10]; (F) pyridine/acetic acid/water, 1:10:89 (pH 3.6); (G) 60 mM sodium borate (pH 8.1).

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2.2. *Standard incubation*

The incubation mixture contained 120 mM Tris–HCl (pH 9.2), 8 mM Mg^{2+}, 4 mM EDTA, 20 mM 2-mercaptoethanol, 25 μM UDP-[U-^{14}C]glucose (20–100 000 cpm) and 100–500 μg membrane protein, in 50 μl final vol. After incubation for 60 min at 30°C, the reaction mixture was fractionated in solvent system (A), or treated with 1 N NaOH [1], or subjected to phenol/water extraction [9,11].

2.3. *Mild acid hydrolysis*

Authentic sophorose, cellobiose and maltotriose (1 mg each) were added to 300 μl [^{14}C]polymer solution which was then made 0.5 N in HCl and heated for 20 min at 100°C. The hydrolysis products were fractionated in solvent system (B) using various oligosaccharide standards. The distributions of radioactivity were determined by liquid scintillation counting and the [^{14}C]sugar fractions were eluted with water and lyophilized.

3. Results

3.1. *Formation of an alkali-soluble product*

Membrane fractions of *Acetobacter xylinum* were prepared by the procedures in [1,12]. In both cases, the incubation with UDP-[U-^{14}C]glucose under the conditions in [1] or under the present conditions led

to a small amount of ^{14}C-labeled product which was degraded by cellulase (from *Aspergillus niger*, Calbiochem). However, ~90% of the ^{14}C-labeled material which failed to migrate in solvent system (A) was solubilized by 1 N NaOH (5 min, 100°C, N_2 atmosphere) and, after neutralization, appeared in the void volume of a column of Sephadex G-100. This product could also be solubilized with 1% (w/v) sodium dodecylsulphate (5 min, 100°C), and it appeared in the aqueous phase of a phenol/water extraction [9,11].

3.2. *Properties of the transferase reaction*

The alkali-soluble product was formed in similar yields by membrane fractions from the cellulose-producing strain and the non-producing strains NRC 17007 and NRC 17008 of *A. xylinum*. The glucosyl transfer reaction was linear with time up to 2 h and was optimal at pH 9.2 and 38–40°C.

Heat treatment (5 min, 100°C) led to complete inactivation. There was an absolute requirement for Mg^{2+} and the optimal concentration of Mg^{2+} was 4–8 mM. The app. K_m-value for UDP-glucose was 25 μM and V was 50 pmol glucose incorporated/min X mg protein. The transfer reaction was remarkably insensitive to the detergent, Tween 80 (up to 4%, v/v) and to the antibiotics, nisin and bacitracin (each tested up to 4 mg/ml). The antibiotic moenomycin inhibited by a non-specific effect which was relieved by addition of Tween 80. The detergent, Triton X-100, was also inhibitory (50% inhibition at 0.1% (w/v) Triton X-100).

3.3. *Properties of the non-cellulosic product*

The major in vitro product failed to migrate upon prolonged paper chromatography in solvent systems (A) and (B), upon high-voltage paper electrophoresis in buffer systems (F) and (G) or upon polyacrylamide gel electrophoresis in the presence of 1% (w/v) sodium dodecylsulphate [9,13]. All further studies were performed with the ^{14}C-labelled product fraction isolated by phenol/water extraction, followed by extensive dialysis and chromatography on Sepharose 4-B [9] where it eluted near the void volume.

The [^{14}C]polymer was resistant towards digestion by the following enzymes, α-amylase (hog pancreas), β-amylase (sweet potato), α-glucosidase (yeast), β-glucosidase (almond), α-galactosidase (coffee bean), β-galactosidase *(Escherichia coli)*, amyloglucosidase *(Aspergillus niger)*, cellulase (fungal and *Trichoderma viride*), pectinase (fungal), crude β-glucuronidase *(Helix pomatia)*, hyaluronidase (sheep testis) and pronase *(Streptomyces griseus)*. After mild alkaline treatment (1 N NaOH, 22 h, 25°C; designed to break ester linkages) the polymer remained inert against cellulases and α-amylase.

Strong acid hydrolysis (2 N HCl, 100°C, 90 min) liberated a single [^{14}C]monosaccharide which by the previous methods [10], in particular, its complete oxidation with glucose oxidase, was identified as D-glucose. The mild acid hydrolysis procedure (see section 2) led to the release of D-[^{14}C]glucose and a series of [^{14}C]oligosaccharides which were isolated by preparative paper chromatography. The material remaining at the origin of the chromatogram contained 60% of the initial radioactivity. This material was eluted and again subjected to the mild acid hydrolysis procedure so that a second series of oligosaccharides could be obtained. Of the inital amount of radioactivity 80% was recovered in the form of D-[^{14}C]glucose and [^{14}C]oligosaccharides which were then characterized by the methods in [10].

The disaccharide fractions contained 20% of the initial total radioactivity. They were stable to α-glucosidase but were degraded to D-[^{14}C]glucose by the crude and the purified β-glucosidase preparations [10]. The trisaccharide (15% of the initial total radioactivity) and the higher oligosaccharide fractions remained unattacked by either α- or β-glucosidase. High-voltage paper electrophoresis of the reduced disaccharides in the presence of molybdate indicated the presence of β-1,2- or β-1,6-glucosidic linkages and excluded the presence of β-1,3- and β-1,4-glucosidic linkages (fig.1A). Paper chromatography in solvent systems (C) and (D; fig.1B) indicated that the [^{14}C]-disaccharide fractions consisted of only sophorose. The [^{14}C]trisaccharide fractions had significantly higher electrophoretic (fig.1A) and chromatographic (fig.1B) mobilities than the available trisaccharide standard, maltotriose. In this respect they resembled the [^{14}C]disaccharides which had higher mobilities than maltose.

3.4. *Comparison with the lipid-linked trisaccharide*

An unidentified lipid-linked trisaccharide has been isolated after the incubation of an *A. xylinum* enzyme

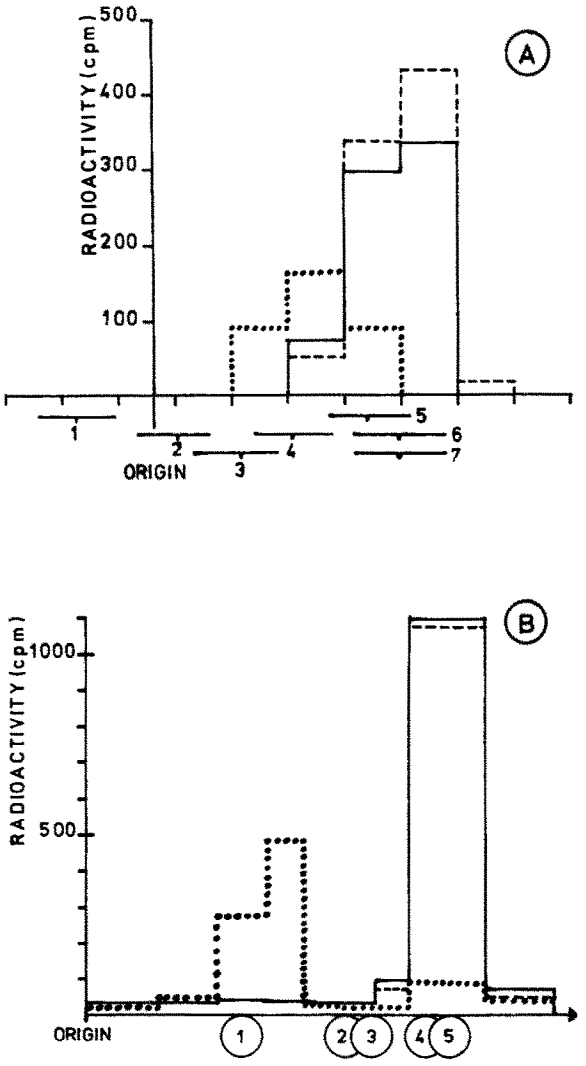

Fig.1. Electrophoretic and chromatographic properties of the [^{14}C]disaccharide fractions isolated by paper chromatography after the first (————) and the second (– – – – – – – – – –) acid hydrolysis of the non-cellulosic [^{14}C]polymer. The corresponding [^{14}C]trisaccharide fractions are represented by a single symbol (. . .). The distributions of radioactivity (cpm) are plotted against the positions (drawn to scale) of the reference compounds used. (A) High-voltage paper electrophoresis [buffer system (E)] of the borohydride-reduced sugars. The following standards were employed: (1) laminaribiitol (β-1.3); (2) cellobiitol (β-1.4); (3) maltotriitol; (4) maltitol (α-1.4); (5) picrate (visible standard). (6) gentiobiitol (β-1.6); (7) sophorositol (β-1.2). (B) Paper chromatography [solvent system (D)]. The following standards were employed: (1) maltotriose; (2) gentiobiose; (3) cellobiose; (4) maltose; (5) sophorose.

preparation with UDP-[U-^{14}C]glucose [10]. This trisaccharide has now been found to withstand the treatment with either α- or β-glucosidase under the previous conditions [10]. After borohydride reduction, most of the lipid-derived [^{14}C]trisaccharide was found in the position of the [^{14}C]trisaccharides of fig.1A.

4. Discussion

Membrane preparations from *A. xylinum* have been shown to utilize UDP-glucose primarily for the formation of a high molecular weight, non-cellulosic polymer. The disaccharide fractions liberated from this polymer by a stepwise acid hydrolysis procedure consisted of only sophorose (β-1,2-D-glucopyranosyl-D-glucose). Trisaccharide fragments were also isolated and their electrophoretic and chromatographic mobilities indicated a possible structural relationship with sophorose (sophorotriose?). The unusual resistance of the trisaccharides and higher oligosaccharides as well as the polymer itself towards various degradative enzymes may be due to β-1,2-glucosidic linkages. A well-characterized β-1,2-glucan from *Agrobacterium* has been reported to be inert against a wide variety of degradative enzymes [14]. A chemical characterization of the present polymer has not been possible because only a very small amount of a polymer with similar properties could be isolated from cells of *A. xylinum* [9].

β-1,2-Glucans are rarely occurring polysaccharides which have so far only been isolated from species of *Agrobacterium* [15] and of *Rhizobium* [16,17]. A polymer fraction whose proposed structure consists of a β-1,4-glucan with single glucosyl-groups attached in 1,2-linkages to every third glucose residue has been isolated from the extracellular culture fluid of *A. xylinum* [18]. However, it appears unlikely that the present non-cellulosic polymer arose from single β-1,2-glucosyl transfer steps to a β-1,4-glucan backbone. Partial acid hydrolysis should lead to the release of trisaccharides whose reducing glucose residues are bound in β-1,4-linkages. This is not consistent with the fact that the reduced [^{14}C]trisaccharide fractions had a significantly higher electrophoretic mobility than cellobiitol (fig.1A).

The formation of a β-1,2-glucan from UDP-glucose

has been achieved with a membrane preparation from *Rhizobium japonicum* [16]. A number of properties of the *Rhizobium* enzyme resemble those of the present *Acetobacter* transferase preparation. The similarity between the trisaccharide components of the acid-labile glucolipid fraction of *Acetobacter* [10] and of the non-cellulosic polymer may indicate a precursor/product relationship, although other possibilities exist. For example, gram-negative bacteria generally contain periplasmic oligosaccharides with β-1,2- and β-1,6-linked glucose residues derived from UDP-glucose [19]. Sugars linked to a lipid carrier may participate in the biosynthesis of these oligosaccharides [20].

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