STRUCTURAL FEATURES OF THE β-GLUCANS ENZYMATICALLY SYNTHESIZED FROM URIDINE DIPHOSPHATE GLUCOSE BY WHEAT SEEDLINGS

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1. Introduction

The chemical structure of the β-glucan (or glucans) synthesized from UDPGlc* by enzyme extracts from plants is still controversial. Some recent papers [1–3] suggested that the glucosyl chains were essentially cellulose-like, that is β-(1 → 4)-linked, whereas in other experiments [4–6], evidence was presented that the polysaccharide biosynthesized from UDPGlc was in fact a laminaran, i.e. β-(1 → 3)-linked, although a fraction of this material was resistant to alkaline treatment. In these and other papers [5–8] it was shown that the solubility in alkali was not an absolute differential characteristic of the structure of linkages between β-glucosyl residues. On the other hand, in experiments of incorporation of radioactive glucosyl into alkali-insoluble glucans, only the radioactive chains are analyzed and they might be linked to a primer polymer, the structure of which might be different.

In this paper, we report experiments showing that both structures β-(1 → 3) and β-(1 → 4) may be obtained with the same enzyme system extracted from wheat roots and from the same substrate UDPGlc. The structure of the β-glucan is dependent on the conditions of the enzymic reaction and especially on the concentration of the substrate.

2. Methods

β-Glucan synthetase was prepared as by Flowers et al. [6], with modifications which were found to yield an increased activity. The biological material consisted of wheat seedlings, grown for 24 to 40 hr at 25° on sieve plates. The roots were cropped from the underside of the plates, and either used fresh or kept frozen at −25° under nitrogen gas. The roots (70 g) were crushed for 2 min in a pestle and mortar under liquid nitrogen. All subsequent steps were at 0°–2°. 210 ml of 0.1 M tris-Cl buffer, pH 7.5, containing 5×10^{-3} M EDTA (Na) and 3.5 g of Polyclar AT was added. After homogenization with a glass rod, the slurry was filtered through cheese-cloth and the filtrate was centrifuged 5 min at 1,000 *g*. The supernatant was further centrifuged 15 min at 45,000 *g* and the sediment was washed twice with 100 ml of tris-Cl 0.1 M, pH 7.5 and recentrifuged. The particulate material obtained in this way may be used as a source of enzyme [6]. For the experiments further described, this extract was

* *Abbreviations:*
UDPGlc, GDPGlc: respectively uridyl and guanosyl diphosphate-D-glucose;
Glc-1-P: α-D-glucose-1-phosphate;
Glc-HMD: 2,4-bis-hydroxymethyl-5-*O*-β-D-glucopyranosyl-1,3-dioxane [10];
Glc-Arabinitol: 2-*O*-β-D-glucopyranosyl-D-arabinitol;
Glc-Erythritol: 2-*O*-β-D-glucopyranosyl-D-erythritol;
$(Glc)_2$- or $(Glc)_3$-Erythritol: 2-*O*-β-laminaribiosyl- (or triosyl-)D-erythritol;
Polyclar AT: insoluble polyvinylpyrrolidone.

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solubilized with 2.2 ml of 1% digitonin in 0.1 M tris-Cl, pH 7.5, containing 0.3 M sucrose. The mixture was shaken occasionally during 10 to 15 min and centrifuged 15 min at 45,000 *g*. This treatment was repeated and the combined supernatants were centrifuged 1 hr at 200,000 *g*. The final supernatant was the source of glucan synthetase, which was incubated as soon as possible after the preparation. The total preparation time did not exceed 5 hr [9].

The incubation medium was as follows: Glc-1-P 1.7 μmoles; $MgCl_2$ 13.6 μmoles; UDP-U-^{14}C-Glc 1.75 μCi, concentration as specified for each experiment, and enzyme extract containing 1.7 mg protein, in the tris-digitonin-sucrose buffer. The total volume was 0.70 ml. The mixture was incubated 30 min at 25° with the addition of 2 mg of insoluble cellodextrins as primer and carrier and immediately inactivated by the addition of 5 ml of 0.5 N NaOH followed by immersion in a boiling water-bath for 5 min. The insoluble material was recovered by centrifugation and the alkali treatment was repeated twice. The pellet was washed repeatedly with water to neutrality and the residue was further treated for measurements of radioactivity and analysis.

Cellodextrins added during incubations weakly activated the incorporation and were useful as carriers in the extraction step. Glc-1-P also acted as an activator. Although the enzyme extract contained UDPGlc-pyrophosphorylase (EC 2.7.7.9), the glucosyl of Glc-1-P was not incorporated into the alkali-insoluble fraction in the absence of UTP.

3. Results and discussion

UDPGlc was incubated in the medium previously described at two different concentrations (table 1, A and B). In both experiments, the radioactivity incorporated into polysaccharides was recovered essentially from the alkali-insoluble fraction. Only 10% of the incorporated radioactivity was found in the alkali-soluble fraction, which was not further studied in the present work.

By total acid hydrolysis of the alkali-insoluble material, no radioactive compound other than glucose was found to be present in pellets A or B, which therefore contained one or several radioactive glucans. In another experiment, after alkali treatment and washing, each of the insoluble residues was subjected to a partial hydrolysis for 2 hr at 20° in fuming HCl [6]. The acid was eliminated by evaporation under vacuum at 30° and the liquid brought to neutrality by stepwise additions of methanol to the evaporation vessel. The hydrolyzed material was water-soluble. A standard aliquot was plated for radioactivity counting.

A noticeable feature of the results in table 1 is that when the concentration of UDPGlc substrate was increased 75-fold, the amount of glucose incorporated into the alkali-insoluble fraction was raised 300 times. This result is not consistent with what is expected from a one-enzyme reaction, unless the substrate itself has an activating effect.

Evidence for the β-structure of the glucosidic linkages in the glucans A or B was substantiated (1) by the insolubility of A or B in alkali; (2) by the activity of β-glucosidase on the disaccharides derived by partial acid hydrolysis from A or B; (3) the products of this hydrolysis were subjected to paper chromatography and the paper strips scanned for radioactivity (fig. 1). Chromatogram A exhibited a series of peaks which cochromatographed with glucose, cellobiose, cellotriose, and there was a non-mobile spot. In contrast, a series of radioactive spots which cochromatographed with glucose, laminaribiose, etc. was present on chromatogram B, besides a small amount of cello-

Table 1
Incorporation of glucosyl from UDPGlc into β-glucans by a 30 min incubation with soluble wheat extract.

	Exp. A	Exp. B
Concentration of UDP-^{14}C-Glc (M) (containing 85 X 10^{-4} cpm)	4×10^{-5}	3×10^{-3}
*Radioactivity incorporated (cpm):**		
1) alkali-soluble fraction**	1,400	8,500
2) alkali-insoluble fraction	17,300	70,000
Glucosyl incorporated in alkali-insoluble fraction (nmoles):		
1) per g of roots	0.086	26.6
2) per mg of protein in extract	0.30	93.0

* Counts in the whole digests, obtained as detailed in Methods.
** Alkali-soluble, 80% ethanol-insoluble fraction.

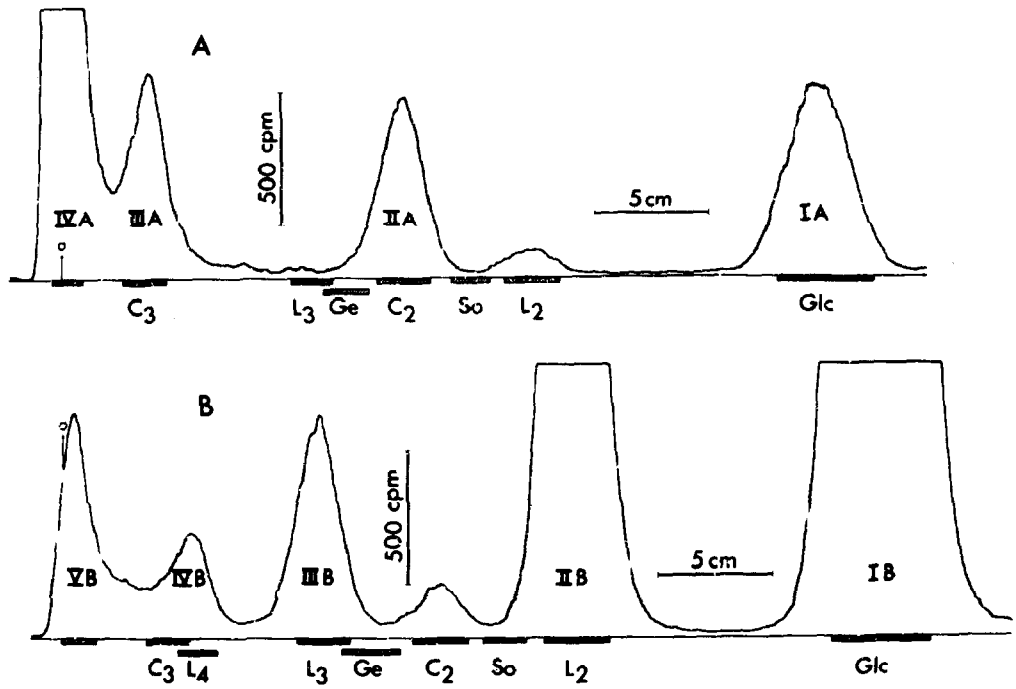

Fig. 1. Radiochromatographic scannings of partial hydrolysis products of alkali-insoluble glucans A and B synthesized from UDPGlc.

Conditions of incubation and hydrolysis: see text and table 1. Chromatography on Whatman 3 MM paper; solvent system: propanol-ethyl acetate-water, (7:1:2, v/v).

Nature of peaks: see table 2. Under each scanning curve, positions of standard sugar spots are indicated: Glc = glucose; L_2, L_3, L_4 = laminaribiose, -triose, -tetraose; C_2, C_3 = cellobiose, -triose; So = sophorose; Ge = gentiobiose. Starting spot (o) on the left.

biose. This situation was reproducible in another solvent system: butanol-acetic acid-water (4:1:2, v/v).

The radioactive disaccharides, trisaccharides and non-mobile peaks were eluted from the paper. The products, mixed with authentic oligosaccharides as carriers, were subjected to a Smith degradation [10] by metaperiodate oxidation, borohydride reduction and mild acid hydrolysis. The products were further fractionated and identified by paper cochromatography (table 2). Oligosaccharides A contain a major amount of β-(1 → 4)-linkages whereas oligosaccharides B are essentially β-(1 → 3)-linked, except the non-mobile peak which contained 66% of β-(1 → 4)-linkages: we suggest that an acid resistant core, β-(1 → 4)-linked, is present in B.

Although these experiments do not lead to any conclusion on the ratio of β-(1 → 4) to β-(1 → 3) glucosidic linkages in the native glucans A or B, it seems that both polysaccharides contain both types of linkages, but their structures must be vastly different: A is much richer than B in β-(1 → 4)-bonds.

In the next experiment, the native radioactive glucans A and B were directly subjected to a Smith degradation and the products analyzed as described by Goldstein et al. [10] by paper chromatography (table 3). The results are in agreement with the preceding conclusions since glucan A contained more than 50% of β-(1 → 4)-linkages and B less than 12%. Some of the β-(1 → 4)-bonds in A are clustered, as shown by the presence of a large percentage of erythritol in the degradation products. The result in table 3 also indicated that the β-(1 → 3) and β-(1 → 4)-bonds in A are not at random in the chains.

Owing to the low proportion of glycerol found in the Smith degradation products of both A and B glucans, it seems unlikely that these glucans contain many branching points or β-(1 → 2) or β-(1 → 6) linked glucosyl.

Table 2
Analysis of partial hydrolysis products of radioactive β-glucans A and B by Smith degradation.

Products of partial hydrolysis	II A	III A	IV A	II B	III B	V B
% Total counts in polysaccharide	11.4	13.3	55.2	20.4	4.8	3.5
*Products of Smith degradation**:						
Glycerol	1. (1)	1. (1)	1.	1. (1)	1. (1)	1.
Erythritol	1.08 (1)	1.23 (2)	1.96	–	–	1.1
Arabinitol	–	–	0.09	0.93 (1)	–	traces
Glc-Erythritol	–	0.1	1.09	–	–	0.74
Glc-Arabinitol	–	–	–	–	1.48 (2)	–
Non-Mobile spot	–	–	0.19	–	–	0.69
Tentative identification	Cellobiose	Cellotriose major product	mixed β-oligoglucans (1→3)-(1→4)	Laminaribiose	Laminaritriose major product	mixed β-oligoglucans (1→3)-(1→4)

* Numerical values: moles of glycosyl in fragment before Smith degradation/moles of glycerol. The amount of each radioactive fragment was measured by integration of the counts on paper with a 4 π Scanner Tracerlab counter.
Figures in parenthesis are theoretical values for the postulated di- or trisaccharides.

Table 3
Structural analysis of β-glucans A and B by paper chromatography.

	Native glucan A		Native glucan B	
	Smith degradation products*			
	% soluble	% total	% soluble	% total
Water-soluble				
Glycerol	2.1		3.90	
Erythritol	25.6		4.65	
Glc-HMD**	9.0		2.12	
Glc-Erythritol	35.2	95.4	9.10	19.75
$(Glc)_2$-Erythritol	7.7		3.85	
$(Glc)_3$-Erythritol	9.5			
Non mobile spot	10.9		76.50	
Water-insoluble	–	4.6		80.25
Calculated %:				
β-(1→3)-linkages	46.3	48.2 ⩾ % ⩾ 46.3	88.20	97.7 ⩾ % ⩾ 88.2
β-(1→4)-linkages	53.7	53.7 ⩾ % ⩾ 51.8	11.80	11.8 ⩾ % ⩾ 2.3
Average D.P.		52.5		130

* Numerical values as in table 2.
** See abbreviations.

In summary, it seems possible to synthesize glucans of both the lichenan (A) and the laminaran (B) structures from UDPGlc in the presence of wheat-enzyme extracts. The structure of glucan A may be related to the one proposed by Peat et al. [11] and Goldstein et al. [10] for the oat gum. Glucan A might be a mixture of an alternating β-(1 → 3)–β-(1 → 4) glucan and another randomly structured glucan containing about 60% of β-(1 → 4)-bonds. In contrast with the structure of A, B seems to be an authentic laminaran, possibly mixed with a small amount of glucans of the A type. According to the concentration of the substrate, either A or B is the major product. This difference in the structures of the glucans produced could be explained if the soluble wheat extract contains two enzymes, at least, one responsible for the biosynthesis of β-(1 → 4)-linkages, with a very high affinity for the substrate UDPGlc, the other enzyme catalysing the biosynthesis of β-(1 → 3)-links with a low affinity for UDPGlc. Both enzymes may also have unknown effectors which modify their respective affinities. The presence of sucrose in the incubation medium might favor the synthesis of laminaran, as suggested by Thomas et al. [12]. However, parallel results were obtained with our particulate enzyme preparations which contained no sucrose.

We are aware that the glucans thus obtained from UDPGlc should be comparable with the compounds synthesized by the use of GDPGlc as a glucosyl donor in the enzymatic reaction. GDPGlc is a rather ineffective substrate in the presence of wheat extracts [9] and we lack, at this stage, sufficient information on the structure of the product.

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