Solubilization of β -glucan synthases from the membranes of cultured ryegrass endosperm cells

Robert J. HENRY and Bruce A. STONE

Department of Biochemistry, La Trobe University, Bundoora, Vic. 3083, Australia

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β -Glucan synthases were solubilized by treating membrane preparations from suspension-cultured ryegrass (*Lolium multiflorum*) endosperm cells with detergents. Of the seven detergents tested only digitonin and octyl glucoside dissociated active synthases from the membranes. The digitonin-solubilized enzymes produced 1,4- β -glucans and 1,3:1,4- β -glucans, whereas the digitonin-insoluble enzymes produced, in addition, 1,3- β -glucans. Chromatography of the digitonin-solubilized β -glucan synthases on DEAE-Sephadex resulted in their partial purification. The octyl glucoside-solubilized enzymes produced more 1,3- β -glucans than did the membrane-bound preparations. These results suggest that the 1,3- β -glucan synthase is a separate enzyme and is not involved in 1,3:1,4- β -glucan synthesis. Digitonin not only dissociated synthases from the membranes, but also stimulated synthase activity. This effect may be related to the inhibition by digitonin of glucosyl transfer from UDP-glucose to form steryl glucosides.

Mixed-linkage 1,3:1,4- β -glucans are important components of the cell walls of the Gramineae (Wilkie, 1979). These glucans represent a family of related molecules in which the glucopyranosyl residues are joined by 1,3- and 1,4- β -glucosidic linkages to form a linear polymer. Characteristically about 30% of the residues are 1,3-linked and the remainder 1,4-linked. Large segments of these molecules (80–90%) are comprised of cellotriosyl and cellotetraosyl sequences joined by single 1,3-linkages, but longer runs of 1,4-linkages (Woodward *et al.*, 1981) have also been detected. In glucans from some sources less abundant sequences of consecutive 1,3-linkages are found (Fleming & Manners, 1966; Igarashi & Sakurai, 1966). The mechanism of synthesis of these 1,3:1,4- β -glucans is unknown.

Cell walls from suspension-cultured ryegrass (*Lolium multiflorum*) endosperm cells contain 49% 1,3:1,4- β -glucans (Smith & Stone, 1973; Anderson & Stone, 1978), and mixed-membrane preparations from these cells produce 1,3-, 1,4- and 1,3:1,4- β -glucans from UDP-glucose (Smith & Stone, 1973; Cook *et al.*, 1980). The influence of reaction conditions on the synthesis of individual β -glucans suggests that more than one β -glucan synthase is involved (Henry & Stone, 1982), but whether the 1,3:1,4- β -glucan is synthesized by the concerted

action of 1,3- and 1,4- β -glucan synthases or by a separate specific enzyme has not yet been established. We have approached this problem by attempting the dissociation and subsequent fractionation of β -glucan synthases from ryegrass membranes.

Materials and methods

Chemicals

Digitonin, octyl glucoside and sodium dodecyl sulphate were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.); Triton X-100 and cetylpyridinium chloride were from BDH Chemicals (Poole, Dorset, U.K.); sodium deoxycholate was from Merck (Darmstadt, West Germany); octaethyleneglycol mono-n-dodecyl ether was from Nikko Chemical Co. (Tokyo, Japan). UDP-[14 C]-glucose and GDP-[14 C]-glucose were from Amersham Australia Pty. Ltd. (Sydney, N.S.W., Australia), and the unlabelled compounds were from Sigma Chemical Co.

Preparation of particulate enzymes

Mixed-membranes sedimenting between 1000 and 100 000 g were prepared from suspension-cultured ryegrass (*Lolium multiflorum*) endosperm cells as described by Smith & Stone (1973).

Treatment of membranes with detergents

The detergent was added as a solid or a concentrated solution to the particulate enzyme preparation, and the mixture was stirred gently for 1 h at 4°C. The extent of solubilization was estimated by centrifuging the detergent-treated enzymes at 100 000 g for 1 h and then assaying the supernatant and pellet to determine detergent-soluble and detergent-insoluble enzyme activity respectively. The pellet was resuspended in an equivalent volume of 20 mM-Mops (4-morpholinepropanesulphonic acid)/NaOH buffer, pH 7.5, containing 20 mM-MgCl₂ and 2 mM-dithiothreitol by using a Dounce homogenizer.

Enzyme assays

Assay mixtures with particulate or detergent-treated enzymes in 50 mM-Mops buffer, pH 7.5, containing 20 mM-MgCl₂, 20 mM-cellobiose and 10 μ M- or 1 mM-UDP-glucose were incubated at 20°C for 15 min. Each assay mixture contained 3 kBq of UDP-[¹⁴C]glucose in a total volume of 0.4 ml. The synthesis of β -glucans was assayed by determining the incorporation of radioactivity into 66% (v/v)-ethanol-insoluble products (Smith & Stone, 1973).

Characterization of β -glucan synthase products

The products of β -glucan synthases were characterized by hydrolysis with specific β -glucan hydrolases (Cook *et al.*, 1980). The reaction mixture was freeze-dried, and washed twice with 10 ml of chloroform/methanol/water (10:10:3, by vol.), then successively with 66% (v/v) ethanol containing 0.85 mM-EDTA, 66% (v/v) ethanol, 70% (v/v) ethanol and finally acetone. The β -glucan composition of the insoluble residue was determined by incubating three replicates with either the 1,3:1,4-

β -glucan endo-hydrolase (EC 3.2.1.73) from *Bacillus subtilis* (Anderson & Stone, 1975), the 1,3- β -glucan exo-hydrolase (EC 3.2.1.58) from *Euglena gracilis* (Barras & Stone, 1969) or the 1,4- β -glucan endo-hydrolase (EC 3.2.1.4) from *Streptomyces* sp. (Cook *et al.*, 1980) that had been freed of 1,3- β -glucan hydrolase. After 72 h under the conditions described by Cook *et al.* (1980), hydrolysates were analysed by gel filtration on Bio-Gel P-2 (Henry & Stone, 1982).

Results

Comparison of detergents

The effects of seven detergents (1%, w/v) on the solubility of membrane protein and β -glucan synthases are compared in Table 1. The percentage of total membrane protein solubilized by the detergents ranged from 32% for digitonin to 96% for sodium dodecyl sulphate.

The synthesis of 66% ethanol-insoluble β -glucans from 10 μ M- and 1 mM-UDP-glucose by the 100 000 g pellets was generally decreased by extraction with detergents. Of the detergents tested, only digitonin and octyl glucoside extracted active synthases into the 100 000 g supernatant, and these two detergents were investigated further.

Effects of digitonin

The effect of digitonin concentration on the proportion of total activity in the 100 000 g supernatant is shown in Fig. 1. Increasing concentrations of digitonin increased the proportion of total activity in the 100 000 g supernatant, especially at 10 μ M-UDP-glucose. Treatment with 10% digitonin solubilized 40% of the activity with 1 mM-UDP-glucose. Treatment with 20% digitonin did not greatly increase solubilization, and a second extraction with

Table 1. Comparison of the effectiveness of detergents in solubilizing β -glucan synthases and membrane protein. For full experimental details see the text. The total protein concentration was 5.2 mg/ml, and that of the detergent was 1% (w/v). All values are means for four replicates, with correction for boiled controls.

Detergent	Protein in 100 000 g supernatant (%)	Glucose incorporated into products insoluble in 66% ethanol (pmol/s)			
		10 μ M-UDP-glucose		1 mM-UDP-glucose	
		100 000 g pellet	100 000 g supernatant	100 000 g pellet	100 000 g supernatant
No detergent	10.4	0.43	0	85.7	0
Digitonin	31.7	0.96	0.32	219	29.9
Octyl glucoside	39.8	0.04	0.02	44.5	7.4
Triton X-100	36.0	0	0	29.0	0
Octaethyleneglycol mono-n-dodecyl ether	32.8	0	0	11.8	0
Sodium deoxycholate	39.2	0	0	1.9	0
Cetylpyridinium chloride	52.2	0.01	0.02	0.91	3.8
Sodium dodecyl sulphate	95.7	0	0	0.35	1.9

Fig. 1. Concentration-dependence of digitonin solubilization of β -glucan synthases
A particulate enzyme preparation (3.8 mg of protein/ml) was treated with various amounts of digitonin and centrifuged at 100 000 *g* for 1 h. For full experimental details see the text, β -Glucan synthase activity in 100 000 *g* pellet: ●, with 1 mM-UDP-glucose as substrate; ○, with 10 μ M-UDP-glucose as substrate. β -Glucan synthase activity in 100 000 *g* supernatant: ■, with 1 mM-UDP-glucose as substrate; □, with 10 μ M-UDP-glucose as substrate.

10% digitonin solubilized an additional 10% and 7% of the activity with the low and high UDP-glucose concentrations respectively.

In addition to dissociating synthases from the membranes, digitonin also activated the β -glucan synthases when added directly to the normal assay mixture (Table 2). At 1% digitonin there was a 90% increase in incorporation from 10 μ M-UDP-glucose and a 30% increase from 1 mM-UDP-glucose. These increases were also found both in the 100 000 *g* pellet and supernatant fractions after centrifugation (Table 1). Some depression of the activity in the 100 000 *g* pellet was observed at digitonin concentrations above 1%, especially at the higher UDP-glucose concentration (Fig. 1).

Although digitonin activated β -glucan synthases, it was an effective inhibitor of the formation of chloroform/methanol-soluble products, particularly from 10 μ M-UDP-glucose (Table 2).

Characterization of β -glucans produced by digitonin-treated membranes

The β -glucans produced by mixed-membrane fractions after treatment with 10% digitonin were characterized by hydrolysis with specific β -glucan hydrolases (Cook *et al.*, 1980; Henry & Stone, 1982). No glucose was released when the β -glucans produced by the digitonin-solubilized synthases at 10 μ M- or 1 mM-UDP-glucose were incubated with the 1,3- β -glucan exo-hydrolase, indicating that no

Table 2. Influence of digitonin on UDP-glucose glucosyl-transferases

For full experimental details see the text. The total protein concentration was 4.0 mg/ml in the assay mixture. All values are the means for four replicates.

Substrate Products	Glucose incorporated (pmol/s)	
	10 μ M-UDP- glucose	1 mM-UDP- glucose
Insoluble in 66% ethanol		
No digitonin	0.73	193
1% (w/v) digitonin	1.38	250
Soluble in chloroform/methanol/ water (10 : 10 : 3, by vol.)*		
No digitonin	0.88	9.25
1% (w/v) digitonin	0.06	3.25

* Chloroform/methanol/water extract washed with 0.5% KCl.

1,3- β -glucan was present. When the β -glucans formed at 10 μ M-UDP-glucose were incubated with the 1,3:1,4- β -glucan endo-hydrolase, tri- and tetra-saccharides were released (Fig. 2). The 1,4- β -glucan endo-hydrolase released mono- and di-saccharides in addition to tri- and tetra-saccharides (Fig. 3). These results are consistent with the known specificity of the hydrolases and indicate that both 1,3:1,4- and 1,4- β -glucans are present.

The products formed by the 100 000 g supernatant and pellet from the digitonin-treated membranes at both 10 μ M- and 1 mM-UDP-glucose are compared in Table 3.

Fig. 2. Hydrolysis of the products of 10%-digitonin-solubilized β -glucan synthases with 1,3:1,4- β -glucan endo-hydrolase from *Bacillus subtilis*

The 66%-ethanol-insoluble β -glucans produced from 10 μ M-UDP-glucose by a 10%-digitonin-solubilized enzyme were incubated with 1,3:1,4- β -glucan endo-hydrolase from *Bacillus subtilis* at 40°C for 72 h. The hydrolysate was separated on a column of Bio-Gel P-2 at 60°C and radioactivity in 1.7 ml fractions was measured by scintillation counting. For full experimental details see the text. Numbers refer to the degree of polymerization of oligosaccharides.

Activity with GDP-glucose

Incorporation from 10 μ M-GDP-glucose by particulate preparations has been shown to be 42% of incorporation from 10 μ M-UDP-glucose (Henry & Stone, 1982). Both digitonin-soluble and insoluble

Fig. 3. Hydrolysis of the products of 10%-digitonin-solubilized β -glucan synthase with 1,4- β -glucan endo-hydrolase from *Streptomyces* sp.

The 66%-ethanol-insoluble β -glucans produced from 10 μ M-UDP-glucose by a 10%-digitonin-solubilized enzyme were incubated with 1,4- β -glucan endo-hydrolase from *Streptomyces* sp. at 40°C for 72 h. The hydrolysate was separated on a column of Bio-Gel P-2 at 60°C, and radioactivity in 1.7 ml fractions was measured by scintillation counting. For full experimental details see the text. Numbers refer to degree of depolymerization of oligosaccharides.

Table 3. Products of β -glucan synthases

For full experimental details see the text. All values are means for two replicates.

Enzyme source	Products formed from 10 μ M-UDP-glucose* . . .	% of total β -glucan		
		1,3- β -Glucan	1,4- β -Glucan	1,3 : 1,4- β -Glucan
10%-digitonin-treated membranes				
100 000 g pellet		22	30	48
100 000 g supernatant		—	52	48
100 000 g supernatant purified on DEAE-Sepharose		—	57	43
Products formed from 1 mM-UDP-glucose . . .				
10%-digitonin-treated membranes				
100 000 g pellet		54	39	7
100 000 g supernatant		—	77	23
1%-octyl glucoside-treated membranes				
100 000 g pellet		65	33	2
100 000 g supernatant		66	27	7

* Very little β -glucan of any type was produced from 10 μ M-UDP-glucose by 1%-octyl glucoside-treated membranes.

fractions from ryegrass membranes showed only slight activity with GDP-glucose as substrate. Incorporation from $10\mu\text{M}$ -GDP-glucose was 16% of that from $10\mu\text{M}$ -UDP-glucose with digitonin-insoluble preparations and 11% with digitonin-soluble preparations.

Purification of digitonin-solubilized β -glucan synthases

When the 100000g supernatant from 10% digitonin-treated membranes was chromatographed on Sepharose 4B and Sephadex G-200, components with β -glucan synthase activity were distributed across the profile and no purification was achieved. However a 10-fold purification was obtained by DEAE-Sepharose chromatography (Fig. 4). β -Glucan synthase was eluted as a single peak and more than 80% of the activity was recovered. The profiles obtained at $10\mu\text{M}$ - and 1mM -UDP-glucose were coincident. The partially purified preparation retained the capacity to produce both 1,4- and 1,3:1,4-glucans, as determined by hydrolysis of the products with specific β -glucan hydrolases. The

proportions of each of the β -glucan products are shown in Table 3.

Effects of octyl glucoside

The effect of glucoside concentration on the solubilization of β -glucan synthases is shown in Table 4. In some preparations 1% octyl glucoside solubilized almost 50% of the activity with 1mM -UDP-glucose, but at 10% octyl glucoside incorporation from UDP-glucose at both substrate concentrations was strongly inhibited.

When added directly to the assay mixture 1% octyl glucoside inhibited incorporation from $10\mu\text{M}$ -UDP-glucose, but more than doubled the activity with 1mM -UDP-glucose (Fig. 5).

Characterization of β -glucans produced by octyl glucoside-treated membranes

In contrast with the digitonin-solubilized preparations, the β -glucans produced by octyl glucoside-solubilized synthases at 1mM -UDP-glucose released glucose as the main product when treated with the 1,3- β -glucan hydrolase (Fig. 6). 1,3- β -

Fig. 4. DEAE-Sepharose chromatography of digitonin-solubilized β -glucan synthases

A 10%-digitonin-solubilized β -glucan synthase preparation was chromatographed on a $1.5\text{cm} \times 0.15\text{cm}$ column of DEAE-Sepharose equilibrated with 20mM -Mops buffer, pH 7.5. Enzyme was eluted with a linear gradient from 20mM -Mops buffer, pH 7.5, to 20mM -Mops buffer, pH 7.5, containing 1.0M -NaCl. For full experimental details see the text. ●, β -Glucan synthase activity with 1mM -UDP-glucose as substrate; ○, β -glucan synthase activity with $10\mu\text{M}$ -UDP-glucose as substrate; □, protein.

Fig. 5. Influence of octyl glucoside on total β -glucan synthase activity

A particulate enzyme (11.5 mg/ml) was treated with various amounts of octyl glucoside. For full experimental details see the text. ●, β -Glucan synthase activity with 1 mM-UDP-glucose as substrate; ○, β -glucan synthase activity with 10 μ M-UDP-glucose as substrate.

Table 4. Solubility of octyl glucoside-treated enzymes
For full experimental details see the text. The total protein concentration was 8.8 mg/ml. All values are the means for four replicates.

Concn. of octyl glucoside (% w/v)	Fraction	Glucose incorporated into products insoluble in 66% ethanol (pmol/s)	
		10 μ M-UDP-glucose	1 mM-UDP-glucose
0	100 000 g pellet	0.085	19.5
	100 000 g supernatant	0.010	0.89
0.1	100 000 g pellet	0.089	20.3
	100 000 g supernatant	0.011	0.88
1.0	100 000 g pellet	0.062	47.1
	100 000 g supernatant	0.012	43.5
10	100 000 g pellet	0.006	0.60
	100 000 g supernatant	0.008	0.41

Glucan represented 66% of the synthase products, the remainder being both 1,4- and 1,3:1,4- β -glucans (Table 3). Very little glucan was produced from 10 μ M-UDP-glucose.

Stability of detergent-solubilized β -glucan synthases

The digitonin- and octyl glucoside-solubilized enzymes retained activity when stored at -70°C or for several hours at 4°C . Overnight storage of digitonin-solubilized synthases at 4°C resulted in almost complete loss of activity.

Fig. 6. Hydrolysis of products of octyl glucoside-solubilized β -glucan synthases with 1,3- β -glucan exo-hydrolase from *Euglena gracilis*

The 66%-ethanol-insoluble β -glucans produced from 1 mM-UDP-glucose by a 1%-octyl glucoside-solubilized enzyme were incubated with 1,3- β -glucan exo-hydrolase from *Euglena gracilis* at 60°C for 72 h. The hydrolysate was separated in a column of Bio-Gel P-2 at 60°C , and radioactivity in 1.7 ml fractions was measured by scintillation counting. The elution volume of the peak labelled 1 was characteristic of a monosaccharide on this column. For full experimental details see the text.

Discussion

The results described in the present paper demonstrate that both digitonin- and octyl glucoside-

solubilized preparations from ryegrass membranes produce β -glucans from UDP-glucose. Digitonin, the detergent most widely used to solubilize membrane-bound β -glucan synthases, has been used effectively with preparations from mung bean (*Phaseolus aureus*) (Feingold *et al.*, 1958), white lupin (*Lupinus albus*) (Flowers *et al.*, 1968), wheat (*Triticum vulgare*) (Péaud-Lenoël & Axelos, 1970), oat (*Avena sativa*) (Tsai & Hassid, 1971) and the fungus *Saprolegnia monoica* (Fevre, 1979). However, digitonin did not solubilize enzymes from *Euglena gracilis* (Marechal & Goldemberg, 1964) or pea (*Pisum sativum*) (Ray *et al.*, 1969; Maclachlan *et al.*, 1979). Other detergents have been used less frequently. Heller & Villemez (1972) solubilized enzymes from *Phaseolus aureus* with Triton X-100. In contrast, enzymes from oats (Tsai & Hassid, 1971) and peas (Ray *et al.*, 1969; Maclachlan *et al.*, 1979) were inactivated by this detergent. Deoxycholate solubilized the 1,3- β -glucan synthase from *Euglena gracilis* (Marechal & Goldemberg, 1964), but the detergent had to be removed by dialysis to restore the activity. In the present work no attempt has been made to remove detergents before assay of activity.

In addition to solubilizing synthases, digitonin also stimulated β -glucan synthesis by the total preparation and by the 100 000 g pellet (Tables 1 and 2). A similar finding has been reported by Flowers *et al.* (1968) for synthases from *Phaseolus aureus* and *Lupinus albus*. This stimulation may be related to the inhibition by digitonin of the enzymic glucosylation of sterol acceptors by the ryegrass preparation (Table 2). Digitonin also inhibited steryl glucoside synthesis by preparations from spinach (*Spinacea oleracea*) leaves (Garcia & Mudd, 1980). At low UDP-glucose concentrations the competition between β -glucan synthases and sterol glucosyltransferase for substrate may limit β -glucan synthesis. This competition would be alleviated by digitonin inhibition of sterol glucosylation. In contrast with the inhibiting action of digitonin, other detergents such as Triton X-100 actually stimulate UDP-glucose:sterol glucosyltransferases (Quantin *et al.*, 1980). The enhancement of steryl glucoside synthesis would result in increased competition for UDP-glucose and may be one reason for the depression of β -glucan synthesis observed in the presence of Triton X-100 and other detergents (Table 1).

Poor stability of detergent-solubilized preparations has been reported for a number of systems (Feingold *et al.*, 1958; Stafford & Brummond, 1970; Tsai & Hassid, 1971). However, both the digitonin- and octyl glucoside-solubilized β -glucan synthases from ryegrass retained activity for several hours at 4°C and for much longer at -70°C.

The low β -glucan synthase activity of digitonin-

solubilized enzymes with GDP-glucose as substrate is consistent with the lower activity of the total membrane-bound enzymes with GDP-glucose (Henry & Stone, 1982). However, Chambers & Elbein (1970) found synthase activity in digitonin-treated particles from *Phaseolus aureus* with GDP-glucose as substrate but very little activity with UDP-glucose. Enzymes from *Phaseolus aureus* capable of utilizing GDP-glucose have been solubilized by using digitonin (Liu & Hassid, 1970) and Triton X-100 (Heller & Villemez, 1972), but the product of these synthases may have been a polymer containing both glucose and mannose rather than glucose alone (Heller & Villemez, 1972).

The only previous attempt to fractionate β -glucan synthases was made by Tsai & Hassid (1971). Using a particulate preparation from *Avena sativa* that produced 1,3-, 1,4- and 1,3:1,4- β -glucans, they were able to separate a digitonin-solubilized preparation into fractions that formed glucans enriched in 1,3- β -linkages and others that formed β -glucans enriched in 1,4- β -linkages. The synthesis of 1,3:1,4- β -glucans by the fractions was not reported.

In the experiments reported in the present paper we have been able to measure the amounts of 1,3- β -glucan, 1,4- β -glucan and 1,3:1,4- β -glucan products. The synthesis of 1,4- β -glucans and 1,3:1,4- β -glucans, but not 1,3- β -glucans, by digitonin-solubilized preparations from ryegrass endosperm suggests that the enzymes producing these β -glucans are distinct from the 1,3- β -glucan synthases. The independent synthesis of 1,3- β -glucans was also indicated by the selective stimulation of their formation in preparations solubilized with octyl glucoside. Moreover, these observations are consistent with the pH-dependence of β -glucan synthesis by the ryegrass mixed-membrane system. 1,3- β -Glucan synthesis is optimal at pH 7 or lower, whereas 1,4- and 1,3:1,4- β -glucan synthesis is greatest at pH 8 or higher (Henry & Stone, 1982). Taken together, these results indicate that 1,3- β -glucan synthases are not involved in 1,3:1,4- β -glucan synthesis in the ryegrass system. Although the non-involvement of 1,3- β -glucan synthases in 1,3:1,4- β -glucan synthesis is clear, the role of 1,4- β -glucan synthases has not been evaluated.

Brett & Northcote (1975) and Hopp *et al.* (1978) have proposed the possible participation of glycosylated lipid intermediates in the transfer of glycosyl residues from UDP-glucose to form β -glucans. Such pathways require several enzymes and lipid intermediates, and, if this type of mechanism is involved in the synthesis of the ryegrass 1,3:1,4- β -glucans, digitonin must be dissociating all the components of the pathway from the membrane to give a complex that remains functional after DEAE-Sepharose chromatography. This seems unlikely, and leaves the possibility that the synthase responsible for 1,3:1,4-

β -glucan synthesis transfers glucosyl residues from UDP-glucose directly to the growing glucan chain. This conclusion is further supported by the failure of bacitracin, an inhibitor for dephosphorylation of polyprenol pyrophosphates, to inhibit β -glucan synthesis (Henry & Stone, 1982), and by the absence of radioactive compounds with the properties of polyprenol phosphate sugars in reaction mixtures (R. J. Henry & B. A. Stone, unpublished work).

1,3:1,4- β -Glucan synthesis may be compared with that of hyaluronic acid, an alternating copolymer of 3-substituted β -N-acetylglucosaminyl and 4-substituted β -glucuronyl residues. Hyaluronic acid synthesis apparently involves transfer of glycosyl residues from UDP-glucuronic acid and UDP-N-acetylglucosamine to the growing chain by membrane-bound enzymes without the participation of lipid intermediates (Sugahara *et al.*, 1979). In one sense the synthesis of 1,3:1,4- β -glucans is simpler, since glucose is the sole monomeric sugar involved, but in another sense is more complex, since the 1,3- and 1,4-linkages do not alternate. Unless a template mechanism is involved, the synthase must control the order in which the linkages are generated. One possibility is that the growing polymer itself could determine the sequences. Pattee (1961) has described a model for non-template-directed sequence generation in biological macromolecules that may be extended to 1,3:1,4- β -glucans. In such a model the stereochemical association of the active site of the synthase with the terminal linkage sequence on the nascent β -glucan establishes the identity of the next linkage formed. However, before a detailed mechanism for linkage sequence generation can be proposed, a more precise definition of the arrangement of linkages in the 1,3:1,4- β -glucan products and further characterization of the synthase will be necessary.

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