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REVIEW

Mechanisms involved in the biosynthesis of polysaccharides

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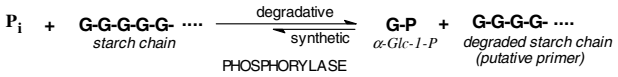
Abstract: The mechanisms for the biosynthesis of three polysaccharides are presented: (i) starch synthesized by starch synthase and adenosine diphospho glucose; (ii) dextran synthesized by *Leuconostoc mesenteroides* B-512FMC dextransucrase and sucrose; and (iii) *Acetobacter xylinum* cellulose synthesized by cellulose synthase, uridine diphospho glucose, and bactoprenol phosphate. All three enzymes were pulsed with substrates, containing ^{14}C-glucose and chased with the same nonlabeled substrates. When the polysaccharides were isolated, reduced, and hydrolyzed, the pulsed reactions gave ^{14}C-glucitol, which was significantly decreased in the chase reaction. These experiments definitively show that all three polysaccharides are biosynthesized by the addition of glucose to the reducing-ends of the growing polysaccharides and not by the addition to the nonreducing-ends of primers. Additional evidence indicates that glucose and the polysaccharides are covalently attached to the active-sites of the enzymes. A two catalytic-site insertion mechanism at one active-site is proposed for the biosyntheses. Two of the polysaccharides are α-linked glucans, starch and dextran, and cellulose is a β-linked glucan, known for several years to require a bactoprenol lipid phosphate intermediate. It is shown how this intermediate is involved in determining that β-linkages are synthesized. Other β-linked polysaccharides: bacterial cell wall peptidomurein, *Salmonella* O-antigen polysaccharide, and *Xanthanomonas camprestris* xanthan, are heteropolysaccharides, with the later two also being hetero-linked polysaccharides, with the β-linkage at the reducing-end of the repeating unit. All three require bactoprenol lipid phosphate intermediates and are biosynthesized by the addition of the repeating units to the reducing-end of a growing polysaccharide chain, with the formation of a β-linkage.

Key words: starch; dextran; cellulose; starch synthase; dextransucrase; cellulose synthase; reducing-end synthesis.

Abbreviations: ADPGlc, adenosine diphospho glucose; CP, conversion period, which is the theoretical time necessary to convert the substrate into product for the amount of enzyme present; d.p., degree of polymerization; α-Glc-1-P, α-D-glucose-1-phosphate; P_i, inorganic phosphate; UDPGlc, uridine diphospho glucose.

Mechanism of the biosynthesis of starch chains in starch granules by starch synthase

The first postulated biosynthesis of polysaccharides was reported by Cori & Cori (1939) studying the reaction of glycogen phosphorylase, with which they found that starting with α-D-glucose-1-phosphate (α-Glc-1-P), the glucose was transferred to the nonreducing ends of the glycogen molecule. Shortly thereafter Hanes (1940) reported a similar reaction for potato phosphorylase, which also transferred glucose from α-Glc-1-P to the nonreducing-ends of amylose and amylopectin. Phosphorylases had previously been known to react with glycogen and starch and inorganic phosphate (P_i) to remove glucose from the nonreducing-ends of the chains to give α-Glc-1-P. Phosphorylases were then recognized to catalyze these reactions with equilibrium constants close to 1, but with the equilibrium being somewhat more favorable for degradation, than for synthesis (Swanson & Cori 1948). The reaction was formulated for starch chains as the following:



It was found that starting with α-Glc-1-P and a starch chain, the reaction rapidly slowed down as the concentration of P_i increased. It was further found that the synthetic reaction did not occur *in vivo*, as the concentration of P_i in plant and animal tissues was 20- to 40-fold higher than the concentration of α-Glc-1-P (Trevelyan et al. 1952; Ewart et al. 1954; Liu & Shannon 1981), and the *in vivo* conditions for phosphorylase greatly favored degradation, rather than synthesis. It was from the Cori & Cori (1939), Hanes (1940) and Swanson & Cori (1948) studies with phosphorylase, however, that the concept of a *required primer* for the biosynthesis of polysaccharides was formulated and it has remained, with a paucity of experimental evidence, in the minds of many people, since then and even today (Leloir et al. 1961; Bocca et al. 1997; Ball et al. 1998; Tomlinson & Denyer 2003; Ball & Morell 2003; Moulis et al. 2006).

Table 1. ^{14}C-glucitol obtained from a 30 min pulse of starch granules with ADP-[^{14}C]Glc and chase with ADPGlc for 120 min.[a]

Starches	Starch synthase activity in the starch granules (nmol Glc/h/100 mg of starch granules)	Pulse ^{14}C-glucitol (counts)[b,c]	Chase ^{14}C-glucitol (counts)[b,c]	Number avg. d.p[d] of pulsed starches	Number avg. MW[d] of pulsed starches
Maize	272	5240	2580	827	133,992
Waxy maize	160	3480	2180	436	70,650
Taro	184	3050	520	462	74,862
Rice	144	2280	1300	467	75,672
Wheat	202	1750	1040	476	77,130
Potato	450	4240	2250	524	84,906
Barley	126	1960	300	127	20,592
Rye	122	500	160	441	71,460

[a] Data taken from Mukerjea et al. (2002).
[b] The different starches were pulsed and chased for a minimum of 2–3 times and a maximum of 5 times.
[c] Each sample was counted in a liquid scintillation spectrometer for 10 min or 10,000 counts, whichever came first, and the background subtracted.
[d] d.p. = number average degree of polymerization = total cpm ÷ cpm of glucitol. Number average MW = (d.p. × 162) + 18.

In the 1960's, some 20 years after the phosphorylase experiments, it was found that adenosine diphospho glucose (ADPGlc) was the high-energy donor of glucose for the biosynthesis of starch chains, rather than α-Glc-1-P, and that the active starch synthesizing enzymes, starch synthase and starch branching enzyme, were entrapped inside the starch granules (De Fekete et al. 1960; Leloir et al. 1961; Recondo et al. 1961). When ADP-[^{14}C]Glc was incubated with starch granules, ^{14}C-glucose was incorporated into starch. When this labeled starch was reacted with the exo-acting enzyme, β-amylase, ^{14}C-labeled maltose was obtained. This experiment has been widely considered proof that starch chains are biosynthesized by the addition of glucose from ADPGlc to the nonreducing-ends of starch primers. This assumption, however, is not necessarily correct in that if starch chains were being synthesized *de novo* from the reducing-end rather than from the nonreducing-end of a primer, the synthesized chains would have every glucose residue in the chains labeled, and the subsequent reaction with β-amylase would also give ^{14}C-labeled maltose.

Because of this and the lack of experiments showing that starch chains were actually synthesized by the addition of glucose to the nonreducing-ends of the putative primers, a pulse reaction with ADP-[^{14}C]Glc and a chase reaction with nonlabeled ADPGlc were carried out with eight different kinds of starch granules. After the starches were solubilized, reduced with $NaBH_4$, and completely hydrolyzed by glucoamylase, the pulsed starches gave ^{14}C-glucitol and the chased starches gave a significant decrease in ^{14}C-glucitol (Table 1). These experiments definitely showed that glucose was being added to the reducing-ends of the growing starch chains, as it would have been impossible to obtain and chase ^{14}C-glucitol if the glucose was being added to the nonreducing-ends of primers (Mukerjea et al. 2002). In this same study, experiments also showed that a glucose and a starch chain were covalently linked to the active-site of starch synthase, during chain elongation. Further, it was shown that a significant size of starch chains was synthesized in the pulse reaction for the eight starches (Table 1) and both ^{14}C-amylose and ^{14}C-amylopectin were formed, something that had never been shown for any of the primer studies that have been conducted to date.

When putative primers, maltose, maltotriose, and maltopentaose were added with ADPGlc to either granule bound or soluble starch synthase, it was found that the major product formed was the next higher maltodextrin homologue. For example, maltose gave maltotriose, maltotriose gave maltotetraose, and maltopentaose gave maltohexaose. In another study, higher homologues beyond the first homologue were produced up to degree of polymerization (d.p.) 6, but in much lower, exponentially decreasing amounts (Denyer et al. 1999).

Mukerjea & Robyt (2005) examined the effect of adding the putative primers (maltose, maltotriose, and d.p. 12 amylodextrin) in a second experiment to three kinds of starch granules from maize, wheat, and rice, with ADPGlc. The reactions were examined in the absence and presence of increasing concentrations of the three putative primers. All of the added putative primers were found to inhibit starch synthesis; the inhibition increased, as the concentrations of the putative primers were increased (see, Figure 1 for the inhibition of maize starch by maltose and maltotriose), contrary to what would be expected for the synthesis of starch by the addition of glucose to primers. The putative primers did undergo a reaction to primarily form the next higher homologue, similar to what previously had been observed by Damager et al. (2001).

Recently, we have isolated, stabilized, and purified potato soluble starch synthase and potato soluble starch branching enzyme, free of any carbohydrate primers (R. Mukerjea & J.F. Robyt, unpublished, results on the purification and characterization of potato starch synthesizing enzymes). Soluble starch synthase synthesized an amylose chain from ADPGlc and the addition of soluble branching enzyme to soluble starch

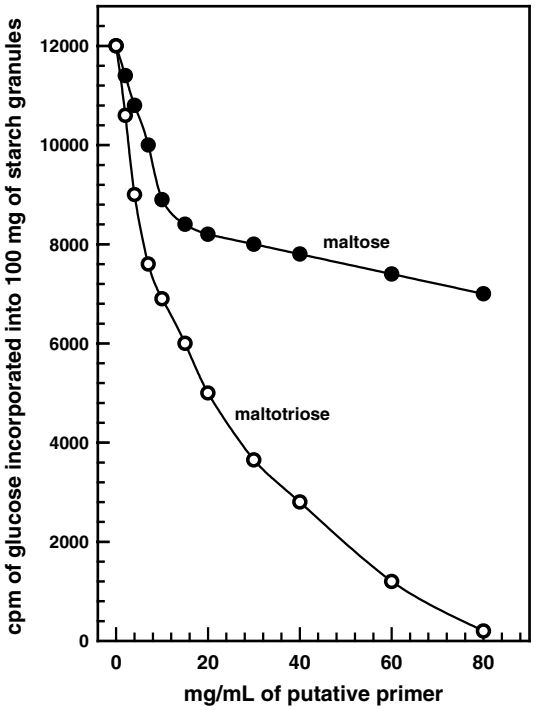


Fig. 1. Inhibition of maize starch synthesis by the putative primers, maltose and maltotriose.

synthase gave an α-(1→6) branched, amylopectin component, further indicating that primers are not involved in the biosynthesis of starch. From the results of these experiments, it was proposed that glucose from ADPGlc is added to the reducing-ends of the growing starch chains by the two-catalytic-site, insertion mechanism, at a single active-site (Fig. 2).

Mechanism of dextran biosynthesis by *Leuconostoc mesenteroides* B-512FMC dextransucrase

The mechanism for the biosynthesis of dextran by *L. mesenteroides* B-512F dextransucrase was first reported by Robyt et al. (1974), using Bio-Gel P2 immobilized-dextransucrase and a pulse reaction with ^{14}C-sucrose and a chase reaction with nonlabeled sucrose. ^{14}C-glucitol was obtained from the pulsed reaction and it was decreased 100-fold in the chase reaction. Recently, Robyt et al. (2008) reinvestigated the dextransucrase reaction, again using pulse and chase experiments, but with soluble B-512FMC dextransucrase. Two volumes of ethanol at 0℃ were added to the pulse and chase reactions to stop the reaction and precipitate the dextrans. The precipitated dextrans were washed 5-times with 67% ethanol to remove any unreacted ^{14}C-sucrose, and ^{14}C-fructose, -glucose, and -isomaltose that might be present. The washed dextrans were treated with dry acetone 5-times and once with dry, absolute ethanol to remove the last traces of water. A vacuum was pulled on the precipitates for 15 min to remove traces of acetone and ethanol. Equal amounts (10 mg) of the dried dextrans were dissolved in water and reduced with $NaBH_4$. Unreacted $NaBH_4$ was destroyed with 4 M TFA and the dextrans were completely hydrolyzed by 4 M TFA for 30 min at 121℃ in an autoclave. Both ^{14}C-glucitol and -glucose were obtained for both pulsed and chased dextrans and were separated by descending paper chromatography. The pulse reaction gave 3,270 dpm ^{14}C-glucitol and the chase reaction gave 1,670 dpm ^{14}C-glucitol. These experiments definitively show that glucose was being added to the reducing ends of the dextran chains. If glucose had been added to the nonreducing-ends of primers (glucose or isomaltodex-

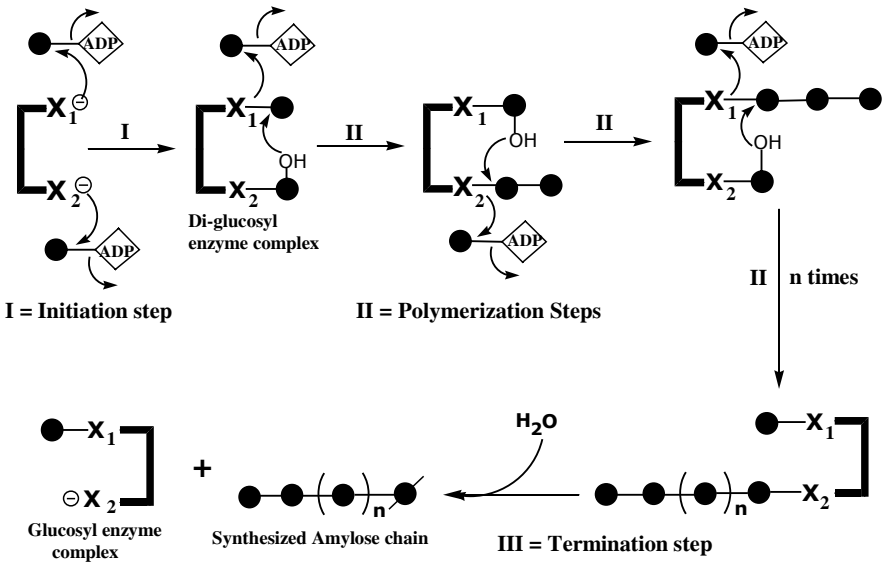


Fig. 2. Mechanism for the biosynthesis of starch chains by the addition of glucose to the reducing-ends in a two catalytic-site, insertion mechanism in which X_1 and X_2 are the catalytic groups at the active-site, analogous to the carboxyl groups of Asp551 and Asp622 at the active-site of dextransucrases.

trins), it would have been impossible to obtain ^{14}C-glucitol in a pulse reaction and to decrease it with non-labeled sucrose in a chase reaction. Similar results were also obtained for *Streptococcus mutans* dextransucrase and mutansucrase that synthesized dextran and mutan, respectively (Robyt & Martin 1983). Mutan is an α-(1→3) linked glucan that is very water insoluble. Diston & Mayer (1984) also found that *Streptococcus sangius* dextransucrase synthesized dextran by adding glucose to the reducing-end of dextran.

When the pulsed B-512F immobilized-dextransucrase was incubated with glucose, two products were formed and released from the immobilized-enzyme: a low molecular weight product, isomaltose, and a high molecular weight product, dextran (Robyt & Walseth 1978). This experiment showed that high-energy glucose and dextran were covalently attached to the active-site of dextransucrase, during the synthesis of dextran and were released by the reaction with glucose. Parnaik et al. (1983) also found that both glucose and dextran were covalently attached to *S. sangius* dextransucrase.

The two-site insertion mechanism was first proposed for the synthesis of dextran by dextransucrase in a review by Ebert & Schenk (1968), although without supporting experimental evidence. Experimental evidence and further elaboration of the two catalytic-site, insertion mechanism was provided by Robyt et al. (1974) and Robyt & Walseth (1978) and has been confirmed by Robyt et al. (2008).

Dextransucrase has been known for over 50 years to also catalyze secondary, transglycosylation reactions in which glucose is transferred primarily to the C6-OH at the nonreducing-ends of low molecular weight carbohydrates, mono-, di-, and tri-saccharides present or added to the sucrose digest (Robyt & Walseth 1978; Robyt & Eklund 1983; Robyt 1995) and also to other non-carbohydrate molecules having primary and secondary alcohol groups (Yoon et al. 2002). Maltose, isomaltose, and glucose, among 30 or more carbohydrates are known to accept glucose at the active-site of glucansucrases to give a series of isomaltodextrin "acceptor products" in decreasing amounts, as the size of the products increase (Robyt & Eklund 1983; Fu & Robyt 1990, 1991; Su & Robyt 1993). The major product is the first oligosaccharide homologue: e.g., isomaltose from glucose, isomaltotriose from isomaltose, panose from maltose, etc. Two carbohydrate acceptors gave only one product: fructose gave the disaccharide, leucrose, and lactose gave a trisaccharide, with glucose attached to the C2-OH of the reducing-end, glucose moiety. Cellobiose, an analogue of lactose, gave a series of isomaltodextrins attached to the C2-OH of the reducing-end glucose moiety.

Moulis et al. (2006) recently rejected the two-site insertion mechanism for B-512F dextransucrase. Using a C- and N-terminal truncated B-512F dextransucrase that was cloned in *E. coli*, their putative evidence was primarily based on a high-pressure liquid chromatography-pulse amperometric detection analysis of the products at the end of the reaction in which isomaltose and isomaltotriose were the major low molecular weight products, along with exponentially declining amounts of higher oligosaccharides down to miniscule amounts of d.p. 20–25 and nothing larger. They also gave some hypothetical arguments against the two-site, insertion mechanism. They proposed that dextransucrase first hydrolyzes sucrose, giving glucose and fructose, a minor reaction of dextransucrase known for many years. They then proposed that dextransucrase uses both glucose and sucrose, as initiator primers for the elongation of dextran, which continues by the addition of glucose to the nonreducing-ends of the isomaltodextrin chain.

Robyt et al. (2008) in a kinetic study of the B-512FMC dextransucrase synthesis of dextran analyzed the products of d.p. 2–100 by fluorescence assisted capillary electrophoresis (O'Shea et al. 1998; Yoon & Robyt 2002). The primary low molecular weight products at any one of the reaction times were glucose, fructose, and leucrose. In the early stages of the reaction, 0.20 conversion period (CP), a relatively small amount of isomaltose was formed, along with exponentially decreasing amounts of isomaltodextrins, down to a minuscule amount of isomaltotetraose; at 0.5 CP, the major isomaltodextrin products was isomaltose, with exponentially decreasing amounts of isomaltodextrin homologues down to a miniscule amount of isomaltohexaose; at 1.00 CP, the major isomaltodextrin product was still isomaltose, with exponentially decreasing amounts of isomaltodextrins down to d.p. 13; at 2.00 CP, the major isomaltodextrin product was still isomaltose, with exponentially decreasing amounts from d.p. 3 down to a miniscule amount of d.p. 24. Analysis was carried out to d.p. 100, but nothing larger than d.p. 24 was observed. The number average molecular weights of the dextrans synthesized at the four CP were 172,000 ± 1,500, 178,000 ± 2,000, 239,000 ± 3,500, and 240,000 ± 3,500, respectively. This analysis definitively shows that high molecular weight dextran is not formed by the primer, nonreducing-end mechanism. The formation of low molecular weight isomaltodextrin products are by the secondary acceptor reactions of glucose and isomaltodextrins and do not contribute to the synthesis of dextran, as claimed by Moulis et al. (2006). If they had, the opposite result of exponentially increasing amounts of isomaltodextrins, as a function of the conversion of sucrose, ending with high molecular weight dextran, should have been observed. An additional experiment, the addition of ^{14}C-labeled glucose to a dextransucrase-sucrose digest only gave ≈ 0.02% of the glucose incorporated into the dextran, indicating that it was not acting as a primer and was acting as a weak acceptor to release covalently-linked dextran from the active-site of dextransucrase. Also treatment of B-512FMC dextransucrase-synthesized dextran with 0.01 M HCl and also with invertase did not produce any fructose, which would have been expected, if sucrose was a primer for the synthesis of dextran (Robyt et al. 2008). These experiments definitively show that glucose and sucrose are not initiator primers and that

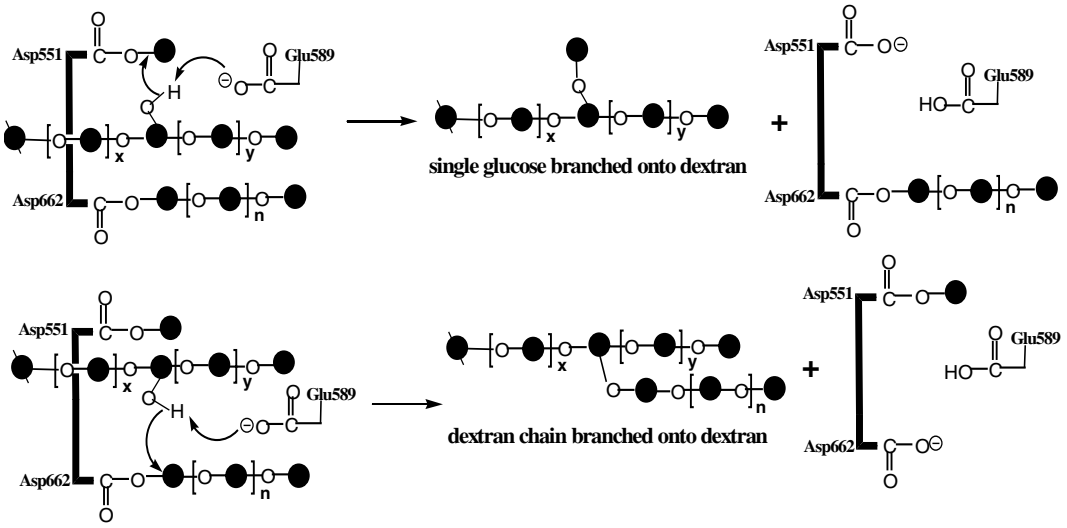


Fig. 3. Biosynthesis of the branch linkages of dextran by dextransucrase acceptor reactions with exogenous dextran chains, reacting with glucose units and/or dextran chains covalently attached to the two catalytic-groups at the active-site of B-512FMC dextransucrase, showing the involvement of the three conserved amino acids (Asp551, Asp622, and Glu589).

isomaltodextrins also are not primers for the synthesis of dextran by dextransucrase.

Moulis et al. (2006) also stated that dextransucrase can only have one active-site for the synthesis of dextran, because there is only one set of the three conserved amino acids (Asp551, Glu589, and Asp622) found at the active-sites of all glycoside hydrolase family 70 enzymes, including glucansucrases, and therefore the synthesis of dextran cannot involve two active-sites, as proposed for the two-site insertion mechanism. Two active-sites, however, was never proposed for the two-site, insertion mechanism. What was proposed was that there were two catalytic groups with two sucrose binding-sites that were involved in the insertion mechanism at one active-site for the synthesis of dextran. This really is not particularly unusual, as there are many enzymes that have two or more sub-sites (catalytic sites and/or substrate binding sites) at one active-site where catalysis occurs. Robyt et al. (2008) now have shown how the three conserved amino acids at one active-site can participate in the two catalytic-site, insertion mechanism. In the reaction, Asp551 and Asp622 act as the two catalytic groups that form covalent intermediates with glucose and dextran, and Glu589 acts as an acid-base catalyst, facilitating the transfer of protons to give the formation of the covalent linkages with the carboxylate groups and the insertion of glucose between the catalytic carboxyl group and the dextran chain, with the formation of the α-(1→6) linkage to the dextran chain. The two catalytic-site, insertion mechanism has been further confirmed (Su & Robyt 1994) by an equilibrium dialysis study with the sucrose analogue, 6-deoxy-sucrose, in which two sucrose binding sites and one acceptor binding site was found for a single dextransucrase molecule.

Robyt et al. (2008) further show that the molecular size of dextran is inversely proportional to the concentration of the enzyme. This indicated that the elongation of dextran is a highly processive reaction in which glucose is rapidly added to the reducing-end of the covalently linked, growing dextran chain. The dextran chain is extruded from the active-site of the enzyme, until it is released by an acceptor reaction with water or a carbohydrate acceptor, such as glucose, isomaltose, or an exogenous dextran chain (see, below for the branching of dextran). From these experiments, Robyt et al. (2008) proposed that the dextran chains are synthesized by the two catalytic-site, insertion mechanism and that the synthesis does not occur by the nonreducing-end, primer mechanism proposed by Moulis et al. (2006).

Robyt & Taniguchi (1976) showed that dextransucrase catalyzes the formation of branch linkages by an acceptor reaction in which released exogenous dextran chains act as acceptors, attacking either the covalently linked glucose to give a single glucose α-(1→3) branch or attacking the covalently linked dextran at the active-site of dextransucrase to give a long dextran chain, attached by an α-(1→3) branch linkage (see Figure 3 for the mechanism), unlike that of starch biosynthesis, which utilizes a separate starch branching enzyme to form α-(1→6) branch linkages.

In summary, the mechanism for the biosynthesis of α-(1→6) linked dextran chains and the biosynthesis of the α-(1→4) linked starch chains are very similar, involving the addition of glucose to the reducing-end of the growing glucan chains, which are covalently attached to the active-site of the synthesizing enzymes, utilizing a two catalytic-site, insertion mechanism at a single active-site (Fig. 2).

Mechanism for the biosynthesis of *Acetobacter xylinum* (bacterial) cellulose

Cellulose is an abundant naturally occurring polysaccharide making up approximately 50% of all plant cell walls. It is also produced by a few species of bacteria that synthesize relatively pure cellulose as an extracel-

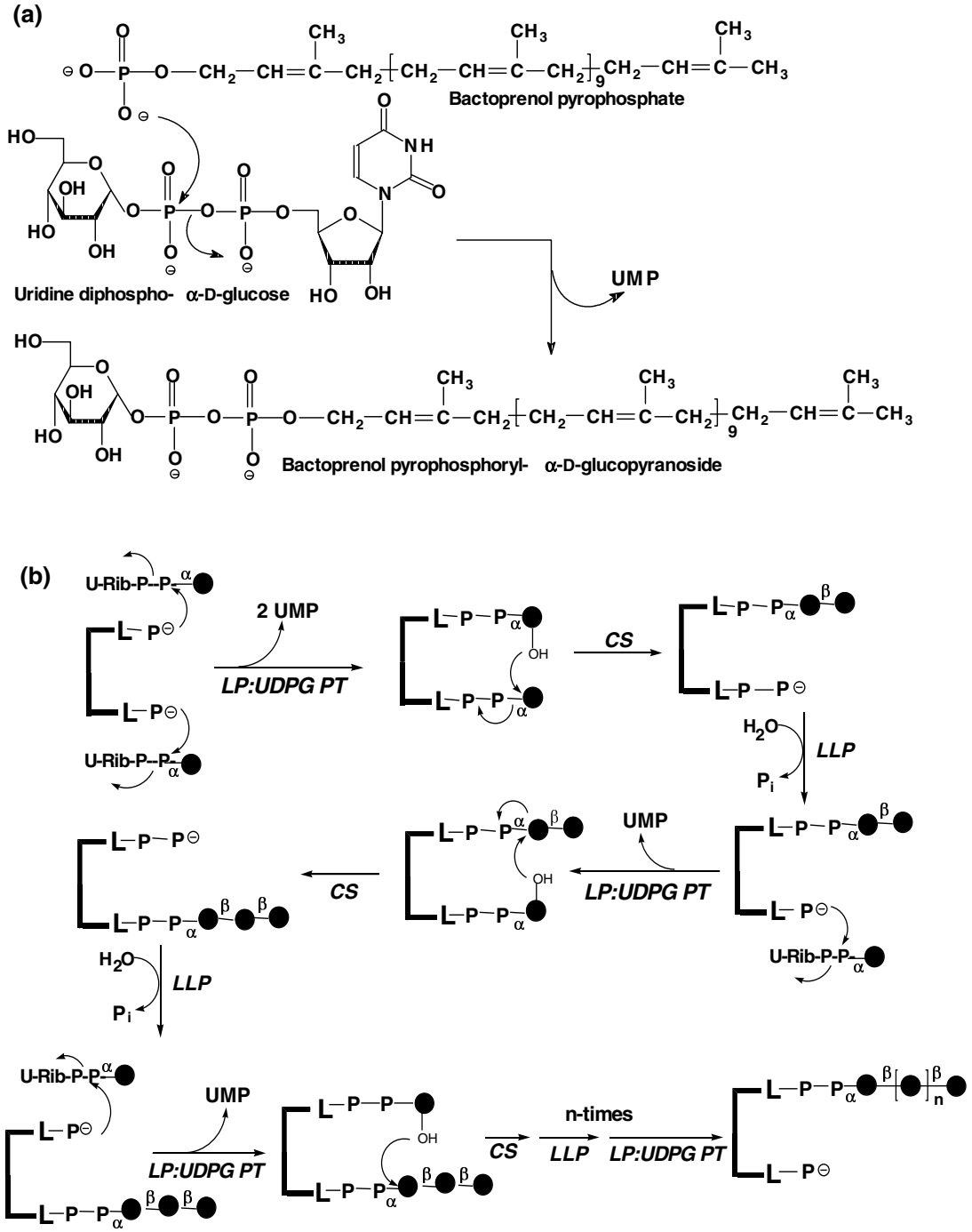


Fig. 4. Mechanism for the biosynthesis of cellulose by cellulose synthase embedded in a membrane. (a) Reaction of UDPGlc with bactoprenol phosphate to give bactoprenyl pyrophosphate α-D-glucose, the high-energy glucose species. (b) Biosynthesis of the cellulose chain by reaction of bactoprenyl pyrophosphoryl-α-D-glucose transfer of glucose to the reducing-end of a growing cellulose chain and the formation of β-1,4 linkages. L = bactoprenyl lipid, P = phosphoryl, Rib = D-ribose, U = uridine, ● = glucopyranose units, *LP:UDPG PT* = lipid phosphate: UDPGlc phosphotransferase, *CS* = cellulose synthase, and *LPP* = lipid pyrophosphate phosphatase.

lular product that is extruded from the surface of the cell (Haigler 1991).

Several strains of *Acetobacter xylinum* synthesize cellulose from uridine diphospho glucose (UDPGlc) by the enzyme, cellulose synthase. Sequence analysis of the enzyme indicates that it is an anchored membrane protein (Saxena et al. 1990). Efforts to study the biosynthesis of cellulose in plants have not been successful, due to the inability to obtain active cellulose synthase and demonstrate the synthesis *in vitro*. It has been proposed that the cellulose chain is elongated from the reducing-end (Saxena et al. 1995). This was based on deductions made from a comparative study of the sequence of several different polysaccharide synthesizing and hydrolyzing enzymes; direct experimental evidence was not presented. A few years later (Koyama et al. 1997) proposed that *A. xylinum* cellulose was synthesized by the addition of glucose from UDPGlc to the nonreducing ends of

the cellulose chains. This was based on the silver staining of the reducing-ends of the cellulose chains and the microdiffusion-tilting electron crystallographic analysis of the cellulose fibers. The evidence here also was not very convincing as it was sketchy, indirect, nonquantitative, and arrived at primarily from reasoning by analogy. To resolve these two opposing positions, the *de novo* synthesis of cellulose by resting *A. xylinum*-cells and *A. xylinum*-membrane preparations was studied by Han & Robyt (1998), using UDP-[^{14}C]Glc pulse and UDPGlc chase reactions. It was found that the synthesized cellulose was tightly associated extra-cellularly with the membrane of the bacterial cells. The cellulose chains could be released from the cells and the membrane preparation by treating them at pH 2, 100℃ for 20 min. The cellulose chains that were released from pulse and chase reactions were purified and separated from low molecular weight compounds by gel chromatography on Bio-Gel P4 (fine). The pulsed and chased products from the resting cells, after reduction and acid hydrolysis gave a ratio of ^{14}C-glucitol to ^{14}C-glucose of 1:11, and after chasing, the ratio decreased to 1:36. The pulsed cellulose from the membrane gave a ratio of ^{14}C-glucitol of 1:12 and after chasing, a ratio of 1:43. These results resolved the conflict, as ^{14}C-glucitol could only be obtained by the addition of ^{14}C-glucose to the reducing-ends of cellulose and ^{14}C-glucose could only be chased into the cellulose chains by nonlabeled UDPGlc, if cellulose was being elongated by the addition of glucose to the reducing-end. If cellulose had been added to primers and elongated by addition to the nonreducing ends, no ^{14}C-glucitol could be formed from UDP-[^{14}C]Glc.

Evidence for the involvement of a lipid pyrophosphate in the biosynthesis of cellulose by *A. xylinum* had been found early (Colvin 1959; Garcia et al. 1974; Cooper & St. John Manley 1975; Swissa et al. 1980). The lipid pyrophosphate was found to be an absolutely required component. It was determined to be a polyisoprenyl alcohol (bactoprenol), containing 55 carbons with a pyrophosphate ester linkage to the alcohol group with the pyrophosphate other end attached to glucose (Fig. 4a).

The mechanism proposed for bacterial cellulose biosynthesis by Han & Robyt (1998) involves three enzyme catalyzed reactions: (a) the first reaction is catalyzed by *Lipid pyrophosphate: UDPGlc phosphotransferase* (*LP: UDPGlc-PT*) that transfers Glc-1-P from UDPGlc to bactoprenol phosphate to give bactoprenol pyrophosphate glucose; (b) the second reaction is catalyzed by *cellulose synthase* (*CS*) that produces the polymerization of the glucose residues by a two-catalytic site insertion mechanism, leaving bactoprenol pyrophosphates; and (c) the third reaction is catalyzed by *lipid pyrophosphate pyrophosphatase* (*LPP*) and gives hydrolysis of pyrophosphate to give bactoprenol phosphate that can again attack UDPGlc, giving bactoprenol pyrophosphate glucose that continues to add glucose to a growing cellulose chain, very similar to the syntheses of starch and dextran (Fig. 4b).

Initially, it might be thought that the lipid phosphate intermediate is not necessarily required for the synthesis and that the glucosyl unit and the growing cellulose chain could be directly attached to the cellulose synthase, like starch and dextran are attached to starch synthase and dextransucrase. This kind of attachment, however, would give the glucosyl residue attached to the active-site in the β-configuration. The subsequent reactions of this glucosyl intermediate would give the addition of the glucosyl group to the growing polymer, but the glycosidic linkage would be alpha to give an α-glucan instead of a β-glucan, cellulose chain. The formation of the lipid pyrophosphate glucosyl intermediate has the glucose attached to the pyrophosphate in the α-configuration because of the way it is formed from UDPGlc (Fig. 4a) and then when it reacts with the lipid-phosphate, the α-configuration is retained. But, in the synthesis of the glucan, the α-configuration is inverted to give a β-configuration in the synthesized polysaccharide. The lipid-phosphate and lipid-pyrophosphate also play another role in that they bind to the enzyme/protein at a hydrophobic site at the active-site of cellulose synthase. Glucose is added from UDPGlc to the lipid phosphate inside the cell and then is enveloped by the lipid and carried through the lipid bi-layer membrane to the outside of the cell, where the lipid unfolds and allows glucose to be added to the growing cellulose chain (see, Figure 4b for the mechanism for cellulose biosynthesis). The proposed reducing-end, insertion mechanism for cellulose, thus, also has no need for preformed oligosaccharide- or polysaccharide-primers.

The requirements for a polyisoprenyl phosphate lipid and the three distinct enzymes for the biosynthesis of cellulose explain why the purification of a single cellulose synthesizing enzyme, has met with failure. The synthesis of plant cell wall cellulose, however, most probably follows a similar path as bacterial cellulose, with possibly a slightly different lipid polyisoprenoid phosphate, such as dolichol phosphate, as the covalent intermediate. The biosynthesis of *A. xylinum* cellulose joins several other β-linked polysaccharides that require bactoprenol phosphate and pyrophosphate intermediates and biosynthesize their polysaccharides by the addition of an activated monomer or repeating unit to the reducing-end of a growing polysaccharide chain. Bactoprenol phosphate was also found to be a required cofactor for the synthesis of *Salmonella* O-antigen polysaccharide, peptidomurein bacterial cell wall polysaccharide, and xanthan *Salmonella* O-antigen polysaccharide. Bray & Robbins (1967) was the first to show a polysaccharide to be synthesized from the reducing-end. Five years later, the bacterial cell wall, peptidomurein (Ward & Perkins 1973) and dextran (Robyt et al. 1974) were also shown to be synthesized from the reducing-end. Almost 20 years after that, the mechanism for the biosynthesis of the cellulose analogue, xanthan, was reported (Ilepi et al. 1993) and then cellulose (Han & Robyt 1998) to occur by the addition of the repeating- or monomer-unit, respec-

tively, to the reducing-end. Bacterial cell wall peptidomurein, *Salmonella* O-antigen polysaccharide, and xanthan are all β-linked heteropolysaccharides, with the latter two also being heterolinked polysaccharides with the β-linkage at the reducing-end of the repeating unit. From these studies on relative diverse polysaccharides, it would appear that the primary mechanism for the biosynthesis of polysaccharides is the two catalytic-site, insertion mechanism in which the monomer or repeating unit is added to the reducing-end of a growing polysaccharide chain, all of which are covalently attached at the active-site of their synthetic enzymes.

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