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Biosynthesis of Oligosaccharides and Polysaccharides in Plants

Mechanisms of enzymic synthesis of complex plant carbohydrates are reviewed.

W. Z. Hassid

Plants are the chief producers of carbohydrates in nature by the process of photosynthesis. Most forms of life which are unable to photosynthesize depend either directly or indirectly on the assimilation of carbon dioxide by plants. All the organic substances which arise from photosynthetic processes serve the other forms of life as starting materials for diverse metabolic functions. While there are many ways in which organic substances are decomposed, there is only one reaction, photosynthesis, which for millions of years has counterbalanced death and decomposition.

Monosaccharides are synthesized by green plants, starting with a carboxylation reaction in which D-ribulose-1,5-diphosphate serves as the acceptor of CO_2 for the formation of phosphoglyceric acid (*1*). By a subsequent series of enzymic reactions, a number of phosphorylated monosaccharide derivatives are produced in the photosynthetic carbon dioxide cycle. Some of these phosphorylated sugars, such as D-glucose-6-phosphate and D-fructose-6-phosphate, are hydrolyzed to free sugars, causing in some cases the accumulation of large concentrations of D-glucose and D-fructose in plants.

The phosphorylated monosaccharides produced in the photosynthetic carbon dioxide cycle are partially consumed in respiration with the production of energy which is utilized for the numerous metabolic reactions of the plants. They are also converted by a series of enzymic reactions to sugar nucleotides, chiefly UDP-D-glucose (*2*), and to other sugar nucleotides, such as UDP-D-galactose, GDP-D-glucose, and ADP-D-glucose (*3*). The sugar moieties of these nucleotides are interconverted by various specific epimerases, and serve as donors for the formation of the numerous oligosaccharides and polysaccharides (*4*).

A monosaccharide must be activated to enable the enzyme to transfer it to an acceptor for the synthesis of an oligosaccharide or to lengthen the chain by subsequent transfers for the formation of a polymer. From the thermodynamic point of view, nucleoside diphosphate sugars are superior donors for complex saccharide formation, because they have the higher negative free energy of hydrolysis ($\Delta F°$) than other glycosyl compounds (*5*). Uridine diphosphate-D-glucose has a relatively high negative $\Delta F°$ of hydrolysis of -7600 calories; although it has never been determined for other sugar nucleotides, it is assumed that the nucleoside diphosphate sugars, containing bases other than uracil or sugars other than D-glucose, have approximately the same $\Delta F°$ values. The most important reaction for complex saccharide formation appears to involve sugar nucleotides.

Oligosaccharides

Sucrose, the most abundant oligosaccharide in higher plants, was first synthesized in vitro by a bacterial enzyme obtained from *Pseudomonas saccharophila* from α-D-glucose-1-phosphate and D-fructose (*6*). However, in this reaction the equilibrium favors the breakdown rather than the synthesis of sucrose. Various attempts by a number of investigators to synthesize sucrose by an enzyme from a plant source from α-D-glucose-1-phosphate and D-fructose failed. Leloir and his collaborators (*7*) later found that the donor of D-glucose for sucrose formation was not α-D-glucose-1-phosphate but the sugar nucleotide UDP-D-glucose. The synthesis takes place by two separate enzymes, one utilizing D-fructose and another D-fructose-6-phosphate as the acceptors according to the following two reactions

$$\text{UDP-D-glucose} + \text{D-fructose} \rightleftharpoons \text{sucrose} + \text{UDP} \quad (1)$$

and

$$\text{UDP-D-glucose} + \text{D-fructose-6-phosphate} \rightleftharpoons \text{sucrose phosphate} + \text{UDP} \quad (2)$$

The sucrose phosphate formed in reaction 2 is hydrolyzed by a phosphatase, resulting in the formation of free sucrose.

Since $\Delta F°$ for hydrolysis of UDP-D-glucose is about -7500 calories per mole, formation of the glycosyl bond of sucrose is favored. For most glycosides, synthesis from a nucleotide sugar precursor would proceed with a favorable free-energy change of about

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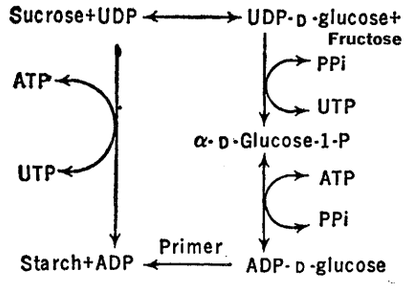

Fig. 1. Reactions involved in the transfer of sucrose to starch.

— 3500 calories per mole. In the case of sucrose synthesis, the formation of sucrose phosphate by reaction 2 is favored by only about —1000 calories, but subsequent hydrolysis of the phosphate group renders this reaction practically irreversible, which would account for the accumulation of sucrose in many plants.

Sucrose phosphate is not readily obtainable in plant tissues. However, several investigators found that, when isolated leaf chloroplasts from different plants were used as an enzyme source with UDP-D-glucose and D-fructose-6-phosphate as substrates, sucrose-6-phosphate was formed (*8*). These as well as other results (*9*) suggest that sucrose-6-phosphate is synthesized first and then hydrolyzed by phosphatase to free sucrose.

Raffinose [*O*-α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranosyl-β-D-fructofuranoside] is found in comparatively low concentration together with sucrose in many higher plants. The D-galactose in this oligosaccharide is attached through an α-glycosyl linkage to the C-6 position of the D-glucose moiety of sucrose. However, attempts to effect a transfer of the D-galactosyl moiety of UDP-D-galactose to sucrose for the formation of raffinose with enzymic preparations from germinated mung bean seedlings were not successful. If raffinose were formed, the germinated seedlings most probably contained glycosidases which hydrolyzed this oligosaccharide.

Bourne *et al.* (*10*) found that raffinose could be synthesized by an enzyme preparation from dormant broad bean (*Vicia faba*) seeds with a mixture of sucrose, α-D-galactose-1-phosphate, and UTP as substrates. This trisaccharide was subsequently synthesized by a direct transfer of the D-galactosyl moiety from UDP-D-galactose-^{14}C to sucrose with an enzyme preparation from mature broad beans (*11*). It appears that ungerminated seeds contain fewer hydrolytic enzymes than the germinated ones, which accounts for the successful synthesis of raffinose.

The synthesis of raffinose and planteose [*O*-α-D-galactopyranosyl-(1 → 6)-*O*-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside] which has been found in the weed *Plantago* and in seeds of *P. ovada*, using D-galactose as the donor in the presence of α-glactosidase preparations, has been demonstrated (*12*).

Although the equilibrium of glycosidase-catalyzed reactions favors hydrolysis, in vivo factors, such as localized high substrate concentrations or rapid utilization of products, may induce oligosaccharide formation. The in vivo synthesis of some complex saccharides from low-energy donors, therefore, should not be entirely overlooked.

Stachyose is a tetrasaccharide, the structure of which is that of raffinose containing an additional D-galactose unit attached through C-1 to C-6 of the terminal D-galactose unit. It would therefore be expected that, analogous to the process of raffinose formation from sucrose, stachyose should have been formed from raffinose and UDP-D-galactose by a transfer reaction. However, attempts to show a transfer of D-galactose to raffinose that would result in stachyose formation could not be achieved. The donor of D-galactose for the formation of this tetrasaccharide is not UDP-D-galactose but galactinol [*O*-α-D-galactopyranosyl-(1 → 1)-*myo*-inositol (*13*). These authors obtained an enzyme from ripening seeds of dwarf beans (*Phaseolus vulgaris*) which transfers D-galactose to raffinose giving rise to stachyose and *myo*-inositol. It was also found that galactinol is a major galactoside constituent in the bean during a certain maturation period and that its formation precedes stachyose.

Although lactose is considered to be an exclusive product of milk synthesized in the mammary gland of mammals, it has been obtained from the ripe fruit of *Achras sapota* (*14*). The presence of lactose has also been reported in *Forsythia anthers* (*15*). There is no information available pertaining to its mechanism of formation in plants.

Starch

This polysaccharide, which is the chief metabolic polymer of higher plants, is readily synthesized by plant phosphorylase in vitro from α-D-glucose-1-phosphate in the presence of a primer, such as a trace of starch or

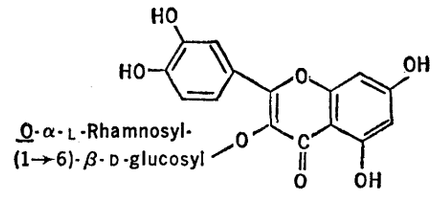

Fig. 2. Rutin

α-1,4-linked oligosaccharide. As in the case of glycogen, it was assumed for many years that phosphorylase was responsible for the synthesis as well as the degradation of the α-1,4-D-glycosyl linkage of starch. However, a number of considerations (*16*) led to the suspicion that, as in glycogen, starch is synthesized in vivo by a transferase from UDP-D-glucose.

Leloir and associates (*17*) found such an enzyme which was associated with starch granules isolated from beans, potatoes, and corn seedlings. This enzyme is closely bound to the starch granule, and attempts to dissociate the activity from the grain have not been successful. Espada (*18*) isolated an enzyme from wheat which catalyzes the reaction

$$\text{ATP} + \alpha\text{-D-glucose-1-P} \rightarrow \text{ADP-D-glucose} + \text{Pi} \quad (3)$$

and showed that this enzyme is different from the one that catalyzes UDP-D-glucose synthesis. Later, Recondo *et al.* (*19*) isolated ADP-D-glucose from corn grains. It was then shown (*17*) that the enzyme which leads to the synthesis of starch is very similar to that involved in glycogen formation, but has a dissimilar specificity with regard to the two nucleoside diphosphate-D-glucose substrates, UDP-D-glucose and ADP-D-glucose, from which these polysaccharides can be synthesized. With the starch granules as a source of enzyme, the D-glucose residues were found to be incorporated ten times faster from ADP-D-glucose than from UDP-D-glucose (*20*). It has been suggested (*4*) that probably both UDP-D-glucose and ADP-D-glucose are involved in equal proportions because, although UDP-D-glucose reacts more slowly, its concentration is approximately five to ten times higher in plant tissues. This would result in about the same rate of transfer.

Frydman and Cardini (*21*) reported a soluble enzyme in sweet corn that synthesizes phytoglycogen from UDP-D-glucose and ADP-D-glucose which has the chemical structure similar to that of the branched amylopectin.

De Fekete and Cardini (*22*) demonstrated a transfer of ^{14}C from sucrose to starch by an enzyme preparation from corn endosperm when the appropriate nucleotides were added to the reaction mixture. To account for these results obtained in vitro, the authors postulated a sequence of reactions (Fig. 1), for which all the enzymes are present in the endosperm extract. Similar results were obtained by Murata *et al.* (*23*) with enzyme systems of ripening rice grain.

Ghosh and Preiss (*24*) have shown that ADP-D-glucose-pyrophosphorylase activity in spinach chloroplasts is stimulated about 50-fold by phosphoglyceric acid. It is possible that during CO_2 fixation the accumulation of 3-phosphoglycerate causes an increase of ADP-D-glucose synthesis by stimulating the concentration of this sugar nucleotide which would enhance the rate of starch synthesis. It therefore appears that activation of starch synthesis probably occurs at the pyrophosphorylase level rather than, as in glycogen, at the transglucosidase level (*25*).

Glycosides

Higher plants contain a polysaccharide consisting of D-glucose residues which is soluble in hot, dilute alkali known as "callose." The D-glucose residues are joined by β-1,3-glucosyl linkages (*26*). Particulate preparations from mung beans are capable of synthesizing this glucan from UDP-D-glucose (*27*). The synthesis of this polysaccharide does not require the addition of any primer. However, when this particulate enzyme is solubilized by extraction with digitonin, it is strongly activated by a number of saccharides, such as laminaribiose, laminaritriose, cellobiose, D-glucose, and other sugars. Because the activators are not incorporated into the polymer (*27*), their action seems to be similar to that of D-glucose-6-phosphate on glycogen synthetase. Leloir (*4*) suggested that the activator probably combines with some specific site on the enzyme surface causing a conformational change which affects the activity of the enzyme.

Callose is predominantly localized in the phloem (*28*) of plants, but it is also readily produced in other plant tissues as a result of injury to the cells (*29*). It is therefore likely that the activation of callose synthesis is of some physiological significance.

An insoluble polysaccharide, paramylon, consisting of β-1,3-linked D-glucose residues is present in the flagella of *Euglena gracilis*. As with callose, the donor is UDP-D-glucose, and no added acceptor is required for its synthesis (*30*). The particulate preparation capable of synthesizing paramylon from UDP-D-glucose can be extracted with deoxycholate, resulting in a solubilized enzyme (*31*).

Glycosides

Glycosides are of widespread occurrence in plants. They all contain a monosaccharide residue or an oligosaccharide residue attached through a β-glycosyl linkage to an "aglycon" which may be an aromatic compound, a lipid, or a nucleic acid. Glycosides do not seem to serve as major food reserves or as structural material; their function in plants is therefore not known.

Dutton and Storey (*32*) demonstrated the synthesis of *o*-aminophenyl-β-D-glucosiduronic acid with a boiled extract that was subsequently shown to contain UDP-D-glucuronic acid and aminophenol (*33*). This finding was the first reported example of transglycosylation from a glycosyl nucleotide shown by the reaction:

UDP-D-glucuronic acid + *o*-aminophenol → *o*-aminophenyl-β-D-glucuronic acid + UDP (4)

Many other acceptors besides *o*-aminophenol may participate in this type of reaction (*34*); these include aliphatic alcohols and carboxylic acids.

D-Glucosiduronic acids are of rare occurrence in plants. However, an enzyme preparation from French bean (*Phaseolus vulgaris*) leaves was shown to catalyze the formation of quercetin-D-glucopyranosiduronic acid from UDP-D-glucuronic acid and quercetin (*35*). In plants, phenolic substances are generally combined with D-glucose, and several systems have been shown to be able to cause these conjugations.

Yamaha and Cardini (*36*) synthesized hydroquinone-β-D-glucopyranoside (arbutin) by the following reaction:

UDP-D-glucose + hydroquinone → UDP + hydroquinone-β-D-glucopyranoside (5)

A diversity of di- and triphenols, but not monophenols, could substitute for hydroquinones as acceptors in the purified enzyme system.

The synthesis of gentiobiosides has also been obtained with an enzyme system from wheat germ which could be separated from the enzyme catalyzing the formation of β-D-glucopyranosides (*37*). As an example, phenyl-β-gentiobiose was obtained by a transfer reaction of D-glucose from UDP-D-glucose to phenyl-β-glucoside. Glucosides containing oligosaccharides with a greater number of monosaccharide residues were also observed to be formed by a crude germ preparation (*38*).

The flavinol rutin (Fig. 2) that occurs in some plants consists of the disaccharide 6-*O*-α-rhamnopyranosyl-D-glucose ("rutinose") which is attached to the 3-hydroxyl group of quercetin by a glycosyl linkage (*39*). This glycoside was shown to be synthesized by the following reactions:

Nucleoside diphosphate-D-glucose + quercetin → 3-quercetin-β-D-glucoside (6)

Nucleoside diphosphate-L-rhamnose + 3-quercetin-β-D-glucoside → rutin (7)

D-Glucose is first transferred from *d*TDP-D-glucose or UDP-D-glucose to form 3-quercetin-β-D-glucoside. Both sugar nucleotides appear to be equally effective as D-glucosyl donors. Rutin (Fig. 2) is then synthesized by a transfer of L-rhamnose to the glucoside from *d*TDP-L-rhamnose or from UDP-L-rhamnose. The lack of specificity for the pyrimidine portion of the base might be due to the existence of one nonspecific enzyme or to a mixture of several specific enzymes.

An enzyme has been obtained from unripe pea seeds which catalyzes the transfer of D-galactosyl residues from UDP-D-galactose to *myo*-inositol to yield 1-*O*-α-D-galactopyranosyl-*myo*-inositol (*40*). This compound, named galactinol, is present in sugar beets (*41*).

Isolated spinach chloroplasts are capable of transferring D-galactose from UDP-D-galactose to an endogenous acceptor producing alkali-labile products which are similar to those isolated from plant material (*42*). The two products identified were β-D-galactosyl glycerol and *O*-α-D-galactosyl-(1 → 6)-*O*-β-D-galactosyl-(1 → 1)-D-glycerol.

Cell Wall Polysaccharides

The material that surrounds the cells of higher plants which forms their skeletal framework must be rigid and capable of withstanding considerable stress. Cellulose, which is characterized by its rigidity and great tensile strength, serves that purpose. It is organized

in densely packed fibers consisting of aggregates of partially crystalline bundles (fibrils), comprised largely of parallel chains of anhydroglucose units. The fibers are intimately associated in an amorphous matrix with other polysaccharides, such as xylan, mannan and hemicelluloses, and pectins. In addition, secondary walls contain noncarbohydrate materials, such as lignin, small amounts of protein, silica, and other substances.

The term cellulose usually has the connotation of fibrous material such as cotton or ramie fibers. No attempt will be made here to deal with the superstructure of the chemical molecules of the polysaccharides, but rather with the biochemical aspect of synthesis from particular substrates.

The case of fibrous cellulose is analogous to that of granular starch. While considerable information pertaining to the biosynthesis of the molecular starch is now available, very little is known about the mechanism of the formation of the starch granule. It will no doubt require much work to elucidate the mechanism by which the molecular chains are combined to form cellulose fibers.

Cellulose

It was known for a long time that the microorganism *Acetobacter xylinum* and other types of *Acetobacter* had the ability to produce cellulose membranes from D-glucose and related carbohydrates (*43*). The biosynthesis of cellulose with a cell-free particulate system from *A. xylinum* and UDP-D-glucose-^{14}C was first achieved by Glaser (*44*). However, the fact that this polysaccharide can be formed by a microorganism from UDP-D-glucose does not necessarily prove that it is formed from the same substrate by plants.

In 1964 an enzyme was found in rapidly growing root tissue of mung beans (*Phaseolus aureus*) (*45*) and in other plants, including immature cotton bolls (*46*), capable of utilizing GDP-D-glucose-^{14}C as substrate for the formation of radioactive polysaccharide, in which the D-glucose units are combined by β-,4-glycosyl linkages and which appeared to be indistinguishable by a number of tests from natural cellulose. The enzyme system polymerizing the D-glucose to cellulose showed a high degree of specificity for GDP-D-glucose. None of the ^{14}C-labeled glycosyl nucleotides containing bases other than guanine (uracil, adenine, thymine, and cytidine) could serve as substrate of cellulose.

A pronounced stimulation of incorporation of D-glucose-^{14}C from GDP-D-glucose-^{14}C into an insoluble polysaccharide was observed upon the addition of GDP-D-mannose to the reaction mixture; but this appears to involve the synthesis of a polysaccharide other than cellulose (*45*). Attempts to separate the transferase activity responsible for the synthesis of cellulose from the endogenous acceptor were not successful. Addition of Co^{2+}, Mn^{2+}, Zn^{2+}, or Ca^{2+} enhances the incorporation of the D-glucose into the polymer.

Mung beans and peas contain a pyrophosphorylase which can form GDP-D-glucose from α-D-glucose-1-phosphate and GTP (*46*).

Based on the data obtained with plant preparations the following mechanism for cellulose synthesis was proposed:

$$\text{GTP} + \alpha\text{-D-glucose-1-P} \rightleftharpoons \text{GDP-D-glucose} + \text{PPi} \quad (8)$$

$$n(\text{GDP-D-glucose}) + \text{acceptor} \rightarrow \text{acceptor-}(\beta\text{-1,4-D-glucose})_n + n\text{GDP} \quad (9)$$

However, other workers presented data that suggest UDP-D-glucose may also be an effective donor for cellulose synthesis. Brummond and Gibbons (*47*), using a modified incubation mixture of that used by Barber *et al.* (*45*), reported that an enzyme preparation from *Lupinus albus* is capable of incorporating glucose-^{14}C from UDP-D-glucose-^{14}C into a mixture of polysaccharides ranging from water-soluble to insoluble in alkali. They found that the alkali insoluble fraction (about 7 percent) is synthesized more readily from UDP-D-glucose than from GDP-D-glucose. However, repetition of this work with *Lupinus albus* in the writer's laboratory yielded a polysaccharide similar to the one obtained by Feingold *et al.* (*27*) from mung beans, containing only β-1,3-glucosyl linkages.

Ordin and Hall (*48*) found that particulate preparations from oat coleoptiles could utilize both GDP-D-glucose or UDP-D-glucose as substrate for polysaccharide formation. Upon degradation of the polysaccharide derived from UDP-D-glucose with cellulase, cellobiose and, to a lesser extent, a substance identified as a trisaccharide containing mixed β-1,4- and β-1,3-glucosyl linkages were obtained. However, when GDP-D-glucose was used as substrate, only cellobiose and cellotriose were obtained, indicating the production of a polysaccharide containing only β-1,4 linkages.

Villemez *et al.* (*49*), working with an incubation mixture containing sucrose and albumin, obtained an alkali-insoluble polysaccharide from a particulate enzyme mung bean preparation and UDP-D-glucose-^{14}C as substrate; hydrolysis of this polysaccharide with acid, in addition to cellobiose, produced approximately 10 percent laminaribiose, in which the D-glucose residues were joined by β-1,3-glucosyl linkages. These results appeared to be similar to those obtained with oat coleoptiles (*48*) in which specific hydrolases were used for determination of the glucan linkages.

While Ordin and Hall's results (*48*) with enzyme preparations from oat coleoptiles and UDP-D-glucose as substrate could be substantiated by chemical methods, a more recent investigation (*50*) showed that particulate enzyme preparations from mung beans differed from those previously reported (*49*). The failure to reproduce the results of Brummond and Gibbons (*47*) and those of Villemez *et al.* (*49*) may perhaps be attributed to the appearance of a seasonal factor in these plants which stimulates the activity of the production of an enzyme catalyzing the formation of an insoluble β-(1 $\rightarrow$ 4)-glucan.

Digitonin extracts of particulate enzyme preparations proved to be active in the synthesis of cellulose from GDP-D-glucose. A soluble preparation, obtained from this solution and from which the protein was subsequently precipitated by the addition of ammonium sulfate and then dissolved, did not show any synthetic activity. However, when boiled active mung bean particles were added to the soluble preparation, the synthetase of the soluble preparation became active, producing cellulose. This strongly indicates that the primer for the cellulose synthesis resides in the particles.

The polymer produced from GDP-D-glucose by an enzyme contained a small proportion of mannose, indicating the presence of an epimerase that interconverts GDP-D-glucose and GDP-D-mannose. The reaction of cellulose formation appears to be very rapid. It reaches half of its maximum velocity in less than 2 minutes. With initially low concentrations up to $7 \times 10^{-7} M$ of GDP-D-glucose-^{14}C no further synthesis of polymer occurs after 2 minutes, although approximately 15 per-

cent of substrate remains in the reaction mixture. However, with higher concentrations such as $7 \times 10^{-6}M$, the synthesis of polymer after 2 minutes proceeds at a very low rate, while about 20 percent of the initial amount of GDP-D-glucose added is still present in the reaction mixture at that time. The reaction is virtually complete within 5 minutes. These results cannot be explained at present; they require further investigation.

In spite of some unexplained results concerning the cellulose synthesizing enzyme system in plants, the data, in general, appear to be consistent with the assumption that GDP-D-glucose is the sugar nucleotide from which the β-1,4-linked chain of cellulose is synthesized.

Although the experiments with mung beans in vitro (*45*) indicate that GDP-D-glucose is the direct donor of D-glucose for the formation of cellulose in plants, Colvin's work (*51*) suggests the presence of a glucosyl-lipid precursor within the plant cell which is converted extracellularly to cellulose. The D-glucose is transferred from the nucleoside diphosphate-D-glucose to the glucolipid at or on the cytoplasmic membrane, and the D-glucose is then translocated outside the cell by the lipid moiety; that is, the glucolipid acts as a carrier from within the cell to the external medium where the D-glucose is polymerized to cellulose.

Other Cell Wall Polysaccharides

It has been observed that the presence of GDP-D-mannose in the incubation mixture used for the synthesis of cellulose from GDP-D-glucose-^{14}C causes the formation of an alkali-insoluble polysaccharide which is not cellulose (*45*). It was also shown that when GDP-D-mannose-^{14}C alone is used as substrate, a radioactive glucomannan is synthesized and that Mg^{2+} is required for its formation. The addition of unlabeled GDP-D-glucose to the reaction mixture containing GDP-D-mannose-^{14}C resulted in a marked inhibition of incorporation of D-mannose-^{14}C into an insoluble polysaccharide. The enzyme or enzymes involved in the synthesis of glucomannan apparently have a greater affinity for GDP-D-glucose than for GDP-D-mannose, which would account for the inhibition of D-mannose incorporation by GDP-D-glucose. Several of the oligosaccharides obtained from degradation of the glucomannan contain both glucose and mannose in various proportions, indicating that the glucomannan is not a mixture of cellulose and mannose. The mannose units in this polysaccharide are linked by β-1,4 bonds (*52*).

The fact that this polysaccharide is obtained from GDP-D-mannose as substrate suggests that the particulate enzyme contains an epimerase which converts GDP-D-mannose to GDP-D-glucose. The incorporation of D-glucose-^{14}C from GDP-D-glucose-^{14}C into the polysaccharide is stimulated three- to fivefold by GDP-D-mannose (*53*). D-Mannose incorporation from GDP-D-mannose-^{14}C, on the other hand, is inhibited by GDP-D-glucose.

Experiments in vivo by several investigators (*54*) showed that D-glucuronic acid originated in plants from D-glucose by a series of reactions. The D-glucuronic acid was subsequently decarboxylated to pentose and polymerized to xylan. The mechanism of synthesis of xylan with enzymic plant preparations in vitro agreed with that postulated for that polysaccharide in vivo. Uridine diphosphate-D-xylose has been isolated from plant seedlings (*55*) and synthesized from UDP-D-glucose with enzymes by the following sequence of reactions (*56*):

$$\text{UDP-D-glucose} \xrightarrow[\text{NAD}]{\text{dehydrogenase}} \text{UDP-D-glucuronic acid} \xrightarrow{\text{decarboxylase}} \text{UDP-D-xylose} \quad (10)$$

Particulate preparations from corn shoots (*57*) readily incorporate D-xylose-^{14}C from UDP-D-xylose-^{14}C into a polysaccharide in which the D-xylose residues are combined by β-1,4 xylosyl bonds. This polysaccharide, similar to natural plant xylan, contains a small proportion of arabinose units which have the furanose configuration.

Pectins are complex macromolecular substances that occur in all higher plants. They are found in the cell walls, particularly in the intercellular layers. Some mature fruits and plant juices are also rich in these substances.

The basic building unit of pectins is known to be α-1,4-linked D-galacturonic acid units. These polygalacturonates are associated with other polysaccharides, chiefly D-galactan and L-araban. The carboxyl groups of the D-galacturonic acid in the chain are methylated to various degrees.

It has been shown (*56, 58*) that UDP-D-galacturonic acid is present in higher plants, and that they contain enzymes which lead to the formation of this uronic acid nucleotide by the following pathway:

$$\text{UDP-D-glucose} \xrightarrow{\text{dehydrogenase}} \text{UDP-D-glucuronic acid} \underset{}{\overset{\text{epimerase}}{\rightleftharpoons}} \text{UDP-D-galacturonic acid} \quad (11)$$

A particulate preparation from mung beans (*Phaseolus aureus*) was found to catalyze the formation of a polygalacturonic acid chain (*59*). The synthetic polygalacturonate could be hydrolyzed with *Penicillium chrysogenum* polygalacturonase to D-galacturonic acid, and with an exopolygalacturonic acid transeliminase from *Clostridium multifermentans* to unsaturated 4,5-digalacturonic acid (*60*). The action of these enzymes is known to be specific for degradation of the polygalacturonic acid chain.

Experiments with a number of plants showed that the enzyme reaction for utilization of UDP-D-galacturonic acid as the donor of D-galacturonic acid for polygalacturonate is fairly specific; however, some incorporation of this uronic acid into polygalacturonate was also observed with a particulate enzyme preparation from tomatoes when TDP-D-galacturonic acid and, to a lesser extent, CDP-D-galacturonic acid were used as donors (*61*).

Inasmuch as the galacturonic acid carboxyl groups of pectin are methylated or partially methylated, the opinion prevailed (*62*) that the immediate precursor of pectin is the methyl esterified D-galacturonic acid derivative of nucleoside diphosphate. This assumption, however, could not be substantiated with the mung bean enzymic system, because the methyl galacturonate moiety from UDP-methyl-D-galacturonate failed to incorporate into the polygalacturonic acid chain (*59*). In this system the esterification of the D-galacturonate takes place by a transfer of the methyl group from *S*-adenosyl-L-methionine (*63*), analogous to the case in which the 4-*O*-methyl ether groups are transferred to D-glucuronic acid of hemicellulose (*64*). In this system the esterification of galacturonic acid residues presumably takes place subsequent to the formation of the polygalacturonic acid chain.

myo-Inositol is known to occur in higher plants both in free state and as the hexaphosphate, phytic acid (*65*). The *myo*-inositol ring is enzymically capable of undergoing scission between C-1 and C-6 in plants producing D-glucuronic acid (*66*). This reaction is

similar to the oxidative cleavage demonstrated by Charalampous (*67*) in rat kidney (Fig. 3).

Loewus and his collaborators (*68*) showed that D-glucuronic acid and its lactone are directly converted by detached plant tissues to L-gulonic acid and to D-galacturonosyl residues of pectin. When *myo*-inositol-(2-^{3}H- or 2-^{14}C) was supplied to strawberry fruit or parsley leaves and allowed to metabolize, the D-galacturonosyl residues obtained from the degraded pectin contained most of the radioactivity in C-5. Other experiments (*69*) with parsley leaves produced similar results. It appears that the D-glucuronic acid formed in the plant from the ruptured *myo*-inositol ring undergoes a number of known enzymic reactions, resulting in the formation of UDP-D-galacturonic acid (*3*) which serves as a donor of the D-galacturonosyl acid moiety for pectin formation. *myo*-Inositol thus appears to be a potential source for pectin formation.

Galactan usually occurs in combination with other polysaccharides as side chains of D-polygalacturonic acid of pectic substances. It is also present in a number of coniferous woods as an arabinogalactan, particularly in birch woods (*70*).

A particulate preparation from *Phaseolus aureus* is capable of incorporating galactose-^{14}C from UDP-D-galactose-^{14}C into a water-soluble galactan having a minimum molecular weight of about 4600 (*71*).

Hemicellulose refers to a chemically ill-defined group of plant cell wall polysaccharides that are closely associated with cellulose and are composed of monosaccharides, namely, D-glucose, D-galactose, D-mannose, D-xylose, L-arabinose, and D-glucuronic, D-galacturonic, and D-mannuronic acids. These sugars and glycuronic acids are combined by various types of linkages. Hemicelluloses may be separated into two fractions, A and B (*72*). However, there is no clear demarcation of the structure of the two fractions. Hemicellulose B usually contains a higher proportion of uronic acid, mainly 4-methyl-D-glucuronic acid, than the A fraction. This methyl derivative of D-glucuronic acid is most frequently isolated in combined form as the 4-methyl-aldobiuronic acid, because it is hydrolyzed with difficulty with acid.

The methyl donor for the formation of 4-methyl-D-glucuronic acid of hemicellulose B proved to be *S*-adenosyl-L-methionine (*64*). A particulate preparation from immature corn cobs containing hemicellulose B was found capable of transferring the ^{14}C-labeled methyl group from *S*-adenosyl-L-methionine to a macromolecular acceptor present in the particles. The radioactive product was shown to be hemicellulose B labeled in the 4-methyl-D-glucuronic acid residues. The observation that the *O*-methyl ether groups of plant heteropolysaccharides are introduced at the macromolecular level is similar to the finding that *C*- and *N*-methyl groups of RNA and DNA are also introduced into preformed macromolecules (*73*).

The particulate enzyme preparation from immature corn cobs also contains an enzyme which transfers the D-glucuronic-^{14}C acid group from UDP-D-glucuronic-^{14}C acid into hemicellulose B, in which the radioactivity resides in the two aldobionic acids, glucuronogalactose and glucuronosyl-xylose (*74*).

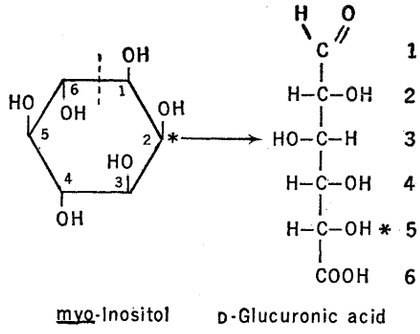

Fig. 3. Oxidative cleavage of *myo*-inositol to D-glucuronic acid. Dotted lines between position 1 and 6 in *myo*-inositol indicate the site of cleavage. The asterisk denotes the site of labeling in *myo*-inositol-2-^{14}C and its location in the product, D-glucuronic acid (*66*).

Synthesis of Polysaccharides in Some Lower Plants

The carbohydrates present in algae and in other lower plants are generally of a different nature than those found in higher plants. Those commonly occurring in the latter are D-glucose, D-fructose, sucrose, various glycosides, starch, cellulose, pectin, and hemicelluloses. In the algae, soluble sugars, such as D-glucose, D-fructose, and sucrose, are either completely absent, or present only in comparatively small amounts. Instead, sugar alcohols, mannitol, dulcitol, sorbitol, and galactosyl glycerols seem to predominate. Of the polysaccharides, such compounds as alginic acid, laminarin, fucosan, and floridian starch are present. The major polysaccharide constituents of red and brown marine algae are perhaps most strikingly exemplified by the sulfated polysaccharides. They account for the bulk of the tissue of some algae (*75*).

In considering the nature of seaweed polysaccharides and their relation to those of land plants, their environment is of particular importance. If the seaweed is not to be broken by vigorous wave action, it must be pliant. In contrast, land plants must maintain a certain rigidity of structure, and they would be expected to contain considerable amounts of cellulose. Indeed, it was found that seaweeds contain a much smaller proportion of cellulose than do land plants. Where cellulose does occur, it appears to be less well oriented and probably less crystalline than in higher plants. In some species the cellulose is entirely replaced by other polysaccharides, such as mannan and xylan, which are relatively insoluble but fibrous with a lower degree of orientation in the fibrillar structure (*76*).

Alginic acid found in the alga *Fucus gardneri* Silva consists of a mixture of D-mannuronic acid and its 5-epimer L-guluronic acid; it is known to be a major constituent of the structural polysaccharides of brown algae. The D-mannuronic acid is always the main component (about 80 percent) of this polymer (*77*).

Enzyme preparations obtained from this alga contained the following enzyme activities—hexokinase, phosphoglucomutase, pyrophosphorylase, GDP-D-mannose dehydrogenase, and mannuronic acid transferase. These active enzymic preparations could only be obtained when the *F. gardneri* homogenate was treated with polyvinylpyrrolidone (PVP). Starting with D-mannose, the enzyme systems carry out several consecutive reactions resulting in the biosynthesis of the D-mannuronic acid chain:

$$\text{D-mannose} \xrightarrow[\text{ATP}]{\text{kinase}} \text{D-mannose-6-P} \xrightarrow{\text{phosphomannomutase}} \alpha\text{-D-mannose-1-P} \quad (12)$$

$$\alpha\text{-D-mannose-1-P} + \text{GTP} \xrightarrow{\text{pyrophosphorylase}} \text{GDP-D-mannose} + \text{PPi} \quad (13)$$

$$\text{GDP-D-mannose} + 2\text{NAD}^{+}\,(\text{NADP}^{+}) + \text{H}_2\text{O} \xrightarrow{\text{dehydrogenase}} \text{GDP-D-mannuronic acid} + 2\text{NADH(NADPH)} + 2\text{H}^{+} \quad (14)$$

$$\text{GDP-D-mannuronic acid} \xrightarrow{\text{transferase}} \text{polymannuronate} \quad (15)$$

In order to synthesize the complete alginic acid polymer, L-guluronic acid (the minor component), which is probably synthesized through interconversion of GDP-D-mannuronic acid by a 5-epimerase, should also be incorporated into the molecule. This, however, has not yet been accomplished.

The final stage of alginic acid synthesis appears to involve a succession of transfers of uronic acid residues from the two GDP-D-uronic acids to an acceptor molecule forming β-1,4-linked polyuronide chains:

$$\left.\begin{matrix}\text{GDP-D-mannuronate}\\ \text{GDP-D-guluronate}\end{matrix}\right\} \xrightarrow[\text{acceptor}]{\text{glucosyl transferase(s)}} \text{alginic acid} \quad (16)$$

The marine red alga, *Porphyra perforata* contains a galactan which on hydrolysis produces approximately an equal proportion of D- and L-galactose isomers. The composition of this polysaccharide consists of D-galactose, 6-*O*-methyl-D-galactose, 3,6-*anhydro*-L-galactose, and sulfate in the ester form in the molar ratio of approximately 1:1:2:1, respectively (*78*).

Porphyra perforata also contains UDP-D-galactose, GDP-L-galactose, UDP-D-glucose, GDP-D-mannose, and UDP-D-glucuronic acid. In addition to a number of known nucleotides, a new nucleotide, adenosine 3′,5′-pyrophosphate, was isolated from this alga (*78*).

Because *P. perforata* contains saccharides composed chiefly of galactose moieties of both D- and L-configuration, together with sugar nucleotides containing the two enantiomorphs of galactose, it may be assumed that these sugar nucleotides are the precursors of the complex galactose compounds. Moreover, inasmuch as GDP-L-galactose is found together with GDP-D-mannose in this red alga, there is reason to believe that the former sugar nucleotide is probably snythesized by a mechanism similar to that by which GDP-D-mannose is converted to GDP-L-fucose (*79*) in which the glycosyl moiety has the L-galactose configuration.

Concerning the origin of the sulfuric acid group in the galactan, it is of interest to compare the structure of "active" sulfate (adenosine 3′-phosphate-5′-phosphosulfonate), which is known to be a sulfate donor (*80*), with that of adenosine 3′,5′-pyrophosphate discovered in *Porphyra perforata.* It is conceivable that the adenosine 3′,5′-pyrophosphate in this alga activates inorganic sulfate to form the "active sulfate" which effects the enzymic sulfation of the polysaccharide.

It may be of interest that while the seaweed polysaccharides alginic acid and fucoidine appear to be derived from the common precursor GDP-D-mannose (*81*), most of the polysaccharide constituents of higher plants, that is, D-galactose, D-glucuronic and D-galacturonic acids, D-xylose, and L-arabinose, are derived from UDP-D-glucose (*5, 82*).

Concluding Remarks

Whereas the sugar diphosphate nucleosides which are present in higher plants contain predominantly the uracil base, sugar nucleotides with other bases such as guanine, adenine, and thymine may occur in the same or other plants. The mung bean plant (*Phaseolus aureus*) which has been subjected to a more intensive study of sugar nucleotide metabolism than any other plant was found to contain mainly uridine diphosphate sugars; practically all the known oligosaccharides, glycosides, and polysaccharides (with the exception of cellulose) were found to be synthesized from sugar nucleotides containing uracil.

Although GDP-D-glucose has been reported from strawberry leaves (*83*), it has not been found in mung beans. The failure to detect it may be attributed to the low level of this sugar nucleotide in the plant. Since cellulose forms the insoluble skeletal framework of the higher plants, its rate of synthesis must be very high; consequently, the sugar nucleotide must be used up as rapidly as it is formed. Pyrophosphorylase, capable of synthesizing GDP-D-glucose from GDP and α-D-glucose-1-phosphate, was found in mung beans and in a number of other plants (*46*).

Experiments in vitro show that in many cases complex saccharides can be formed from sugar nucleotides containing different bases. Although UDP-D-glucose is considered to be the normal substrate for glycogen formation, synthesis of this polysaccharide also proceeds from ADP-D-glucose, but at about 50 percent of the rate observed when UDP-D-glucose is used. On the other hand, starch is formed from ADP-D-glucose at a rate which is approximately tenfold greater than from UDP-D-glucose (*4*). It was shown that two different enzymes are involved in the reactions with the different sugar nucleotide substrates.

Glycolipids are known to be formed by plant enzymes similar to those which produce complex saccharides from sugar nucleotides by transfer reactions. Thus various investigators observed such lipid complexes containing xylose, mannose, and glucose (*84*).

Kauss (*85*) found that a particulate enzyme preparation from mung beans transfers the glucosyl moiety from UDP-D-glucose-^{14}C to a lipid which was shown to be a mixture of β-sitosteryl and stigmasteryl glucosides. These complexes, however, could not be shown to be intermediates in the polysaccharide synthesis reaction.

In the synthesis of homopolysaccharides such as starch, glycogen, callose, or paramylon, the monosaccharides are activated by the formation of nucleotide diphosphate derivatives from which they are transferred to a nonactivated growing polymer. In microbial heteropolysaccharides the mechanism of formation appears to differ and is more complex. Nikado (*86*) working with mutants of *Escherichia coli* and *Salmonella*, and Osborn, Horecker, and their co-workers (*87*) demonstrated that in *S. typhimurium* the "core" of the heteropolysaccharide is synthesized stepwise from UDP-D-glucose, UDP-D-galactose and UDP-*N*-acetyl-D-glucosamine, and the appropriate lipopolysaccharide primers. At at each step a different primer is required, in which the terminal nonreducing monosaccharide is the one to which the new sugar is being added.

Robbins *et al.* (*88*) working with a bacterial polysaccharide, the surface *O*-antigen of *Salmonella newington*, showed that elongation takes place at the reducing end of the growing chains. Sugars are not transferred directly to the antigen from sugar nucleotides, but are transferred first into lipid-linked oligosaccharides. The polysaccharide chain then grows by assembly of these lipid-linked precursors at the reducing, rather than at the nonreducing, end of the polymer. This method of assembly, in which the newly formed chains are transferred to the next subunit, is analogous to the growth of proteins or fatty acids. They suggest that these differences may reflect the more complex requirements of surface bacterial polysaccharides synthesized by membrane-bound enzymes.

Experiments by Villemez *et al.* (*89*) with particulate plant enzymes indicate that the enzymes which synthesize the cell wall polysaccharides probably originate from a single type of organelle.

This hypothesis is supported by density gradient centrifugation which yields a similar distribution of transferring enzymes. Other data also suggest that the most likely source of the cell wall polysaccharide-synthesizing particles is the plasma membrane.

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