Biosynthesis of Food Constituents: Saccharides. 1. Monosaccharides, Oligosaccharides, and Related Compounds – a Review

Jan VELÍŠEK and Karel CEJPEK

Department of Food Chemistry and Analysis, Faculty of Food and Biochemical Technology, Institute of Chemical Technology Prague, Prague, Czech Republic

Abstract

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This review article presents a survey of selected principal biosynthetic pathways that lead to the most important monosaccharides, oligosaccharides, sugar alcohols, and cyclitols in foods and in food raw materials and informs non-specialist readers about new scientific advances as reported in recently published papers. Subdivision of the topics is predominantly via biosynthesis. Monosaccharides are subdivided to sugar phosphates, sugar nucleotides, nucleotide-glucose interconversion pathway sugars, nucleotide-mannose interconversion pathway sugars, and aminosugars. The part concerning oligosaccharides deals with saccharose, trehalose, raffinose, and lactose biosynthesis. The part devoted to sugar alcohols and cyclitols includes the biosynthetic pathways leading to glucitol, inositols, and pseudosaccharides. Extensively used are reaction schemes, sequences, and mechanisms with the enzymes involved and detailed explanations employing sound chemical principles and mechanisms.

Keywords: biosynthesis; monosaccharides; sugar acids; sugar alcohols; cyclitols; sugar phosphates; sugar nucleotides; aminosugars; oligosaccharides; pseudooligosaccharides

The main pathways of saccharides biosynthesis and degradation comprise an important component of primary metabolism that is essential for all living organisms. Saccharides are the first products formed in photosynthesis, and are the products from which plants synthesise their own reserves, structural components of cell walls (Velíšek & Cejpek 2005) as well as a variety of conjugates including glycoproteins, proteoglycans, and glycolipids. These materials then become the foodstuffs of other organisms. Secondary metabolites are also ultimately derived from the metabolism of saccharides by the acetate, shikimate, mevalonate, and 1-deoxy-D-xylulose pathways (Voet & Voet 1990; Dewick 2002).

1 Monosaccharides

1.1 Sugar phosphates and sugar nucleotides

Six-carbon sugars (hexoses) and five-carbon sugars (pentoses) are the most frequently encountered monosaccharides in nature. Photosynthesis[1] produces initially the three-carbon sugar D-glycer-

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aldehyde 3-phosphate (3-phosphoglyceraldehyde), which isomerises to 1,3-dihydroxyacetone phosphate (glyceron phosphate). These two molecules are used to synthesise D-fructose 1,6-bisphosphate. D-Fructose 1,6-bisphosphate is converted into D-fructose 6-phosphate by splitting off phosphate. D-Fructose 6-phosphate has two alternative fates. One molecule of D-fructose 6-phosphate is converted into D-glucose 6-phosphate; the other molecule of D-fructose 6-phosphate disintegrates into D-xylulose 5-phosphate and acetyl phosphate that forms D-erythrose 4-phosphate together with D-glyceraldehyde 3-phosphate. D-Erythrose 4-phosphate is coupled to one molecule of 1,3-dihydroxyacetone phosphate and the result is one molecule of D-sedoheptulose 1,7-bisphosphate (D-*altro*-hept-2-ulose 1,7-bisphosphate). After splitting off one phosphate molecule, the D-sedoheptulose 7-phosphate formed reacts with D-glyceraldehyde 3-phosphate giving rise to D-ribose 5-phosphate and D-xylulose 5-phosphate. These two pentoses form D-ribulose 5-phosphate which is further phosphorylated to D-ribulose 1,5-bisphosphate and starts a new round of the Calvin cycle (Voet & Voet 1990).

The overview of sugar phosphates and sugar nucleotides relevant to the synthesis of cell wall polymers and storage products in higher plants is given in Figure 1 (Reiter 2002).

One of the two molecules of D-fructose 6-phosphate (D-Fru6P) formed in the Calvin cycle is the net yield of photosynthesis. It reversibly isomerises either to D-glucose 6-phosphate (D-Glc6P), by a sequence which effectively achieves the reverse of the glycolytic reactions, or to D-mannose 6-phosphate (D-Man6P). The aldose 6-phosphates (D-Glc6P and D-Man6P) are reversibly converted to the corresponding 1-phosphates (D-Glc1P and

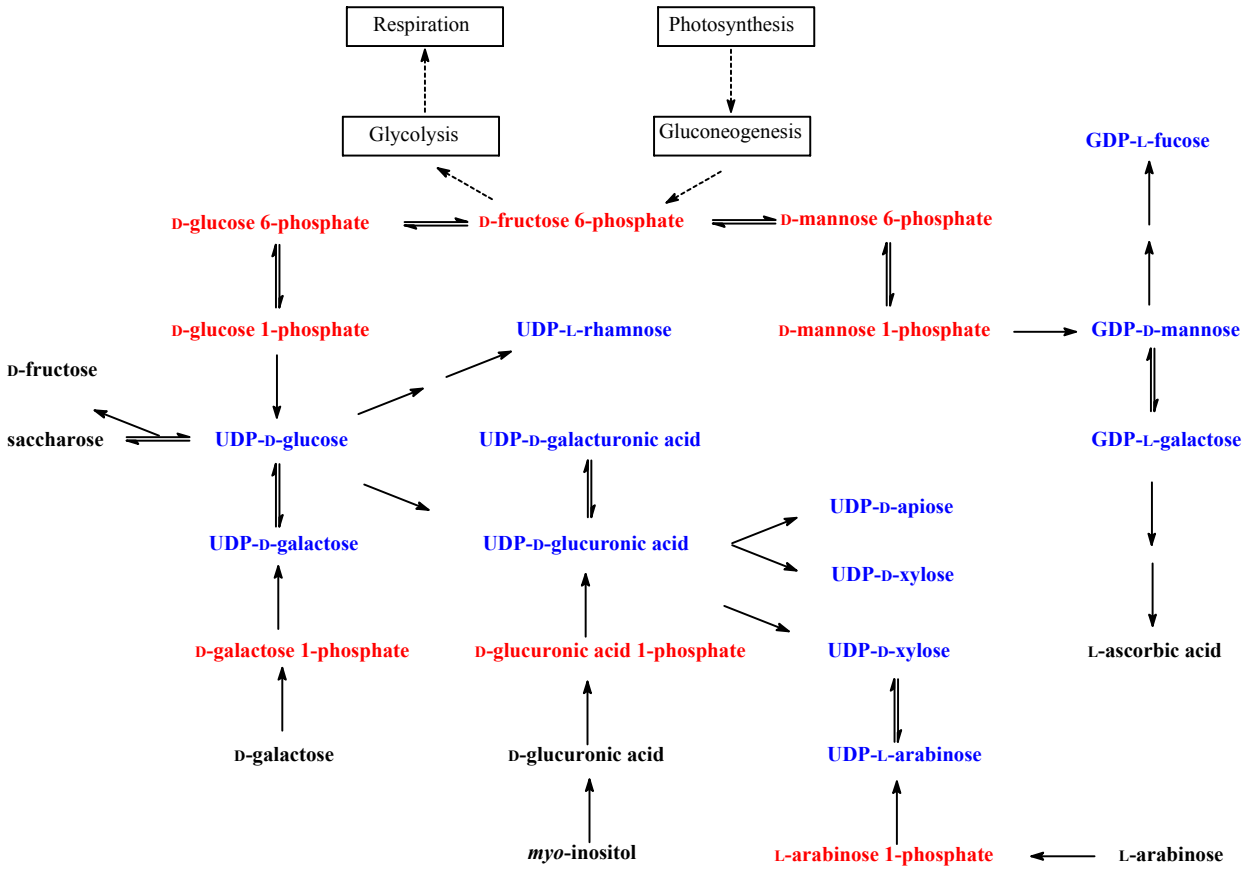


Figure 1

[1]Photosynthesis is the process by which light energy is converted into chemical energy stored in ATP and NADPH. The light-independent Calvin cycle (also known as Calvin-Benson cycle), a series of biochemical reactions taking place in photosynthetic organisms, uses the energy from these energy carriers to convert CO_2 into organic compounds that can be used by the organism.

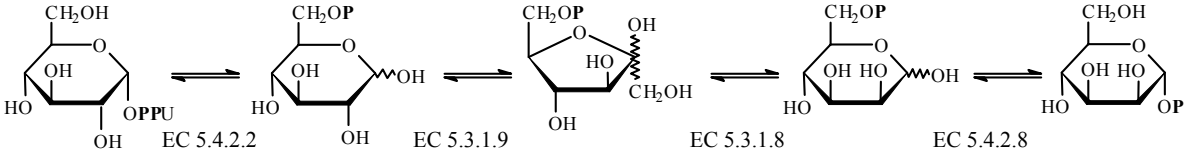


Figure 2

D-Man1P) by suitable phosphomutases (Figure 2). Most of the enzymes involved interact metabolically with glycolysis and gluconeogenesis through the reversible actions of phosphomannose isomerase (EC 5.3.1.8), phosphoglucose isomerase (EC 5.3.1.9), phosphoglucomutase (EC 5.4.2.2), and phosphomannomutase (EC 5.4.2.8) (Figure 2).

Conversions of aldose-aldose and aldose-ketose are base-catalysed reactions that proceed through the open-chain endiolate stabilised by hydrogen bonds (Velíšek 2002) (Figure 3).

These isomerisations are also metabolically frequent reactions catalysed by specific isomerases. For example, the glycolytic enzyme phosphoglucose isomerase (EC 5.3.1.9) catalyses isomerisation of D-Glc6P to D-Fru6P and *vice versa*. The enzyme catalysis obviates the need for a strong base (B:) to achieve the formation of the endiolate anion (Dewick 2002). Transformation of D-Glc6P to D-Glc1P and *vice versa* is a reaction catalysed by phosphoglucomutase (EC 5.4.2.2). The C-1 hydroxyl group of Glc6P attacks the phosphorylated enzyme (its phosphate group is covalently bound to serine) forming the intermediate dephosphoenzyme bound to D-glucose 1,6-bisphosphate (D-Glc1,6P) which leads to the formation of D-Glc1P and the phosphoenzyme is regenerated (Voet & Voet 1990) (Figure 4).

The 1-phosphate sugars are further converted by the reaction with the respective 5´-nucleoside triphosphate (Seifert 2004) to metabolically active forms, nucleotide sugars, such as adenosinediphospho-D-glucose (ADP-D-Glc), cytidinediphospho-D-glucose (CDP-D-Glc), guanidinediphospho-D-glucose (GDPD-Glc), and uridinediphospho-D-glucose (UDP-D-Glc) (Figure 5). The reaction is catalysed by suitable nucleotidylyltransferases that have an important role not only for the initial activation of sugars in the production of nucleotide sugars, but also for the scavenging or recycling of the sugars released from glycosides, oligosaccharides, and polysaccharides. The type of nucleotide employed depends on the biosynthetic pathway and the product that is synthesised.

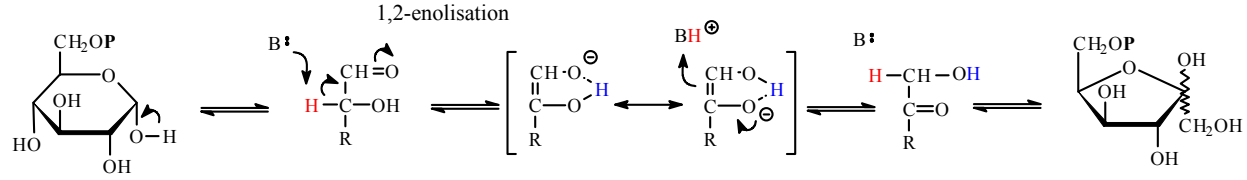


Figure 3

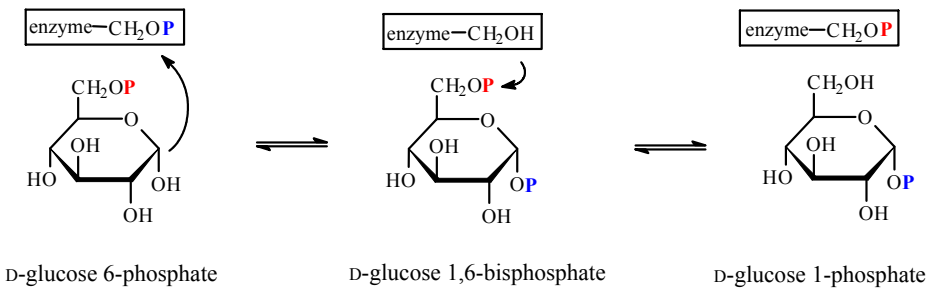


Figure 4

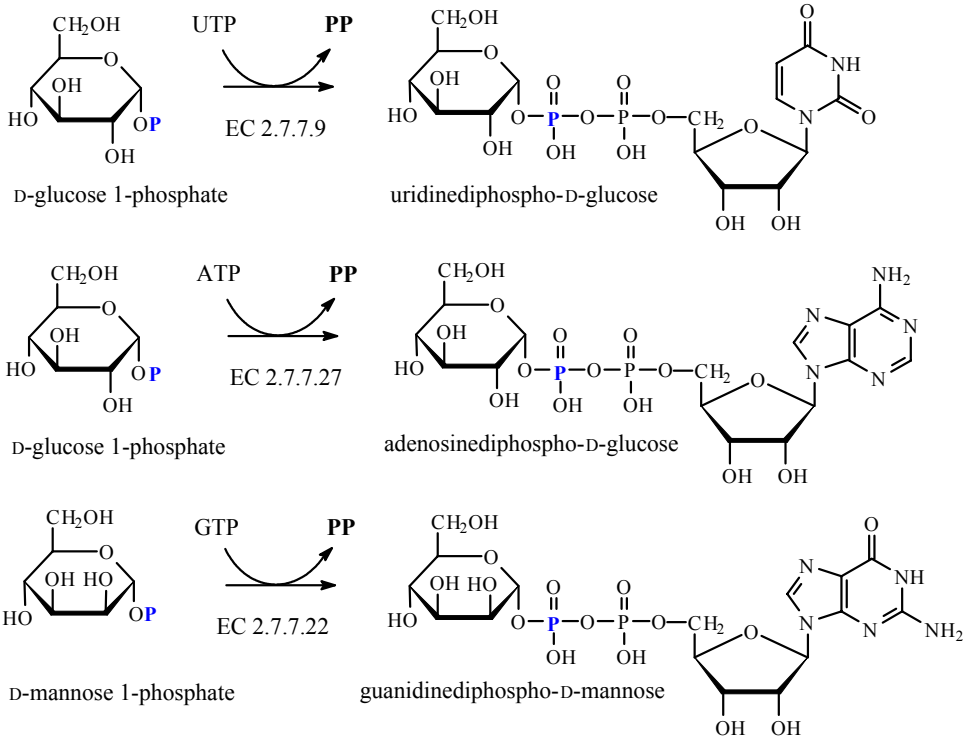


Figure 5

UDP-sugars are the nucleotide sugars most frequently employed.

The two major points of direct biosynthesis of nucleotide sugars involved in the synthesis of the cell wall material from phosphorylated monosaccharides are the synthesis of UDP-D-Glc and the synthesis of GDP-D-Man (Reiter & Vanzin 2001; Reiter 2002; Seifert 2004). UDP-D-Glc is formed from D-Glc1P and uridine-5′-triphosphate (UTP) in a reaction catalysed by UDP-D-glucose pyrophosphorylase (EC 2.7.7.9) and the formation of GDP-D-mannose (GDP-D-Man) from D-Man1P is catalysed by GDP-D-mannose pyrophosphorylase (EC 2.7.7.22)[2]. Analogously, ADP-D-glucose pyrophosphorylase (EC 2.7.7.27) catalyses the conversion of D-Glc1P and adenosine-5′-triphosphate (ATP) into inorganic diphosphoric acid (**PP**) and ADP-D-Glc which is involved in starch biosynthesis (Figure 5). Diphosphoric acid can be hydrolysed to two molecules of phosphoric acid by diphosphatase (EC 3.6.1.1).

In plant cells that grow rapidly and thus synthesise the cell wall material, including those undergoing secondary wall thickening, an alternative pathway for the synthesis of UDP-D-Glc is catalysed by the degradative enzyme sucrose synthase (EC 2.4.1.13). This enzyme converts UDP and saccharose into UDP-D-Glc and D-Fru. The sucrose synthase-catalysed metabolism is thus linked with biosynthetic processes (i.e. cell wall or storage products) (Koch 2004).

Free monosaccharides and alduronic acids formed from homoglycosides (oligosaccharides, polysaccharides) and heteroglycosides by hydrolases and/or by transglycosylation reactions can be transformed to the corresponding phosphates by the sequential action of monosaccharide kinases and nucleotide sugar pyrophosphorylases via so-called salvage pathways (Seifert 2004). Although a general C-6 hexokinase (EC 2.7.1.1) may operate on all available sugars, C-6 kinases for specific sugars do exist. Thus, formation of D-Glc6P from D-Glc is catalysed by hexokinase (EC 2.7.1.1) and, in vertebrates and bacteria, by glucokinase (EC 2.7.1.2), formation of D-Fru6P from D-Fru by fructokinase (EC 2.7.1.4), D-Man6P from D-Man by hexokinase (EC 2.7.1.1) and mannokinase (EC 2.7.1.7).

[2]Analogous pyrophosphorylases act e.g. on D-Gal1P (UTP-hexose 1-phosphate uridylyltransferase, EC 2.7.7.10), D-Xyl1P (UTP-xylose 1-phosphate uridylyltransferase, EC 2.7.7.11) and other sugars.

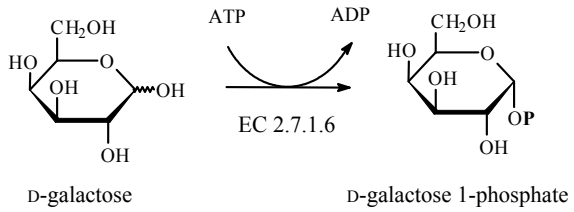


Figure 6

D-Galactose 1-phosphate (D-Gal1P) forms from D-galactose (D-Gal) in a reaction catalysed by galactokinase (EC 2.7.1.6) (Figure 6), the formation of L-fucose 1-phosphate (L-Fuc1P) from L-fucose (L-Fuc) is catalysed by fucokinase (EC 2.7.1.52), L-arabinose 1-phosphate (L-Ara1P) arises from L-arabinose (L-Ara) under the catalysis of arabinokinase (EC 2.7.1.46). D-Glucuronic acid 1-phosphate (D-GlcA1P) forms from D-glucuronic acid (D-GlcA) in a reaction catalysed by glucuronokinase (EC 2.7.1.43) and D-galacturonic acid 1-phosphate (D-GalA1P) forms from D-galacturonic acid (D-GalA) under the catalysis with galacturonokinase (EC 2.7.1.44). For simplicity, only D-Gal and L-Ara salvage pathways are shown in Figure 1 (Seifert 2004).

However, the primary route of synthesis for most nucleotide monosaccharides and the corresponding uronic acids is the modification of the sugar moiety in UDP-Glc or GDP-Man by oxidation, reduction, isomerisation, and/or decarboxylation reactions.

1.2 D-Galactose, uronic acids, pentoses, and L-rhamnose (UDP-glucose interconversion pathway)

The activation of D-Glc via UDP-D-Glc pyrophosphorylase (EC 2.7.7.9) or sucrose synthase (EC 2.4.1.13) yields a cytoplasmatic pool of UDP-D-Glc which functions, e.g., as a donor of glucose for the synthesis of cellulose and glycogen. UDP-D-Glc also serves as a substrate for the synthesis of other nucleotide sugars and nucleotide uronic acids required for the formation of cell wall matrix components, e.g. UDP-D-Gal, UDP-D-glucuronic acid (UDP-D-GlcA), UDP-D-galacturonic acid (UDP-D-GalA), and UDP-L-rhamnose (UDP-L-Rha) (Seifert 2004) (Figure 7).

UDP-D-glucose can be converted to UDP-D-Gal via a freely reversible 4-epimerisation reaction involving an enzyme-bound (UDP-D-glucose 4-epimerase, EC 5.1.3.2) 4-oxo intermediate, UDP-D-*xylo*-hexopyranos-4-ulose (called UDP-4-dehydro-D-glucose in biochemical literature) (Seifert 2004). Most of the UDP-D-Gal is ultimately needed for the synthesis of arabinogalactan-proteins and cell wall polysaccharides including xyloglucan, rhamnogalacturonan I, rhamnogalacturonan II, and the storage polysaccharide galactomannan (e.g. in guar gum). In green tissues, substantial amounts of UDP-D-Gal are also needed for the synthesis of chloroplast galactolipids.

L-Rhamnose (L-Rha) is a predominant 6-deoxyhexose in most higher plants, and represents

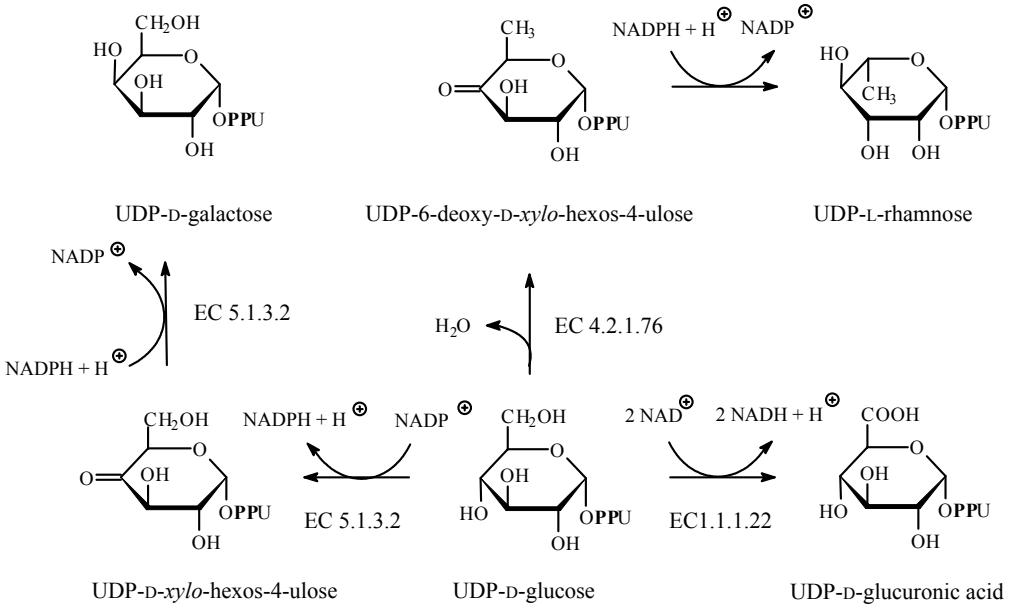


Figure 7

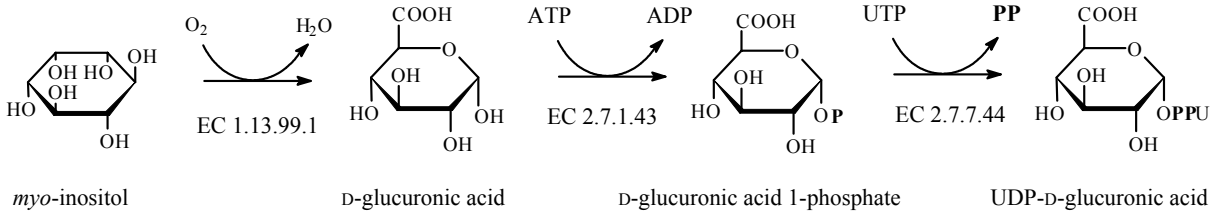


Figure 8

a major component of the pectic polysaccharide rhamnogalacturonan I and rhamnogalacturonan II. L-Rhamnosyl residues are also often conjugated to secondary metabolites yielding the corresponding rhamnosides (Seifert 2004). The irreversible conversion of UDP-D-Glc to UDP-L-Rha proceeds via UDP-6-deoxy-D-*xylo*-hexopyranos-4-ulose (UDP-4-dehydro-6-deoxy-D-glucose or UDP-4-dehydro-D-chinovose) by L-rhamnose synthase, which hypothetically consists of sequentially acting UDP-D-glucose 4,6-dehydratase (EC 4.2.1.76) and UDP-6-deoxy-D-*xylo*-hexos-4-ulose 3,5-epimerase-4-reductase (UDP-4-oxo-6-deoxy-D-glucose 3,5-epimerase-4-reductase).

Higher plants incorporate large amounts of D-GluA residues into cell wall glucuronoarabinoxylans. D-GluA forms irreversibly from UDP-D-Glc by UDP-D-glucose dehydrogenase (EC 1.1.1.22) (Figure 7) (Dumville & Fry 2000). Biogenesis of UDP-D-GlcA can also occur via an alternative pathway from *myo*-inositol, which involves the action of *myo*-inositol oxygenase (EC 1.13.99.1) and the sequential action of D-glucuronokinase (EC 2.7.1.43) and UDP-glucuronic acid pyrophosphorylase (EC 2.7.7.44) (Figure 8).

UDP-D-glucuronic acid represents a major branched-point in the biosynthesis of several other nucleotide sugars (Seifert 2004). Higher plants incorporate large amounts of D-GalA residues in the backbones of pectic material and the interconversion between UDP-D-GlcA and UDP-D-GalA is freely reversible (Figure 9). It is catalysed by UDP-D-galacturonic acid 4-epimerase (EC 5.1.3.6). The 4-epimerisation reaction proceeds via a 4-oxo intermediate, UDP-D-*xylo*-hex-4-ulopyranosic acid (UDP-4-dehydro-D-glucuronic acid), which is stereospecifically reduced by the enzyme prosthetic group NAD(P)H + H^+ to UDP-D-GalA.

UDP-D-glucuronic acid also serves as the precursor for the synthesis of UDP-D-xylose (UDP-D-Xyl), UDP-L-arabinose (UDP-L-Ara), and UDP-D-apiose (UDP-D-Api) (Figure 9). UDP-D-glucuronate decarboxylase (synonymous with UDP-D-xylose synthase, EC 4.1.1.35) converts UDP-D-GluA into UDP-D-Xyl in an essentially irreversible reaction. The interconverting enzyme uses an $NAD(P)^+$ cofactor to generate a transient 4-oxo intermediate, UDP-D-*xylo*-hex-4-ulopyranosic acid (UDP-4-dehydro-D-glucuronic acid). This intermediate loses CO_2 in an elimination reaction typical of β-oxo

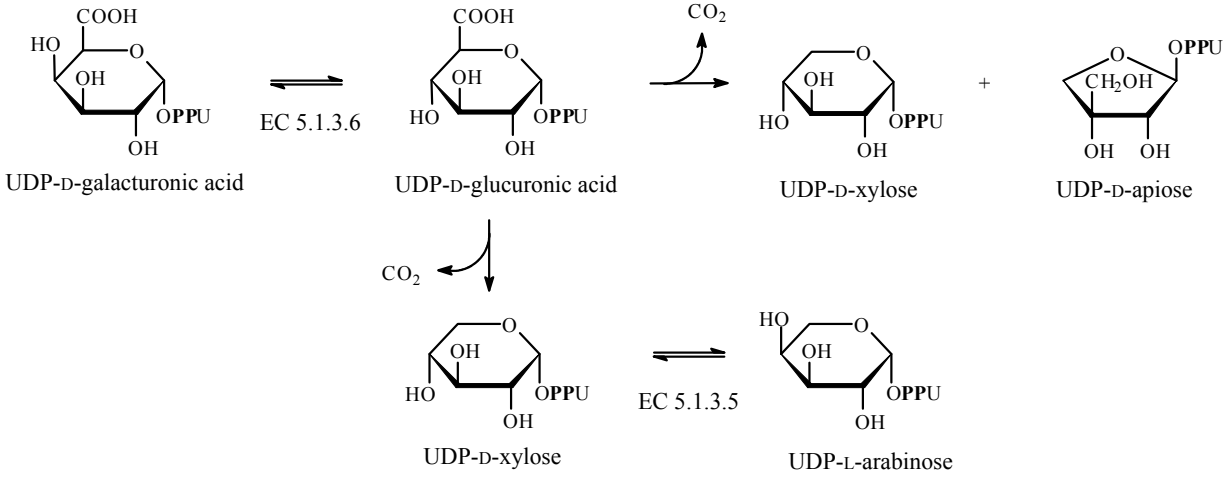


Figure 9

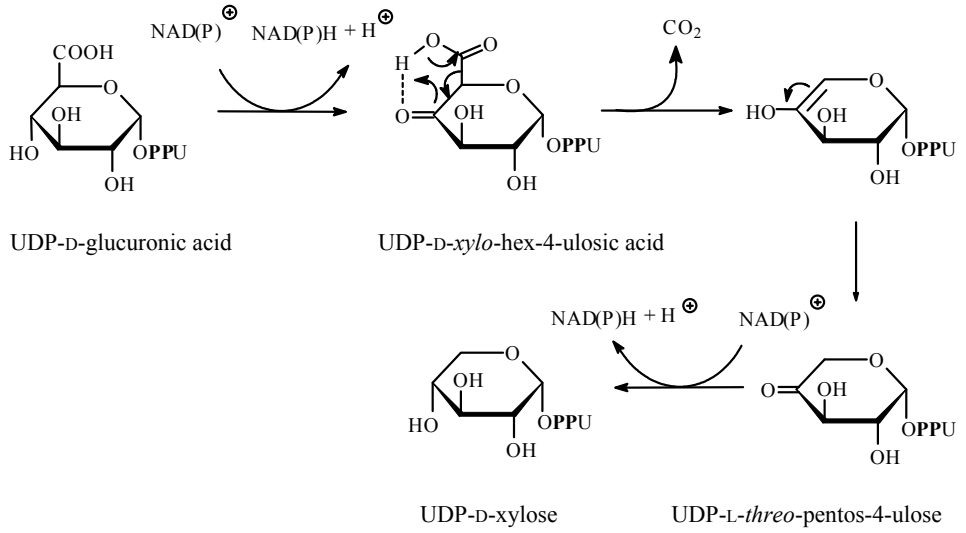


Figure 10

acid and forms UDP-L-*threo*-pentopyranos-4-ulose (UDP-4-dehydro-D-xylose), which is then stereospecifically reduced to yield UDP-D-Xyl (Figure 10).

The second bifunctional decarboxylase UDP-D-apiose/UDP-D-xylose synthase (also referred to as UDP-D-glucuronate cyclase) converts UDP-D-GlcA into approximately equimolar amounts of UDP-D-Xyl (Figure 9) and UDP-D-Api (Mølhøj *et al*. 2003). In most higher plants, D-Api is specifically found in complex polysaccharide rhamnogalacturonan II. This transformation proceeds via UDP-L-*threo*-pentopyranos-4-ulose (Figure 11). It is believed that it may involve aldol cleavage between C-2 and C-3 followed by isomerisation and aldol condensation between C-2 and C-4. The aldehyde group at C-3 of the resulting intermediate would be converted to hydroxymethyl group by the transiently reduced $NAD(P)H^+$ cofactor of the enzyme.

UDP-D-xylose is reversibly converted to UDP-L-Ara by UDP-D-xylose 4-epimerase (EC 5.1.3.5) via a common 4-oxo intermediate UDP-L-*threo*-pentos-4-ulose (UDP-4-dehydro-D-xylose) (Seifert 2004).

1.3 L-Galactose, L-gulose, and L-fucose (GDP-mannose interconversion pathway)

GDP-D-mannose (GDP-D-Man) is an activated form of D-mannose for the incorporation into *N*-linked glycans and mannose-containing cell wall components such as glucomannans and galactomannans. GDP-D-mannose also serves as a substrate for nucleotide sugar interconversion enzymes yielding GDP-L-fucose (GDP-L-Fuc) and GDP-L-galactose (GDP-L-Gal) (Figure 12). The latter compound serves as the donor for glycosyltransferases but it also plays a key role in the biosynthesis of L-ascorbic acid (Seifert 2004).

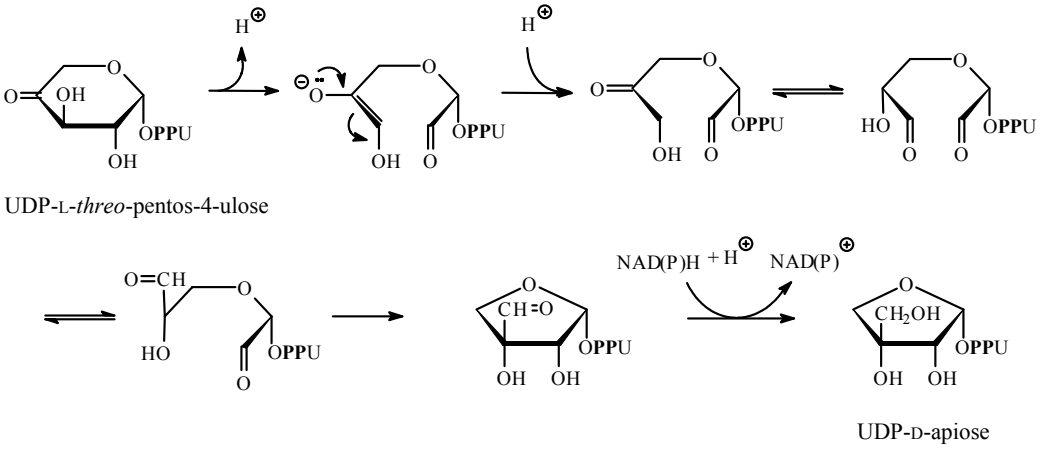


Figure 11

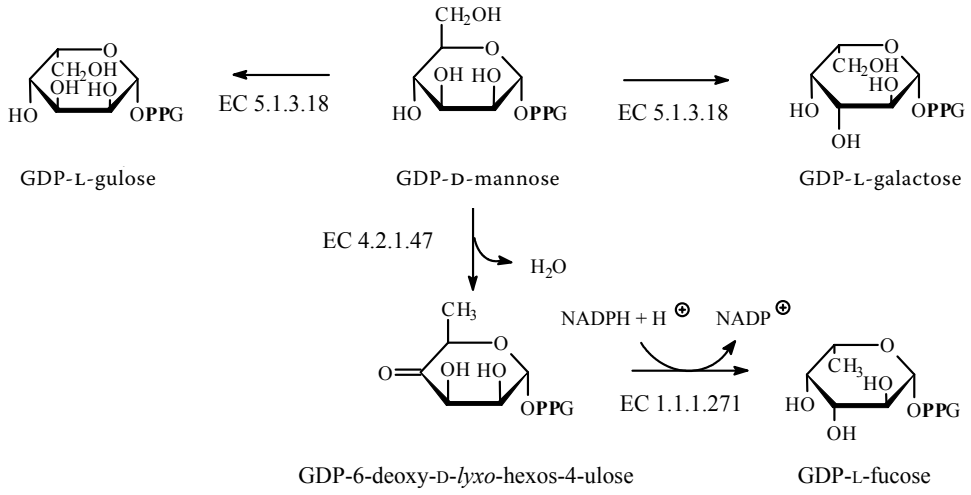


Figure 12

GDP-L-fucose formation requires subsequent activities of GDP-D-mannose 4,6-dehydratase (EC 4.2.1.47) and a bifunctional GDP-L-fucose synthase (EC 1.1.1.271). The intermediate, UDP-6-deoxy-D-*lyxo*-hexopyranos-4-ulose (4-dehydro-6-deoxy-D-mannose), formed by the 4,6-dehydratase reaction, undergoes a 3,5-epimerisation followed by a C-4 reduction yielding L-Fuc. The fucosylated side chains of xyloglucans are believed to enhance the rate of formation of strong xyloglucan-cellulose interactions.

GDP-L-galactose, on the other hand, is formed by GDP-D-mannose 3,5-epimerase (EC 5.1.3.18), which also generates GDP-L-gulose (GDP-L-Gul). This conversion represents a 3,5-epimerisation via enol intermediates. L-Galactose is incorporated into xyloglucans and *N*-linked glycans.

1.4 Aminosugars

1.4.1 Aldosamines and related compounds

Aminosugars such as D-glucosamine (2-amino-2-deoxy-D-glucose), D-galactosamine (2-amino-2-deoxy-D-galactose), and D-mannosamine (2-amino-2-deoxy-D-mannose) and their *N*-acetyl derivatives occur as principal components of various biologically active oligosaccharides and biopolymers (Voet & Voet 1990; Dewick 2002; Velíšek 2002). For instance, *N*-acetyl-D-glucosamine (GlcNAc) is a building unit of milk oligosaccharides, chitin, peptidoglycans of bacterial cell walls (mureins), and glycosaminoglycans (mucopolysaccharides) that function in various biological systems as components of proteoglycans (hyaluronic acid and dermatan sulfate). *N*-Acetyl-D-galactosamine (GalNAc) is a building unit of proteoglycans (chondroitin sulfate and dermatan sulfate).

The starting compound for the synthesis of *N*-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose, UDP-GlcNAc) is D-Fru6P (Figure 13). It is first converted to D-glucosamine 6-phosphate in a reaction with L-glutamine catalysed by glucosamine 6-phosphate synthase (EC 2.6.1.16)[3]. D-Glucosamine 6-phosphate is acetylated and forms *N*-acetyl-D-glucosamine 6-phosphate using acetyl-CoA as the acetyl group donor (glucosamine 6-phosphate *N*-acetyltransferase, EC 2.3.1.4), *N*-acetyl-D-glucosamine 6-phosphate is converted to *N*-acetyl-D-glucosamine 1-phosphate by phosphoacetylglucosamine mutase (EC 5.4.2.3).

α-D-Glucosamine 1,6-phosphomutase (EC 5.4.2.10) catalyses the isomerisation of D-glucosamine 6-phosphate to D-glucosamine 1-phosphate in the pathway leading to bacterial cell wall peptidoglycan and lipopolysaccharide biosyntheses. D-Glucosamine 1-phosphate is then acetylated to *N*-acetyl-D-glucosamine 1-phosphate by D-glucosamine 1-phosphate *N*-acetyltransferase (EC 2.3.1.157). *N*-acetyl-D-glucosamine 1-phosphate reacts with UTP yielding UDP-*N*-acetyl-D-glucosamine (UDP-GlcNAc pyrophosphorylase, EC

[3]The reverse reaction, i.e. hydrolysis of *N*-acetyl-D-glucosamine to D-fructose 6-phosphate and ammonia is catalysed by glucosamine 6-phosphate deaminase (EC 3.5.99.6); hydrolysis of *N*-acetyl-D-glucosamine 6-phosphate to D-glucosamine 6-phosphate is provided by *N*-acetylglucosamine 6-phosphate deacetylase (EC 3.5.1.25).

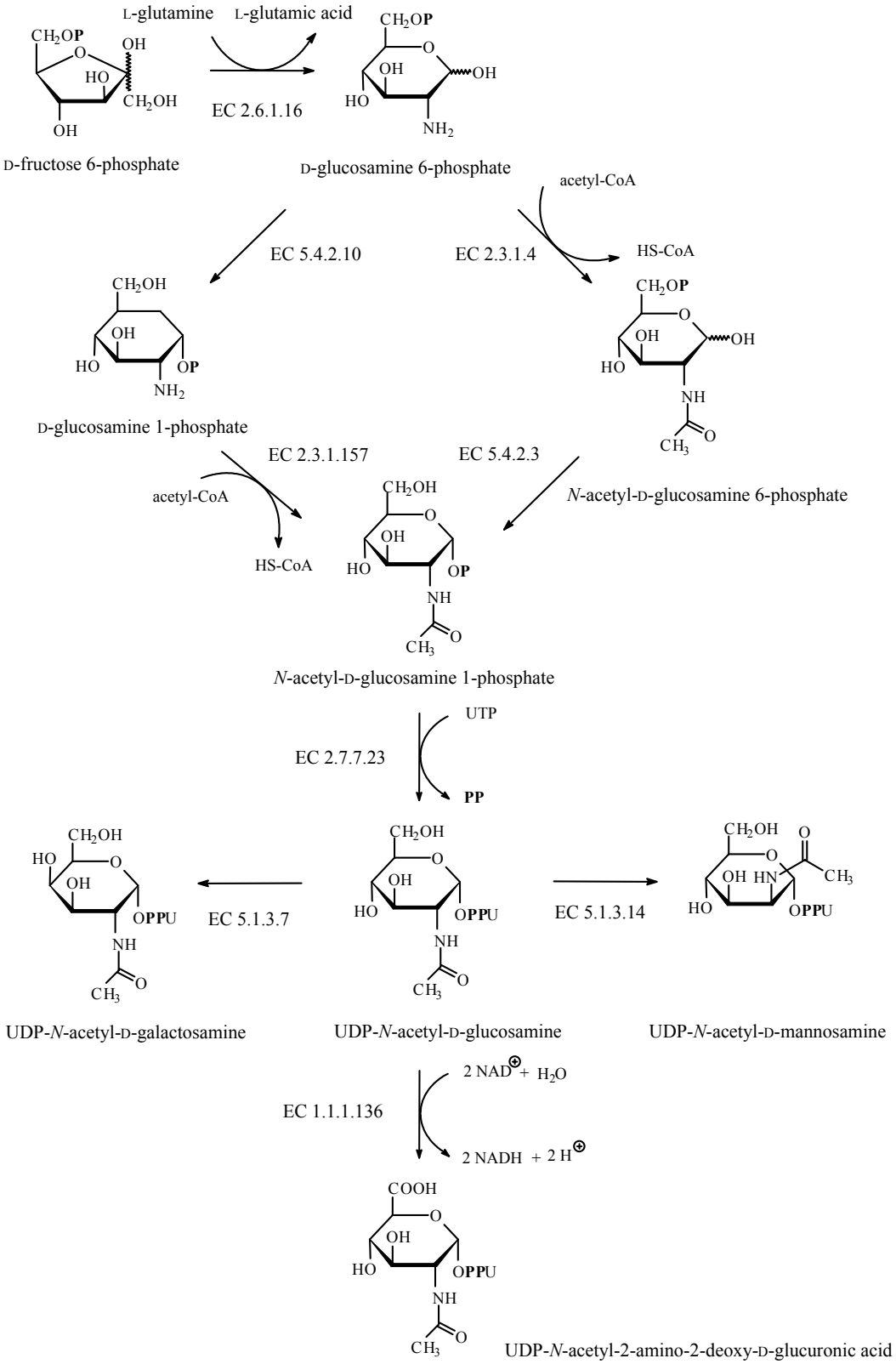


Figure 13

2.7.7.23). This activated form of *N*-acetyl-D-glucosamine can be further converted to UDP-*N*-acetyl-D-glucuronic acid (UDP-*N*-acetylglucosamine 6-dehydrogenase, EC 1.1.1.136), UDP-*N*-acetyl-D-galactosamine, i.e. UDP-2-acetamido-2-deoxy-D-galactose (UDP-*N*-acetylglucosamine 4-epimerase,

EC 5.1.3.7), and UDP-*N*-acetyl-D-mannosamine, i.e. UDP-2-acetamido-2-deoxy-D-mannose (UDP-*N*-acetylglucosamine 2-epimerase, EC 5.1.3.14). Finally, various transferases are involved in the biosynthesis of biopolymers containing aminosugars (Voet & Voet 1990).

1.4.2 Acetylmuramic acid

Bacterial cell walls contain an unusual saccharide, *N*-acetyl-β-muramic acid, i.e. 2-acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose (β-MurAc), bound in peptidoglycan structures called murein (Dewick 2002; Voet & Voet 1990). The peptidoglycan chains are composed of alternating β-(1→4) linked *N*-acetyl-D-glucosamine and *N*-acetylmuramic acid residues that are cross-linked via peptide structures (through the lactyl group of the *N*-MurAc to link the peptide via amide/peptide bond). The peptidoglycan building stone, UDP-*N*-MurAc, forms from UDP-*N*-acetyl-D-glucosamine and phosphoenolpyruvic acid via the intermediate UDP-*N*-acetyl-3-*O*-(1-carboxyvinyl)-D-glucosamine (UDP-*N*-acetyl-D-glucosamine 1-carboxyvinyl transferase, EC 2.5.1.7), which is reduced to UDP-*N*-MurAc by UDP-*N*-acetylmuramate dehydrogenase (EC 1.1.1.158) (Figure 14).

1.4.3 Acetylneuraminic acid

Sialic acids are a family of nine carbon α-oxo acids that play a wide variety of biological roles in higher animals and some microorganisms. Sialic acids comprise about 50 members which are derivatives of neuraminic (5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic) acid (Neu). They carry various substituents at the amino or hydroxyl groups. The amino group of Neu is acetylated or glycolated, while at all non-glycosidic hydroxyl residues one or various acetyl groups may occur. In mammals, sialic acids are found at the distal ends of cell surface conjugates, and thus are major determinants of specific biological functions such as cellular adhesion, formation or masking of recognition determinants and stabilisation of glycoprotein structures (Schauer 2004). In certain strains of pathogenic bacteria, they occur as a homopolysaccharide (polysialic acid) with α-(2→8) and/or α-(2→9) ketosidic linkages in capsular polysaccharides that mask the organism against the immune system (Revilla-Nuin *et al*. 1998).

N-acetylneuraminic (5-acetamido-3,5-di-deoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic) acid (Neu5Ac), *N*-glycolylneuraminic acid (Neu5Gc), and *N*-acetyl-9-*O*-acetylneuraminic acid (Neu5,9Ac_2) are the three most frequently occurring members of the sialic acids family. Only Neu5Ac is ubiquitous, while the others are not found in all species (Schauer 2004).

N-acetyl-α-neuraminic acid, a constituent of food glycoproteins (e.g. milk κ-caseins) and glycolipids (e.g. gangliosides), forms by aldol-type reaction from *N*-acetyl-D-mannosamine and pyruvic acid (Figure 15).

The reaction is more complex in the enzyme-catalysed version (Lucka *et al.* 1999). The two enzymes initiating the biosynthesis of Neu5Ac from *N*-acetyl-D-mannosamine (mammals), the hydrolysing UDP-*N*-acetylglucosamine 2-epimerase (EC 5.1.3.14) and *N*-acetylmannosamine kinase (Mg^{2+}/K^{+}-dependent enzyme, EC 2.7.1.60), are parts of one bifunctional enzyme that catalyses hydrolysis of UDP-*N*-acetyl-D-glucosamine, its isomerisation to *N*-acetyl-D-mannosamine, and phosphorylation of *N*-acetyl-D-mannosamine to *N*-acetyl-D-mannosamine 6-phosphate. The next step, aldol-type condensation of *N*-acetyl-D-mannosamine 6-phos-

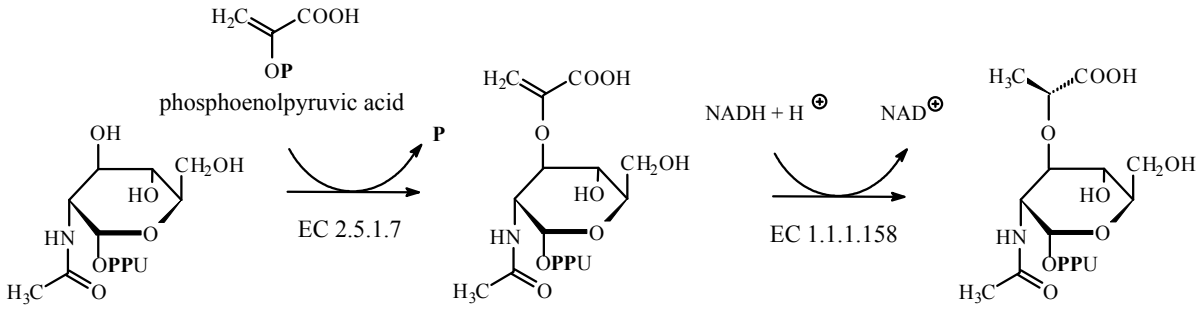


Figure 14

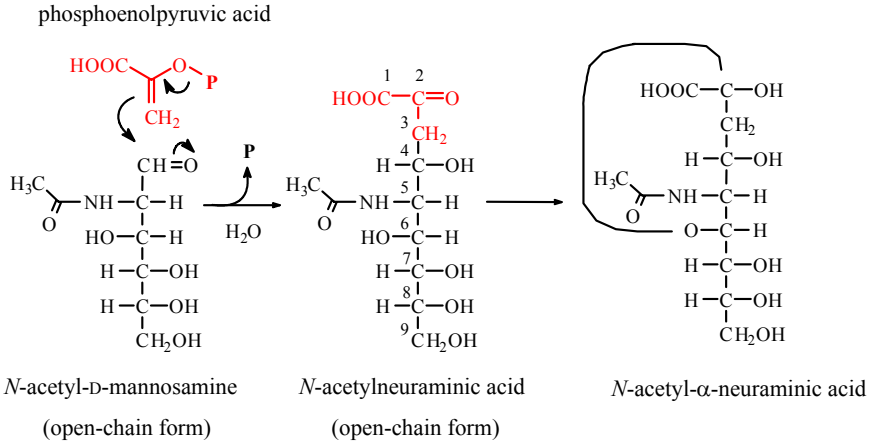


Figure 15

phate (open-chain form) with phosphoenolpyruvic acid to *N*-acetylneuraminic acid 9-phosphate, is catalysed by *N*-acetylneuraminate 9-phosphate synthetase (EC 2.5.1.57). Hydrolysis of this phosphate by *N*-acetyl-neuraminate 9-phosphatase (EC 3.1.3.29) yields Neu5Ac. *N*-Acetylneuraminate synthase (EC 2.5.1.56) generates Neu5Ac from *N*-acetyl-D-mannosamine and phosphoenolpyruvic acid (bacteria) (Figure 16).

2 Oligosaccharides

The formation of glycosides, oligosaccharides, and polysaccharides is dependent on the activation of the respective sugar by its binding to a nucleoside diphosphate. Nucleophilic displacement of the respective nucleoside diphosphate-leaving group by a suitable nucleophile then generates the new sugar derivative[4]. This will be a glycoside

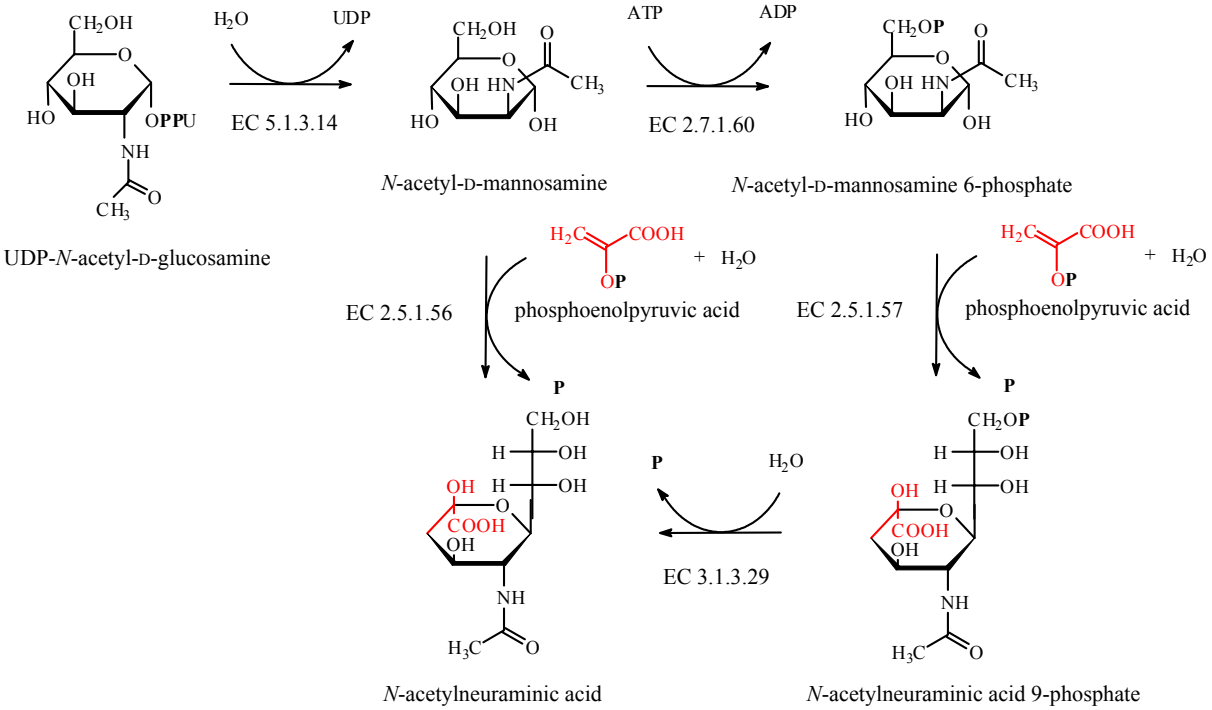


Figure 16

[4]Synthesis of nucleoside triphosphates from nucleoside diphosphates is realised by nucleoside-diphosphate kinases. Many ribo- and deoxyribonucleoside triphosphates can act as donors, the most usual one is ATP. For example, UTP from UDP is formed by uridine diphosphate kinase (EC 2.7.4.6) that carries the phosphate residue from ATP to UDP.

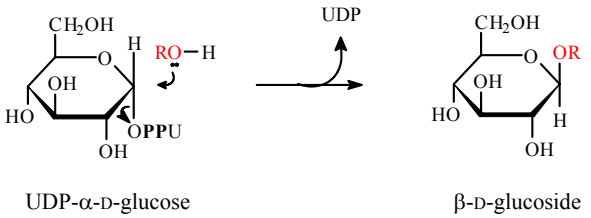


Figure 17

if the nucleophile is a suitable aglycone molecule or oligosaccharide if the nucleophile is another sugar molecule.

This reaction, if mechanistically of S_N2 type, should give an inversion of configuration at C-1 in the electrophile, generating a product with β-configuration and nucleoside diphosphate (Figure 17). Many of the linkages formed between sugar monomers actually have α-configuration, and it is believed that a double S_N2 mechanism operates involving also a nucleophilic group of the enzyme (Dewick 2002) (Figure 18).

2.1 Saccharose

The non-reducing disaccharide saccharose, β-D-fructofuranosyl-(2⟷1)-α-D-glucopyranoside, is one of the most abundant products in nature (Velíšek 2002). Most photosynthetic eukaryotes and some specific prokaryotes synthesise saccharose. In higher plants, saccharose occupies a unique position, comparable only to glucose in the animal world. It is a major product of photosynthesis, with a central role as a primary transport sugar, in the growth, development, storage, signal transduction, and stress (Winter & Huber 2000; Cumino *et al.* 2002).

The biosynthetic enzymes sucrose-phosphate synthase (EC 2.4.1.14) and sucrose-phosphate phosphatase (EC 3.1.3.24), and the degradative enzymes invertase (EC 3.2.1.26) and sucrose synthase (EC 2.4.1.13)[5] have been well characterised in plants and unicellular eukaryotes (Koch 2004). Biosynthesis of saccharose starts with the glucose donor UDP-D-Glc that reacts with the sugar acceptor β-D-Fru6P and forms saccharose 6^F-phosphate and UDP. Saccharose 6^F-phosphate is then hydrolysed to saccharose and phosphate (Figure 19).

2.2 Trehalose

α,α-Trehalose, α-D-fructofuranosyl-(1⟷1)-α-D-glucopyranoside, is another non-reducing disaccharide that occurs in plants and a wide range

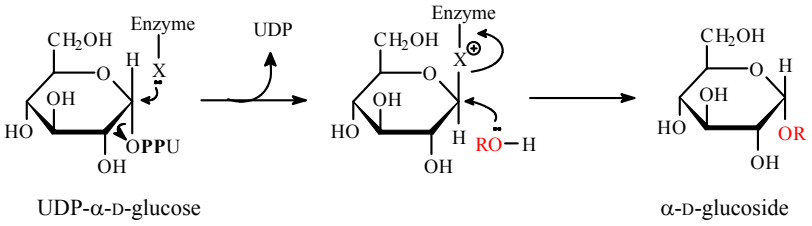


Figure 18

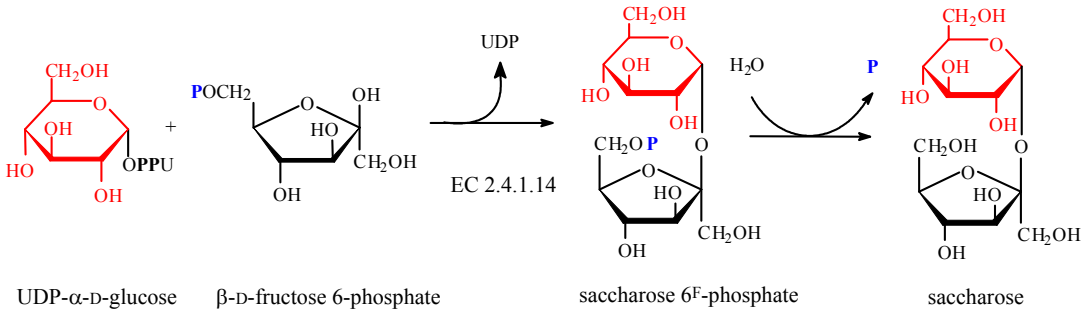


Figure 19

[5]The only known enzymatic paths of saccharose cleavage in plants are catalysed by invertase (EC 3.2.1.26) and reversible sucrose synthase (EC 2.4.1.13) reactions. Invertase-catalysed hydrolysis of saccharose into D-glucose and D-fructose has been associated with cell expansion, whereas sucrose synthase-catalysed decomposition into D-fructose and UDP-D-Glc has been linked with biosynthetic processes such as starch biosynthesis.

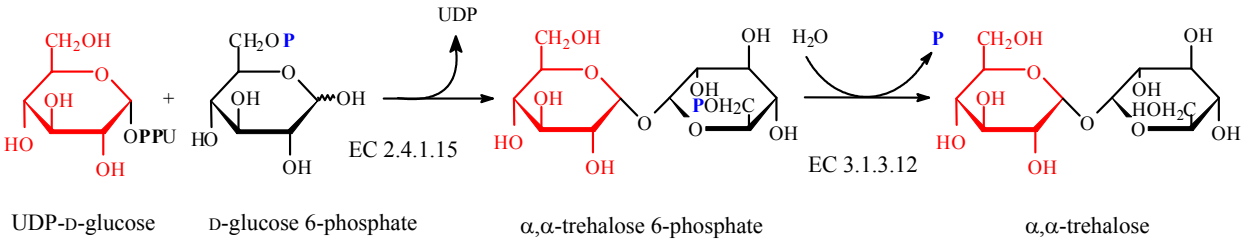


Figure 20

of organisms such as bacteria, fungi, nematodes, and crustaceans. In addition to its function as a storage and transport sugar, α,α-trehalose plays an important role in the protection against stress especially during heat stress and dehydration (Wingler 2002). Biosynthesis of α,α-trehalose is a two-step reaction. It starts with UDP-D-Glc that reacts with D-Glc6P and forms α,α-trehalose 6-phosphate (trehalose 6-phosphate synthase, EC 2.4.1.15). Trehalose 6-phosphate phosphatase (EC 3.1.3.12) catalyses the phosphate hydrolysis to α,α-trehalose (Figure 20).

2.3 Raffinose

Biosynthesis of trisaccharide raffinose, α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl-(1↔2)-β-D-frucofuranoside, starts with UDP-D-Gal that, under catalysis with galactinol synthase (EC 2.4.1.123), reacts with *myo*-inositol and forms pseudooligosaccharide galactinol (1-*O*-α-D-galactopyranosyl-1L-*myo*-inositol) (Loewus & Murthy 2000). The subsequent reaction of galactinol with saccharose catalysed by galactinol-sucrose galactosyltransferase (EC 2.4.1.82) yields raffinose

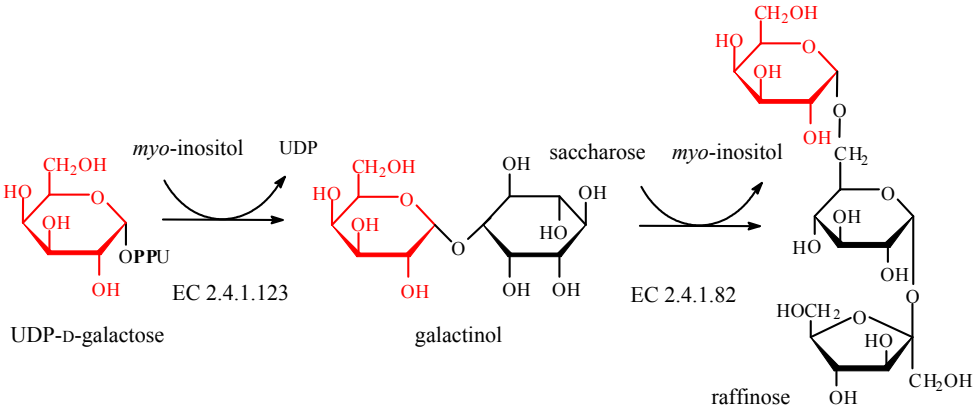


Figure 21

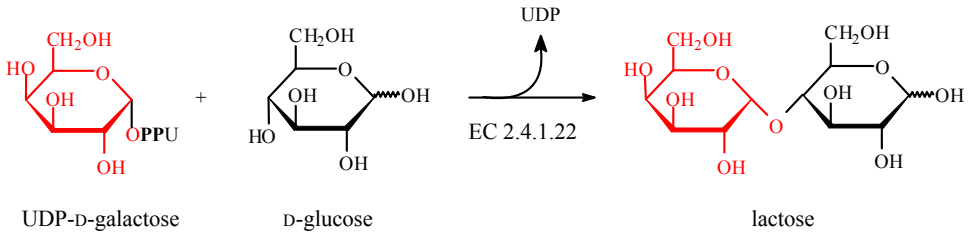


Figure 22

[6]Lactose synthase (EC 2.4.1.22) is a complex composed of two distinct protein components, a catalytic and a regulatory subunit. The catalytic subunit, UDP-galactose-glycoprotein galactosyltransferase (EC 2.4.1.38), does not catalyse the biosynthesis of lactose. It participates in the biosynthesis of oligosaccharide chains of secretory and membrane-bound glycoconjugates (e.g. glycoproteins and glycopeptides) by catalysing the transfer of Gal from UDP-Gal to the terminal *N*-acetyl-β-D-glucosaminyl residue. The regulatory subunit (i.e. the modifier protein α-lactalbumin) in the lactose synthase complex, reversibly binds to UDP-galactose-glycoprotein galactosyltransferase and thus promotes, in the presence of Mn^{2+} ions, glucose binding and facilitates the biosynthesis of lactose.

and *myo*-inositol is released (Figure 21). Another galactosyltransferase (galactinol-raffinose galactosyltransferase or stachyose synthase, EC 2.4.1.67) catalyses galactosyl transfer from galactinol to raffinose resulting in the formation of tetrasaccharide stachyose, α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl-(1↔2)-β-D-frucofuranoside.

2.4 Lactose

Lactose, β-D-galactopyranosyl-(1→4)-D-glucopyranose, the major reducing disaccharide in most mammalian milks, is synthesised from UDP-D-Gal and D-glucose exclusively in the lactating mammary glands (Berger & Rohrer 2003). The reaction is catalysed by the enzyme lactose synthase (EC 2.4.1.22)[6] (Figure 22).

3 Sugar alcohols and cyklitols

3.1 Glucitol

Aldoses can be converted to sugar alcohols by the widely specific aldose reductase (EC 1.1.1.21). This NAD(P)H-dependent enzyme acts e.g. on D-glucose, D-galactose, L-arabinose, D-xylose and some other sugars and forms the corresponding sugar alcohols, i.e. D-glucitol (D-sorbitol), galactitol, L-arabinitol, and xylitol, respectively (Figure 23).

Several other oxidoreductases acting with NAD^+ or $NADP^+$ as acceptors catalyse the oxidation of sugar alcohols to the corresponding parent sugars and *vice versa*. For instance, NAD^+-dependent sorbitol dehydrogenase (EC 1.1.1.14) also acts on D-glucitol (giving D-fructose) and other closely related sugar alcohols. NAD^+-dependent mannitol dehydrogenase (EC 1.1.1.67) acts on D-mannitol giving D-fructose (Dewick 2002).

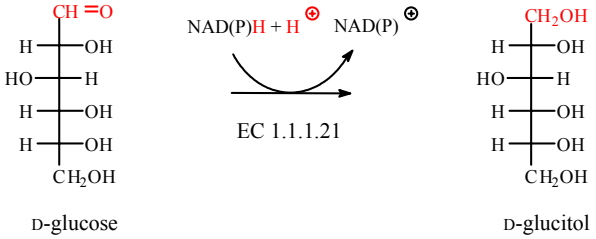


Figure 23

3.2 Inositols

Biosynthetic conversion of D-glucose 6-phosphate to free *myo*-inositol involves two enzymatic steps. The first step is the irreversible cyclisation of D-Glc6P to 1L-*myo*-inositol 1-phosphate (1D-*myo*-inositol 3-phosphate). This reaction is catalysed by inositol 1-phosphate synthetase (EC 5.5.1.4)[7]. The second step, the loss of phosphate catalysed by inositol phosphatase (EC 3.1.3.25), releases free *myo*-inositol (Figure 24). Altogether, this scheme constitutes the sole pathway of *myo*-inositol biosynthesis in cyanobacteria, algae, fungi, plants, and animals, and occupies the central role in their cellular metabolism (Loewus & Murthy 2000).

Functionally, the conversion of D-Glc6P to 1L-*myo*-inositol 1-phosphate involves three sub steps: NAD^+-coupled oxidation of D-Glc6P at C-5, aldol condensation between C-1 and C-6 of D-*xylo*-5-hexulose 6-phosphate (5-oxo-D-glucose 6-phosphate), NADH catalysed reduction of 1L-2-*myo*-inosose 1-phosphate (1D-2-*myo*-inosose 3-phosphate, D-2,3,6/3,5-pentahydroxycyclohexane 2-phosphate) to yield 1L-*myo*-inositol 1-phosphate (1D-*myo*-inositol 3-phosphate).

Only *myo*-inositol is biosynthesised *de novo* from D-Glc6P. Metabolic processing of *myo*-inositol then produces many biologically important products (Loewus & Murthy 2000). The oxidation of *myo*-inositol gives D-GlcA (p. 134), conjugation

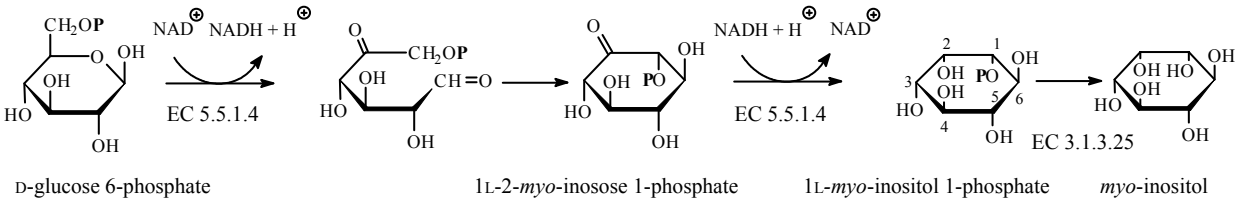
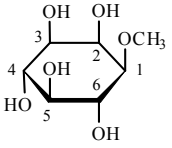
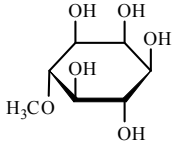


Figure 24

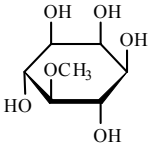
[7]This enzyme requires NAD^+, which dehydrogenates the -CHOH- group to -CO- at C-5 of the glucose 6-phosphate, changing C-6 into an active methylene, able to condense with the –CH=O group at C-1. Finally, the enzyme-bound NADH reconverts C-5 into the -CHOH- form. The enzyme has a preference for the β-anomeric form of D-glucose 6-phosphate.



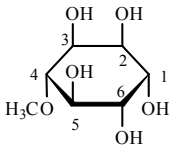
D-bornesitol
(1D-1-*O*-methyl-*myo*-inositol)



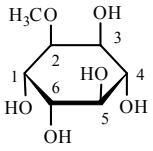
D-ononitol
(1D-4-*O*-methyl-*myo*-inositol)



D-sequoyitol
(1D-5-*O*-methyl-*myo*-inositol)



D-pinitol
(1D-4-*O*-methyl-*chiro*-inositol)



L-quebrachitol
(1L-2-*O*-methyl-*chiro*-inositol)

Figure 25

with UDP-D-Gal forms galactinol (p. 141), the galactosyl donor for biosynthesis in the raffinose and galactopinitol series of pseudosaccharides. The isomerisation of *myo*-inositol produces other stereo-forms of inositol. The methylation of *myo*-inositol and other isomeric (*scyllo*-, *chiro*-, *muco*-, and *neo*-) inositols leads to *O*-methyl inositols (bornesitol, ononitol, sequoyitol, pinitol, quebrachitol, etc.), which participate in stress-related responses, storage of seed products, and the production of inositol-glycosides (Figure 25).

The research on inositol-linked stress-related processes in plants is still in its pioneering stage. For example, the biochemical conversion of *myo*-inositol to D-pinitol is catalysed by *O*-methyl transferase yielding D-ononitol. The enzyme catalysing the next step, epimerisation of C-1 of ononitol, has yet to be examined. This reaction proceeds via 1D-4-*O*-methyl-1-*myo*-inosose as the intermediate and yields D-pinitol as the final product. Furthermore, *myo*-inositol is involved in biosynthesis of phytic acid, phosphatidylinositol, its polyphosphates, and other lipids.

EC (Enzyme Commission) numbers and some common abbreviations

EC (Enzyme Commission) numbers, assigned by IUPAC-IUBMB, were taken from KEGG: Kyoto Encyclopedia of Genes and Genomes, http://www.biologie.uni-hamburg.de. In many structures, the abbreviation **P** is used to represent the phosphate group and **PP** the diphosphate group. At physiological pH, these and some other groups will be ionised, but in pictures the unionised forms are depicted to simplify the structures, to eliminate the need for counter-ions, and to avoid the mechanistic confusion.

Ac	acetyl
ADP	adenosine-5′-diphosphate
Api	apiose
Ara	arabinose
ATP	adenosine-5′-triphosphate
CDP	cytidine-5′-diphosphate
CoA	coenzyme A as a part of a thioester
Fru	fructose
Fuc	fucose
Gal	galactose
GalA	galacturonic acid
GalNAc	*N*-acetyl-D-galactosamine
GDP	guanosine-5′-diphosphate
GTP	guanosine-5′-triphosphate
Glc	glucose
GlcA	glucuronic acid
GlcNAc	*N*-acetylglucosamine
Gul	gulose
Man	mannose
Mur	muramic acid
Neu	neuraminic acid
P	phosphoric acid
PP	diphosphoric acid
Rha	rhamnose
UDP	uridine-5′-diphosphate
UTP	uridine-5′-triphosphate
Xyl	xylose

References

Berger E.G., Rohrer J. (2003): Galactosyltransferase-still up and running. Biochemie, **85**: 261–274.

Cumino A., Curatti L., Giarrocco L., Salerno G.L. (2002): Sucrose metabolism: Anabaena sucrose-phos-

phate synthase and sucrose-phosphate define minimal functions domains shuffled during evolution. FEBS Letters, **517**: 19–23.

Dewick P.M. (2002): Medicinal Natural Products. A Biosynthetic Approach. 2nd Ed. Wiley, New York, USA.

Dumville J.C., Fry S.C. (2000): Uronic acid-containing oligosaccharins: Their biosynthesis, degradation and signalling roles in non-diseased plant tissues. Plant Physiology and Biochemistry, **38**: 125–140.

Koch K. (2004): Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Current Opinion in Structural Biology, **7**: 235–246.

Loewus F.A., Murthy P.P.N. (2000): *myo*-Inositol metabolism in plants. Plant Science, **150**: 1–19.

Lucka L., Krause M., Danker K., Reutter W., Horstkorte R. (1999): Primary structure and expression analysis of human UDP-*N*-acetyl-glucosamine-2-epimerase/*N*-acetylmannosamine kinase, the bifunctional enzyme in neuraminic acid biosynthesis. FEBS Letters, **454**: 341–344.

Mølhøj M., Verma R., Reiter W.-D. (2003): The biosynthesis of the branched-chain sugar D-apiose in plants: functional cloning and characterization of a UDP-D-apiose/UDP-D-xylose synthase from *Arabidopsis*. The Plant Journal, **35**: 693–703.

Reiter W.-D. (2002): Biosynthesis and properties of the plant cell wall. Current Opinion in Plant Biology, **5**: 536–542.

Reiter W.-D., Vanzin G.F. (2001): Molecular genetics of nucleotide sugar interconversion pathways in plants. Plant Molecular Biology, **47**: 95–113.

Revilla-Nuin B., Rodriguez-Aparicio L.B., Ferrero M.A., Reglero A. (1998): Regulation of capsular polysialic acid biosynthesis by *N*-acetyl-D-mannosamine, an intermediate of sialic acid metabolism. FEBS Letters, **426**: 191–195.

Schauer R. (2004): Sialic acids: fascinating sugars in higher animals and man. Zoology, **107**: 49–64.

Seifert G.J. (2004): Nucleotide sugar interconversions and cell wall biosynthesis: how to bring the inside to the outside. Current Opinion in Plant Biology, **7**: 277–284.

Velíšek J. (2002): Chemie potravin I. 2. vydání. Ossis, Tábor.

Velíšek J., Cejpek K. (2005): Biosynthesis of food constituents: Saccharides. 2. Polysaccharides. Czech Journal of Food Science, **23**: 173–183.

Voet D., Voet J.G. (1990). Biochemie. Victoria Publishing, Praha.

Wingler A. (2002): The function of trehalose biosynthesis in plants. Phytochemistry, **60**: 437–440.

Winter H., Huber S.C. (2000): Regulation of sucrose metabolism in higher plants: localization and regulation of activity of key enzymes. Critical Reviews in Plant Sciences, **19**: 31–67.

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Corresponding author:

Prof. Ing. Jan Velíšek, DrSc., Vysoká škola chemicko-technologická v Praze, Fakulta potravinářské a biochemické technologie, Ústav chemie a analýzy potravin, Technická 5, 166 28 Praha 6, Česká republika
tel.: + 420 220 443 177, fax: + 420 233 339 990, e-mail: jan.velisek@vscht.cz