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STARCH AND OTHER POLYSACCHARIDES – MODIFICATION AND APPLICATIONS – A REVIEW

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Following the review concerning the nonenergetic functions of saccharides in plants [Kączkowski, 2002] this review is devoted to the presentation of polysaccharide synthesis breakdown and modification, using available chemical and biological methods, in order to alter the concomitant properties towards the practically required directions. For comparison to starch, as the main plant reserve polysaccharide, other components of this type, obtained at present by the microbial biotechnology methods and applied in clinical, agricultural and nutritional industries, are also shortly reviewed. As variable saccharides are important components of glycosides, some more interesting groups of such compounds are also referred, including cooperating glycosyltransferases (GT) as well as their modification possibilities and application areas; they concern polyketide, flavonoid (anthocyanidin) and cyanogenic glycosides, glucosinolates, and some others.

INTRODUCTION

Many interesting information, concerning the metabolism of saccharides in higher plants has recently been presented in the literature. Some new nonenergetic functions of mono- and oligosaccharides have been reviewed previously [Kączkowski, 2002], but in the following article the main aspects of some polysaccharide functions and modifications are reported. To those of the greatest importance to the human and animal nutrition, some industrial uses, and plant cell wall building belong starch, cellulose and xyloglucans, particularly, when they undergo natural or artificial modifications. As cellulose and other cell wall polysaccharides need more detailed treatment, this review is limited to the storage ones, mainly to starch biosynthesis, degradation and modifications. The latter concern mainly the forms significant for broad practical uses and, to some extent, those substituting this polysaccharide obtained biotechnologically. Of interest are also glycosyltransferases, which can modify the structure of cell wall xyloglucans and the content of low molecular weight glycosides, particularly, when altered products can be formed by the enzyme modification. Examples of such modifications are also reviewed below. Glycosides are particularly interesting because of their recognized bioactivity and organoleptic properties (positive or negative) they give for many edible plant products.

STARCH – STRUCTURE AND BIOSYNTHESIS

Starch – the α-glucan – is the most common storage polysaccharide deposited in seeds, tubers and roots of plants. It is composed of two α-glucan fractions: amylose - α-1-4-glucan, constructed of linear glucose chains bound together by almost only α-1-4-glucoside bonds (containing very few α-1-6-bonds), and amylopectin – branched α-glucan with many branching units bound to the basic α-1-4-glucan chain by the α-1-6-bonds. Both fractions are deposited in granules located in the endosperm; the granules are of variable shape and size.

Saccharide storage. The final step of anabolic route of saccharide metabolism in higher plants, initiated in chloroplasts by the formation of 6-P-Fru and 6-P-Glc, is the deposition of their excess in storage tissues (tubers, seeds, roots), as an α-glucan form – starch. It is known, that so-called transitory starch is partly synthesised already in chloroplasts (during the day), when assimilates are produced in higher excess, but in the dark it is transferred in the sucrose form to storage tissues, where it is deposited for long as the reserve starch. Starch is located in nonphotosynthesising tissues in amyloplasts and harvested as the final agricultural yield. Both starch fractions: amylose and amylopectin differ in the chemical structure and the granule shape and size; the transitory starch (in leaves) has smaller granules, almost exclusively composed of amylopectin, whereas the reserve starch contains ca. 15–35% amylose and 65–85% amylopectin [Ball *et al.*, 1998; Casey *et al.*, 2000], depending on the origin.

Granule structure. It is well known, that natural starch is composed of two fractions: the major one – amylopectin, built of α-1-4-glucan chain, branched by α-1-6-glycoside bonds, and the minor fraction – amylose, composed of almost unbranched α-1-4-glucan chain. The former belongs to the largest biomolecules and is responsible for the semicrystalline dense packing glucans in insoluble granules [Jenkins & Donald, 1995]. Amylose consists of much smaller molecules with very low level of branching; it is noncrystalline (soluble?) granule component and, in contrast to the former, is not amenable to the X-ray diffraction analysis. The interactions between these two fractions are not clear in the granule construction, though it is suggested, that amylose is able to infiltrate the amylopectin crystalline

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structures [Bell *et al.*, 1996], as the small angle X-ray scattering of starches with increased amylose level shows the changes in the ratio of the amorphous to crystalline lamellae in the granule complex [Jenkins & Donald, 1995]. The basic properties of amylose and amylopectin are presented in Table 1; the formation of starch structures is additionally presented in Figure 1.

TABLE 1. Structural properties of amylose and amylopectin.

Characteristics	Amylose	Amylopectin
Weight (% in the granule)	15–35	65–85
% of α-1-6-branches	<1	4–6
Molecular mass (Da)	10^4–10^5	10^7–10^8
Degree of polymerisation	100–1000	1000–10 000
Chain length (Glc units)	3–1000	3–50

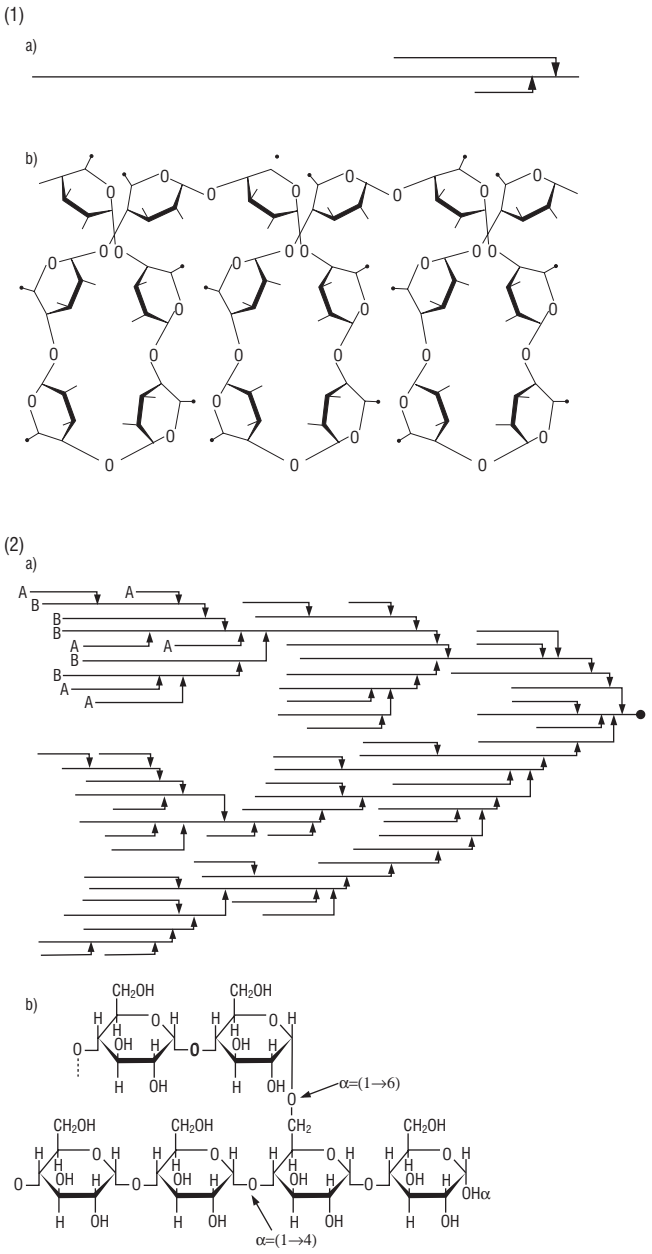

FIGURE 1. Structure of starch fractions: amylose – 1; amylopectin – 2; a) the schematic structures; b) bonds joining together the Glc residues in amylopectin; amylose presents the linear, helically coiled α-1-4-glucan chain with very few branchings.

Starch biosynthesis. The basic substrates for starch synthesis were found to be 1-P-Glc and ATP (or UTP), which participate in the following reaction, catalysed by ATP-Glc-diphosphorylase: 1-P-Glc +ATP → ADP-1-Glc + + P – P.

Active glucose formed is then transferred to nonreducing end of α-glucan chain, elongating it and the new α-1-4--glycoside bond is formed; this reaction is catalysed by α-glucan synthase (starch synthase): ADP-1-Glc + α-glucan-(nGlc) → ADP + α-glucan-(n+1-Glc). The reaction is repeated many times until the chain length becomes satisfactory for further reaction steps and the linear intermediate structure is formed. Next, the transfer of 3–50 glucose residue fragments follows from C-4 to C-6 position placed at the determined Glc unit of linear chain. This is the basic reaction of branching catalysed by α-glucan branching glucosyltransferase (mechanism presented in Figure 2). Thus, the formation of both starch fractions includes the transfer of Glc residues (or α-glucan chain) from the active form (ADP-1-Glc or amylose chain respectively) onto nonreducing OH group of α-glucan chain [Clarke *et al.*, 1999] – in case of amylose elongation – C-4, but at branching, to form amylopectin – C-6. In this process three enzymes participate: ADP-1-Glc diphosphorylase (Glc activation); α-glucan synthase (starch synthase – SS) and α-1-4-glucan (starch) branching glucosyltransferase. All these enzymes were found to exist in many isoforms (particularly starch synthase) of close homology [Ball *et al.*, 1998; Harn *et al.*, 1998; Nelson & Pan, 1995].

Adenylyl-1-Glc phosphorylase (ADP-1-Glc-ase - AGP--ase) occurs in chloroplasts of photosynthesising tissues, and in those unable to that process because of two places of starch synthesis. Chloroplastic enzyme is regulated allosterically (activated by 3-PGA, inhibited by P). On the other hand the regulation of ADP-1-Glc-ase located in seed or tuber cytoplasm is more complex and probably connected with sucrose or Glc import rate; in these tissues the enzyme is located probably in the cytosol only.

Starch synthase (ADP-1-Glc-1-4-α-glucan-4-D-glucosyltransferase) occurs, like the above one, in many isoforms of various localisation. Different isoform structures result from corresponding gene modifications, which controlled their synthesis during evolution; this depends on plant family or even species. In some investigated plants three starch synthases were found: that – bound to the starch large granule surface (GBSS I), that – cytosol soluble, and the third one – bound to small starch granules. For the majority of those forms (differentiated additionally depending on origin), both – the ADP-Glc and UDP-Glc, can serve as the typical substrates. According to amino acid sequences, they are divided into four groups: three soluble: - SS I, SS II and SS III, as well as GBSS I. Genes controlling them demonstrate in dicots high homologies in amino acid sequences, but they are differing of those from monocots [Campbell, 1997]. However starch synthases of all groups, including bacterial ones, as well as glycogen synthase (GS) contain three short, conserved domains: I - Lys-X-Gly-Leu (region of ADP-Glc binding), placed close to N-terminus, as well as two domains close to C-terminus – (SS II and III), which are prolonged by an elastic arm; this arm is lacking in GBSS I. Most commonly all starch synthases reveal maximum activity only, when complexed with a specific protein located also

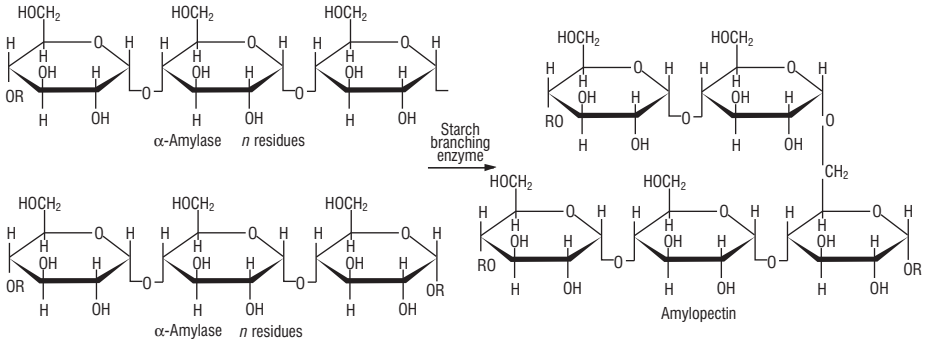

FIGURE 2. Mechanism of branching catalysed by starch branching enzyme (SBE); the transfer of glucan fragment from C-4 to C-6 position to form branched amylopectin.

on the starch granule surface [Martin & Smith, 1995]. This anticipates the more complex regulation of amylose synthesis on the granule surface, but the lowered content of it in some special mutants should result in the inhibited surface protein synthesis [Courey *et al.*, 1998].

The suggestion on amylopectin synthesis inside the starch granules and that of catalysed by GBSS I on their surface, was confirmed also *in vitro* in experiments with the ^{14}C marked ADP-1-Glc [Denyer *et al.*, 1996; Van der Wal *et al.*, 1998] applied to the nutrient in various time combinations. Because of the need to initiate amylose chain synthesis by one of oligosaccharides maltotriose was a necessary factor in some combinations. Those experiments proved that amylopectin is synthesised as the former, building up the inner α-1-4-glucan chains of granule; it gradually undergoes branching to form α-1-6-glycoside bonds and side chains.

Branching and debranching enzymes. Both types of enzymes are taking part in the physiological starch modification during its synthesis and degradation, respectively. Starch branching enzymes (SBE) belong to transferases, because they split the α-1-4-linkage inside the α-glucan chain and transfer the liberated fragment of it (3–50 Glc residues) onto the C-6 hydroxyl group of a strictly determined Glc residue inside this chain to form the α-1-6-bond. The amount and location of such branches contained in an amylopectin molecule influence the chemical and physical properties of starch [Mizuno *et al.*, 1993]. On the basis of amino acid sequences two types of SBE – A and B – were distinguished. The SBEs of type A have lower affinity to amylose than the type B. Another difference is the length of glucan chains used as a substrate to be branched. The type B needs the longer one than the type A and presents to be branched. The type B needs longer glucan chain than type A and has higher branching frequency, when amylose serves as the substrate [Jobling *et al.*, 1999]. Those isoform behaviours may influence the properties of starch obtained, which has already been proved for corresponding maize mutants [Guan & Preis, 1993].

Starch debranching enzymes (SDBEs) are known to be active during seed germination and their function is to hydrolyse the α-1-6-glucoside bonds and it is the first step of starch degradation. Recently however the crucial role of SDBE in starch synthesis was also discovered in some rice and maize mutants accumulating instead of a part of amylopectin – phytoglycogen, the heavily branched, water soluble glucan [Kubo *et al.*, 1999; Mouille *et al.*, 1996]. In their action SDBEs show the similarity to those hydrolases known since years - isoamylase and pullulanase, but they seem to act more specifically. The action of SDBEs is based on the elimination of not all, but only selected branches (a trimming) and may suggest a change of highly branched glucans produced at the starch granule surface by SS and SBE to amylopectin, the latter being transferred inside the growing granules; this is so-called the "trimming model" for starch granule formation [Zeeman *et al.*, 1998].

During the process described, the soluble enzymes: starch synthase, followed by α-1-4,1-6-glucosyltransferase (α-glucan branching – SBE) are taking part; the unique substrate is suggested to be ADP-1-Glc. On the other hand amylose is formed on the granule surface; thus catalysed by insoluble GBSS I [Nelson & Pan, 1995]. If this enzyme is in the contact with amylopectin, its fragments can serve as the primer of α-glucan synthesis, as well, but when amylopectin is lacking, maltotriose is necessary as additional primer [Koch, 1996].

MODIFICATIONS IN STARCH SYNTHESIS

As it is well known to food technologists, starch is not only the basic energy source for plants and animals, as one of the main substrates for foods and feeds, but it also is applied in the industry, *e.g.* textile, paper and plastic [Casey *et al.*, 2000]. Increasing demand for starch results from the improvement of production economy, renewability and degradation ability in the environment, as well as development of a growing number of possible chemical and genetic starch modifications [Visser & Jacobsen, 1993]. The increasing knowledge concerning mechanisms and conditions of starch synthesis (amylose to amylopectin ratio) and significant progress in transgenic plant production enable the production of starches of different composition and structure, thus with modified technological properties [Mueller-Roeber & Kossmann, 1994]. The physico-chemical properties of α-1-4-glucans as polymers are dependent on branching or polymerisation degree (*i.e.* chain lengths). Therefore the simplest way to evaluate those properties is the analysis of the amylose to amylopectin ratio, which is dependent directly on the activity or regulatory factors of participating enzymes – particularly starch synthases (soluble and GBSS I), as well as the branching and debranching enzymes. Therefore they are applied in food and feed industries, but also in paper and adhesive ones.

As mentioned, variable properties of starch are dependent mainly on the activity and regulation ways of enzymes participating in its synthesis. The first enzyme is ADP-Glc diphosphorylase, which is located both: in the chloroplasts and endosperm of tissues unable to photosynthesis. Among the known isoforms of starch synthase, GBSS I is responsible for amylose synthesis and is located on the granule surface, but waxy mutants of maize are lacking of it. Some similar mutants of this kind obtained from many plants, synthesise amylopectin-rich starch, but in lower level; they contain starch synthases SS II or SS III only. Structural changes of starch synthesised in this way consists of lowered branching number and shortened side chains of amylopectin [Moorehead *et al.*, 1999].

There has been mentioned, that among branching enzymes (SBE), which transfer the chains during amylopectin synthesis from C-4 to C-6, two groups were differentiated – A and B [Guan & Preiss, 1993] and their catalytic properties understood. SBE-A show lower amylose affinity than isoforms B, the latter being able to form longer α-glucan chains, but less branching places, than the former. Those kinetic properties may serve as the basis for obtaining starches of anticipated properties [Casey *et al.*, 2000]. Also debranching enzymes (amylo-1-6-α-glycosidases SDBE) which hydrolyze specifically α-1-6 -bonds in amylopectin are suggested to take part in starch biosynthesis. This seems to be similar to isoamylase and pullulanase, which are lacking or severely reduced, *e.g.* in maize and *Chlamydomonas* mutants and synthesize phytoglycogen (heavily branched, water soluble glucan) instead of amylopectin [Mouille *et al.*, 1996]. Admittedly, the mechanism of debranching enzyme action is not fully understood, nevertheless it opens up new possibilities for manipulation directed to form starches of new applicable properties [German *et al.*, 1999].

The knowledge on enzymes participating in starch biosynthesis allowed some trials to be undertaken to replace plant genes with the bacterial or algal ones showing higher productivity of this polysaccharide or susceptibility to some regulatory factors [Casey, 2000]. Those trials concerned the exchange of DNA fragments coding for particular subunits in oligomeric enzymes for yeast subunits, to form much higher thermostability of ADP-1-Glc diphosphorylase of maize hybrid endosperm enzyme [Green & Hannah, 1998; Lloyd *et al.*, 1999]. Main enzymes exploited in those investigations were: the mentioned diphosphorylase and isoforms of starch synthase [Craig *et al.*, 1998]. The effects obtained concerned not only starch level increase, but also that of ADP-Glc affinity, changes in the chain length, amylopectin content, and affinity to some regulators [Edwards *et al.*, 1999]. These and some other effects obtained were related to the properties of synthesised starches, thus enabling them to be applied in some new directions (*e.g.* in the production of ecologically degradable plastics) [Courey *et al.*, 1998].

RESERVE STARCH BREAKDOWN

The reserve starch stored in seeds is degraded almost exclusively by hydrolytic action of amylases, but that stored in potato tubers is decomposed mainly by phosphorolysis, although amylases are also active in those tissues [Beck & Ziegler, 1989]. The main enzyme taking part in seed starch degradation – α-amylase is insufficiently active at the onset of germination and is synthesized (mainly in scutellum and partly also in the aleurone layer) and transported to the endosperm or, in some part, it is liberated with inhibitors of protein nature from a complex [Andrzejczuk-Hybel, 1996]. The necessary factor cooperating in -S-S- bond reduction (complexing enzyme with inhibitor) is the thioredoxin system (Fd/TR-h) [Kobrehel & Buchanan, 1991, 1997; Kączkowski, 2000]. The main regulating factors of α-amylase synthesis known since over 20 years are the plant hormones: gibberelins (GA) and abscisic acid (ABA), the former promoting and ABA – inhibiting the mRNA synthesis at the transcription level [Cornford *et al.*, 1989; Nolan *et al.*, 1987]. The high pI isoforms of α-amylase are additionally regulated by Ca^{2+}, but those of low pI are not; however their secretion is slightly stimulated by this ion.

α-Amylase has the predominant role in starch degradation, whereas β-amylase seems to play the auxiliary function only; it is suggested to be important at the germination start, when the embryo needs much energy from glycolysis. Contrary to α-amylase specific to split the internal α-1-4--glycoside bonds, to form mainly low molecular-weight dextrins (6–10 Glc units), β-amylase breaks each second α-1-4-glycoside bond from the reducing end, forming maltose as a product. This disaccharide is decomposed further to form two Glc units in the reaction catalysed by α-1-4-glycosidase, present also in the endosperm. Glucose, after the activation to 6-P-Glc (ATP, hexokinase), enters the glycolytic route.

β-Amylase exists in resting seeds in two forms: free (soluble) and bound (latent) to some proteins. The former may be significant in the early phases of germination, but the latent one can be liberated from the complex, also by the -S-S- bond reduction, using *in vivo* the thioredoxin system [Kączkowski, 2000]. The large β-amylase isoform obtained, undergoes decomposition to three minor ones by proteolysis [Kreis *et al.*, 1987]. β-Amylase is located also on starch granule surfaces beside scutellum and aleurone layer and in subaleurone, but in the latent form. Its level in cereal seeds is typically very high, compared with the function (limited to the maltodextrin degradation); this enzyme is considered to be in such content and is treated as a storage protein.

Some other enzymes also take part in starch degradation in seeds. For debranching enzymes two functions are attributed: (a) to prevent production of isomaltose and isomaltodextrin during amylopectin degradation; (b) to make it possible to access branched polyglucans to phosphorylase action, where it cooperates in starch degradation. As mentioned, two types of debranching enzymes are distinguished: (a) directly cleaving the α-1-6 interchain linkages (pullulanase and isoamylase) and (b) indirectly acting – combining the amylo-α-1-6-glucosidase with an oligodextrin transferase. The pullulanase and isoamylase are distinguished by the substrate specificity to degrade pullulan (see below) or glycogen (phytoglycogen), respectively [Bell *et al.*, 1996]. The direct debranching is typical for microorganisms and plants, whereas the indirect one – for mammals and yeast. Pullulanase and isoamylase were later found to be isoforms of debranching enzymes (*e.g.* in potato tubers) [Ishizaki *et al.*, 1983].

The starch hydrolysing enzymes *e.g.* β-amylase, when present in the endosperm in the active form can act directly

on starch granules because of cooperation with concomitant enzymes (peptidases or glycosidases) and are regulated by some kinetic or hormonal factors, *e.g.* maltose and glucose, as the end-products; it concerns mainly β-amylase activity. The typical plant hormones – GA and ABA regulate amylase synthesis at the transcription level, increasing or inhibiting the corresponding mRNA formation. At last, protein inhibitors, specific particularly to α-amylase, are also effective regulators of amylase action as the complexing factors, changing them into the latent forms.

Some words should be devoted the other types of enzymes taking part in starch degradation in potato tubers – phosphorylases. Those enzymes are transferring the terminal Glc units from the nonreducing end of polysaccharide primary chain – on the phosphate molecule, to form 1-P-Glc and a shortened by one Glc unit polysaccharide chain. The α-1-6 bonds block the phosphorylase action, therefore they have to be removed before by cooperating isoamylase or glycosyltransferase; the mechanism is presented in Figure 3.

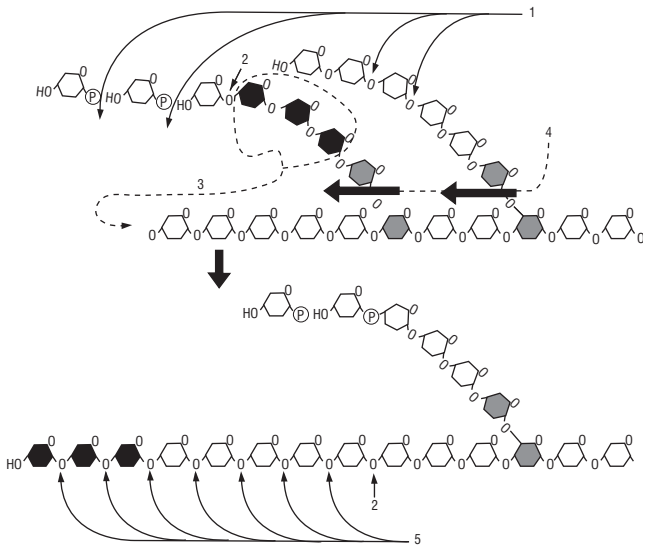

FIGURE 3. Mechanisms of cooperation of less typical enzymes taking part in starch degradation; 1 – typical phosphorylase; 2 – last possible bond degraded by phosphorylase; 3 – oligo-α-1-4,1-6-glucanotransferase; 4 – isoamylase (amylo-1-6-glucosidase); 5 – new positions of phosphorylase action after debranching.

PROPERTIES AND APPLICATIONS OF SOME STARCH COMPETITIVE MICROBIAL POLYSACCHARIDES

As the starch modifications seem to have rather limited possibilities and because of its insufficient production, microbial biotechnology has become regarded in recent years to offer interesting polysaccharide products of industrial scale fermentations. Many microorganisms synthesise specific exopolysaccharides (EPS), which can be attached to the cell surface or are liberated to the extracellular medium in the form of the amorphous slime [Sutherland, 1998]. The physiological functions in the natural media may be associated with virulence (plant pathogens), plant-microbial interactions or protection of microbial cells against desiccation or bacteriophage attack. But, when cultivated in selected medium, many strains are now applied to biotechnological production of polysaccharides, which have been already accepted or intensively investigated. Their industrial applications are due to their unique or superiour physical properties as related to the traditional plant polysaccharides. The low production costs (high efficiency), using vast nutrient components, as well as novel or special application areas are also of significance.

From many now available products, the most important and valuable seem to be: (a) β-D-glucans – in this group most interesting are: bacterial cellulose, β-1-3-D-glucans (curdlan, scleroglucan), xantans; (b) α-glucans – the most similar to starch is pullulan, but also (c) those of mixed bond types – gellan and hyaluronic acids.

Cellulose is produced mainly by bacteria *Acetobacter xylinum* and is an exopolysaccharide, excreted into the medium, where it undergoes rapid aggregation to form microfibrills. It is produced commercially to get pure cellulose I form, composed of 60% Iα- and 40% Iβ type and being lignin- and other noncellulosic material-free. Fibrils form a unique 3–8 nm thick ribbon differing morfologically from all other native celluloses. It is utilized therefore as a wound dressing for patients with various skin injuries, or skin substitute with high mechanical strength, water capacity and oxygen permeability; it is also excellent for construction of acoustic diaphragm membranes (parallel glucan chain orientation), as a binder in the ceramic industry and as a thickener for adhesives.

Many bacteria *e.g. Agrobacterium* or *Rhisobium* species produce EPS β-1-3-glucans, among them curdlan and scleroglucan are distinguished; both types are low molecular weight products of 74 and 18 kDa, respectively. Curdlan is insoluble in cold water, but at heating it forms weak gel, the strength of which increases, when dissolved at over 100°C; the gel obtained (of tripple helix structure) no longer melts and its strength is independent of divalent cations (contrary to alginates), is susceptible to shrinkage or syneresis, but resistant to β-glucanase activity. It is applied for improving food texture (tofu, fish pastes); some sulfated or acetylated curdlans are used in medicine (antithrombotic activity) or in laboratory (chromatography for monosaccharide separation) [Sutherland, 1995].

Scleroglucan is closely related to curdlan, but its M_γ does not exceed 18 kDa and is produced by some fungal species, *e.g. Sclerotium*, as well as by some wood rotting *Basidiomycetes*. They are composed of β-1-3-glucan chain, branched (regularly or not) by 1-6-bonds with Glc side chains (1-3 Glc units). The water solutions are highly viscous, independently of temperature (20–80°C) and have tripple helical conformation. Scleroglucans are, contrary to curdlan, easily degradable by several glucanases, leading to disaccharide gentiobiose. They have high antiviral and antitumour potential, therefore found much interest in the biomedical research, particularly after recognizing of their various host-defense responses; together with curdlan both are biological modifiers acting through mechanisms mediated by the immune system [Sutherland, 1998]. Many biological functions of these polysaccharides are dependent on the molecular mass, branching regularity and frequency, as well as the tertiary molecule conformation (*e.g.* nonspecific stimulation of the immune system).

As mentioned, the most similar microbial polysaccharide resembling starch is pullulan – α-D-glucan, composed of

maltotriose (and some maltotetraose) units (α-1-4-linked) inside each Glc unit, being coupled by α-1-6 glucoside bonds. This polysaccharide is produced by fungi *Aureobasidium pullulans* or *Tramella mesenterica*. Pullulan molecular mass is between 1000–100 000 kDa and is dependent on strain and cultivation conditions. Pullulan is not degraded by most amylases, but the enzyme α-1-4-bond specific pullulanase was isolated, which decomposes this glucan to basic units – maltotriose and maltotetraose. Pullulan is easily water soluble, forms viscous solutions, being stable in presence of many cations, but is unable to form gels. When esterified, it increases viscosity properties, but lowers the enzyme susceptibility. It can be used for production of the new packagings as it is oil resistant, water soluble, and oxygen and flavour permeable material; it is used to packing foods for direct cooking. It is used also as an adhesive and the component at fiber production.

Gellan is synthesized by bacteria *Sphingomonas paucimobilis* as linear chain polysaccharide (*ca*. 500 kDa) composed of four monosaccharide repeat units (Glc_3Rha) in many places bound with acetyl or glyceroil residues. High acylation level prevents crystallization, but induces formation of weak, elastic and thermoreversible gel. Deacylation, on the contrary, causes extensive association, resulting in brittle strong gels with various cations. Changing acyl level alters gel properties to the anticipated degree similar to those of agar, alginate and carrageenan gels. Gellan is popular in many countries *e.g.* in USA and EU where is used for a wide range of foods as a gelling, stabilizing and suspending agent. The gellan gels are stable over a wide pH range already at very low concentrations (0.75%), they give excellent flavour release and can be used in many combinations with other hydrocolloids, to obtain many unexpected effects. Structures of some microbial polysaccharides are presented in Figure 4.

Xanthan, synthesised by *Xanthomonas campestris* is the most popular commercial polysaccharide of microbial origin; its annual production overcomes 20 000 ton. It is comoposed of cellulose-like β-glucan backbone with branching side chains built of D-mannose and D-glucuronic acid. Depending on strains and nutrient conditions, the cultured acylation level and side chain constructions change, both influencing xanthan properties, which may depend also on the secondary double helical conformation adopted in solution. Some strains of *Acetobacter xylinum* synthesise xanthan-like products – acetans – more similar to cellulose. Xanthan in solution shows pseudoplasticity, high viscosity at stress removal, and excellent suspending properties. Therefore they are added into a wide range of foods, also because of rapid flavour release, pleasant "mouth-feel" and compatibility with other food ingredients (proteins, lipids). Xanthans interact also synergistically with plant galacto- and glucomannans (guar gum), to form elastic, cohesive gels, above very low saccharide concentrations and this behaviour also found many applications in food processing. All xanthan properties are highly dependent on acylation level, which is the supplementary method to alter them [Bozzi *et al.*, 1996; Sutherland, 1998].

The incomplete review of microbial saccharide structure and properties demonstrates the wide range of possibilities for biotechnological production and alteration of many different raw materials or half-products, which can successfully substitute plant polysaccharides, including starch or cellulose. The technologies already exploited offer many possible applications in food processing and medicine (antitumour agents, wound dressings) and many other industrial uses. Therefore further rapid developments of microbial saccharide biotechnologies are expected promptly [German *et al.*, 1999; Casey *et al.*, 2000; Sutherland, 1998].

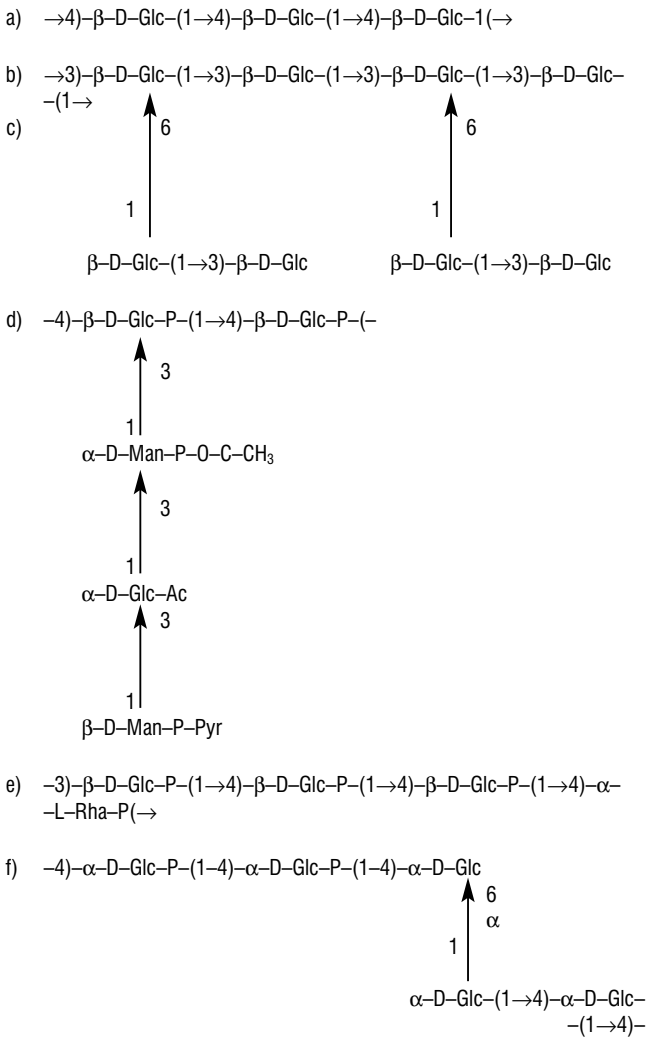

FIGURE 4. Schematic structure of microbial exopolysaccharides (EPS) produced on the industrial scale for practical uses; a) bacterial cellulose; b) curdlan; c) scleroglucan; d) xanthan; e) gellan; f) pullulan.

GLYCOSYLTRANSFERASES IN PLANT METABOLISM

Glycosyltransferases (GT), encoded by extended gene families differing in the amino acid sequences in the catalytic site, are a group of specifically and functionally variable enzymes able to transfer glycosyl residues. Two groups of those enzymes are distinguished: (1) cooperating in the synthesis of many secondary metabolites of glycoside structure [Vogt & Jones, 2000] and (2) endoglycosyltransferases (eGT), which take part in modification of the cell walls [Campbell & Braam, 1999]. Glycoside structure is present also in high molecular-weight structures – lectins (protein--saccharide complexes), in which both components are joined together by glycoside bonds. They play there various regulatory functions, *e.g.* localization of proteins, cell-to-cell interactions, metabolite recognition or interaction with many physiological proceses [Elgavish & Shaanon, 1997].

This group needs more detailed treatment and will not be referred to in this review, similarly to the eGT, which are specific to the cell wall modification [Fry, 1995; Campbell & Braam, 1999].

GLYCOSYLTRANSFERASES IN GLYCOSIDE BIOSYNTHESIS

Enzymes of this group were found recently to be excellent tool for modification of many products of practical uses (*e.g.* medicine, nutrition) [Vogt & Jones, 2000; Mendez & Salas, 2001] because of various specificities (which additionally can be modified by genetic engineering methods). They catalyze generally the transfer of saccharides from active forms (nucleotide phosphate ones) to low molecular-weight acceptors referred to as aglycons, containing at least one OH group. Most common monosaccharides to be transferred onto aglycons are: glucose, galactose and rhamnose (frequently in oligosaccharide forms), as well as many D- and L-deoxyhexoses, but their most common active forms are UDP and ADP derivatives. At last, the best known glycosides are: phenole (mainly flavonoids), glucosinolates, cyanogene, terpenoid and polyketide derivatives.

Many enzymes able to glycosylate flavonoids were isolated and characterised recently; this concerns glycosylation of anthocyanidins and aglycons of other flavonoid groups [*e.g.* Tanaka *et al.*, 1996]. Genetical methods – mRNA isolation, cDNA synthesis and PCR multiplication were mainly used in the investigations. There has been demonstrated, that GTs are in general not very specific, as concerns substrate (*e.g.* OH group location or other structural differences and the saccharide transferred). Similar relations concern the cinamyl acid aglycones (cyanogenic glycosides) and glucosinolates [Halkier & Du, 1997], as well as steroid diterpenes, known to be very specific substrates (*e.g.* to hydroxylation position). Regardless of those examples, molecular activities of GT concerning the artificial substrate glycosylation were found to be more specific, than the natural ones. The common amino acid sequence found to be active in flavonoid GT glycosylation is Pro-Ser-Pro-Cys, located 40 amino acids before C-terminus, which is the binding site for NDP--saccharide. The presence of other, strongly conserved sequences was also observed, *e.g.* motifs: Trp-Ala-Pro-Gln--Val and His-Cys-Gly-Trp-Asn-Ser, with the identity degree of 95% (as concerns total GT sequence identity being 60–80%) [Bairoch, 1991]. It seems to be probable, that Gln and His included in the motifs are responsible for β-conversion, because the natural glycosides generally contain β-glycoside bond configuration [Kapitonov & Yu, 1993]. Basing on incomplete data of GT gene cDNA base sequences, the GT "genetic tree" was constructed [Vogt & Jones, 2000] and enzymes divided into 3 groups overlaping each other to some extent were distinguished.

Finally, it is worth mentioning that trials of induced mutations inside GT gene family were undertaken in order to improve the taste of products and to eliminate toxic properties of some glycosides. As examples, it can be cited the bitter taste elimination from the citrus fruits or toxicity of glycoalcaloids, aglycone stabilisation in the cell, digestion improvement of glycosyl antioxidants [Terao, 1999; Moehs *et al.*, 1997] and sweet taste amplification (steviol) in *Stevia rabaudiana*. Modifications are based also on the inversion of binding place, complexing saccharides with some metabolically active substances (*e.g.* hormones), growth regulation or transoport across cell membrane [Vogt & Jones, 2000].

GLYCOSYLTRANSFERASES COOPERATING WITH POLYKETIDES

One most interesting group of glycosyltransferases is that transferring the saccharide (mono- or oligo-) to the specific OH group in polyketides. Polyketides comprise large group of products, synthesized by microorganisms and plants as secondary metabolites, starting of direct condensation of acetyl residues (transported by CoA-SH). The process is similar to fatty acid formation, but in polyketide synthesis not all C=O groups formed undergo the reduction. Most of polyketides are particularly produced by *Actinomycetes* family, genus *Streptomyces*. They have high diversity of biological activities and include both glycosylated or not, antibiotics (*e.g.* rifamycin, tetracyclins), antitumour drugs (doxorubicin), cholesterol lowering agent (lovastatin), immunosuppressive factors (rapamycin), insecticides and antiparasitic agents. After the biosynthetic routes of bioactive polyketides have been recognized, the new way of their altering (called combinatorial biosynthesis) was introduced, giving theoretically unlimited possibilities for new technologies and product application [Mendez & Salas, 2001; Leadlay, 1997]. It is based on manipulation of the genes for enzymes participating in biosynthetic reactions.

The source of structural diversity of polyketides mentioned are even more broaded by different saccharides attached to specific positions of aglycons. Moreover, saccharides usually participate in the molecular recognition of molecular target, thus they can act as the self-regulating mechanisms [Weymouth-Wilson, 1997]. Further manipulation possibility exists therefore in this group, in the area of saccharide structure and attachment site alteration.

The most abundant and diverse group of secondary saccharides bound with polyketide bioactive glycosides, are 6-deoxyhexoses (6-DO-Hs), which were found in many plants, fungi and bacteria; over 70 6-DO-Hs were detected as components of bioactive polyketides; examples are given in Figure 5 [Stockmann & Pieperberg, 1992]. Recently many enzymes involved in 6-DO-Hs formation were purified and genes coding them were isolated [Mendez & Salas, 2001]. Saccharides are transported to the specific OH group of aglycone from an active saccharide form (Sacch-NDP), which is catalysed by glycosyltransferases (GT). Over 50 encoding genes were isolated from antibiotic producing *Actinomycetes*; all belong to the large specific GT family [Campbell *et al.*, 1999], in which most enzymes share conserved Gly-rich region, close to the C-terminus, characteristic of UDP-glycosyl and UDP-glucuronoyl GTs [Reed, 1993]. The sequence similarity among these enzymes isolated from various sources are rather high, ranging 50 to 83%, or even more identities, depending on sources compared. Recently the GT (GtfB) has been crystallized and the structure determined; it transfers the glucosyl residue from UDP-Glc to the vancomycin aglycone [Mulichak *et al.*, 2001]; the enzyme has a two-domain structure with the deep inter-

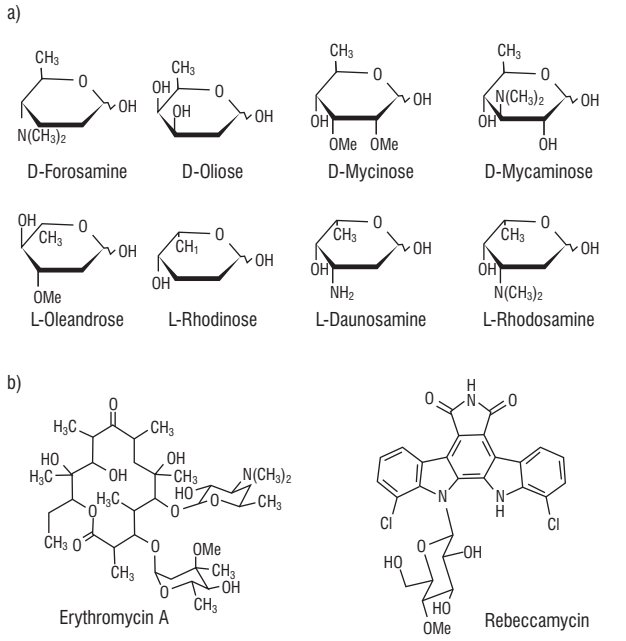

FIGURE 5. Examples of most common components building polyketide glycosides; a) some D- and L- 6-deoxyhexoses and 6-deoxyhexoseamines; b) examples of polyketide aglycones.

domain left, which probably constitutes the UDP-Glc binding site. The transfer of specific GTs (when isolated in a purified form) between species might serve as an additional strategy to promote the incorporation of different saccharides into an aglycone.

GLUCOSINOLATE GLUCOSIDES

It is worth mentioning also from the nutritional point of view, the group of specific glucosyltransfrases able to S-glycosylate the thiohydroxymate to form a group of glucosinolates. These compounds cause a wide range of effects of ecological interactions between plants and pests and they are of nutritional importance (reduction of feeding or nutritional quality of many agriculturally cultivated *Cruciferae* plants). Glucosinolates (formerly called mustard oil glucosides) originate from amino acids and undergo some oxidoreductive processes and double sulfur incorporation (in the SH and SO_4^{2-} forms) and the Glc S-glucosylation [Halkier & Du, 1997]; the simplified route of their synthesis is given in Figure 6.

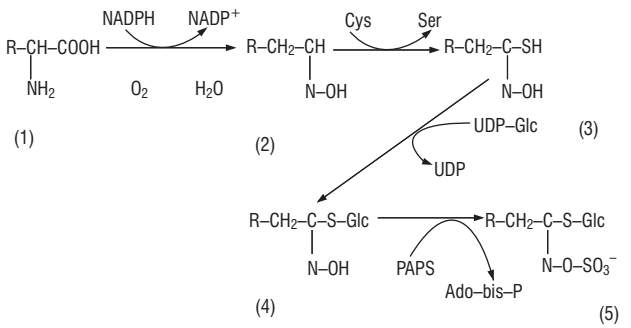

FIGURE 6. The simplified route of glucosinolate biosynthesis; 1 – amino acid; 2 – oxime; 3 – thiohydroxymate; 4 – desulphoglucosinolate; 5 – glucosinolate; PAPS – 3'phosphoadenosine-5'phosphosulphate (3'5'-Ado-bis-P); AMP-3'-P- adenosine-3'-phosphate.

The final step of glucosinolate synthesis is the S-glucosylation by the specific UDP-Glc thiohydroximate glucosyltransferase, which is then followed by sulfate incorporation, catalyzed by the soluble 3'-adenosine-5'-phosphosulfate (PAPS) and enzyme – desulfo-phosphoglucosinolate sulfotransferase. Both enzymes mentioned are well characterized [Reed *et al.*, 1993] and were shown to have high substrate specificity to thiohydroximates. Other enzymes (from *Arabidopsis*) were also characterised and genes encoding their synthesis were determined. The genes, when characterized, seem to be very important tool for initiation of the molecular strategy for modulation of the glucosinolate levels in *Brassica* crops; this can lead to improved flavour or some other nutritional properties [Halkier & Du, 1997], *e.g.* rape breeding for obtaining the glucosinolate-free cultivars.

There is also known and intensively investigated a large group of xyloglucan endoglycosyltransferases, specific to plant cell wall modification during their growth, development and some physiological processes [Nishitani, 1995]. Their action influences the changes in the cell wall extensibility, causes the turgor phenomenon, thickness modification of some fragments, leaf and flower shading, fruit maturation, intercellular channel formation and many others [Campbell & Braam, 1999; Cosgrove, 1998]. This type of GTs however is bound with the cell wall metabolism and will not be referred to here.

CONCLUDING REMARKS

The main topic of this review was to show the large variability of possibilities for modification of plant metabolism in order to obtain the favourable changes in the directions more useful for industry and nutrition. They concern particularly the level and properties of polysaccharides (*e.g.* starch), which are the main energy source harvested as an agricultural yield, but also many other plant (or microbial) compounds of biological activity, in which saccharides are only a part of components. The latter includes glycosides of diverse aglycone composition, but also different saccharides attached. Many saccharides were found to have very important applications: clinical (antibiotics, antitumour or immunosuppressive agents), veterinary or agricultural ones (growth promoters, insecticides, herbicides and antiparasitic agents), food and feed components responsible for the taste and flavour of plant products (of positive or negative character). All those components are formed by glycosylation, which is catalysed by glycosyltransferases and this process has also an important physiological function in stabilisation or compartmentation of glycosides. Therefore the GTs occur in organisms in many specific forms and are coded by different specific gene families.

Many methods for modification of bioactive plant- or microbial products of practical interest have now been undertaken, concerning the level, composition, useful properties and economically profitable applications. Many methods are at present known to reach those effects, the most interesting being the breeding, genetic engineering, and some combinations thereof. The recent progress in molecular biology methods and the achievements noted allow expecting of further success in this area.

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