Stability of polysaccharides against degradation and the techniques used to assess their molecular integrity

Stability studies of polysaccharides turned out to be of huge practical importance. They improved our understanding of important natural processes and helped to design commercial products with longer shelf life. In addition, now we can use degradation mechanisms for our advantages in many pharmacological applications.

Close structural relation between polysaccharides and water

Due to their chemical structure, polysaccharides contain lots of polar groups. Hydroxyl groups are particularly abundant in their structures. Due to this abundance carbohydrates are often described as polyhydroxy compounds. As a result, in aqueous solutions they are able to form an extensive network of hydrogen bonds, both between different polymer molecules and with the molecules of water. Extensive hydration has a very strong influence on the physical and chemical behavior of polysaccharides. It explains a number of unusual and practically important properties characteristic for carbohydrates and polysaccharides, such as ability to form gels. Stability of various polysaccharide preparations also depends on the relation between these polymers and water (Frank, 2013)

Stability of cell-wall polysaccharides

Around 30% of cell wall consists of cellulose which is a β(1-4) linked glucose polymer. The polymer contains between 5,000 and 25,000 glucose residues. In the plant cells polymer threads congregate together forming crystalline fibril structures. Fibrils consist of 36 polymer threads arranged in parallel manner and bound together via network of hydrogen bonds. Fibrils of cellulose interact with hemicellulose. Type I cell walls contain xyloglucan. Its backbone is made of β(1-4) linked glucose residues, most of which are linked with xylose side groups. Xylose moieties can be further substituted. Type II cell walls consist of β(1-4) linked xylan residues with various chain substitutions such as arabinose. Types I and II of cell walls have different pectins contents (type I – 30%, type II – around 10%). Mature plant tissues such as those in woody plants have secondary cell walls with extra layers of lignin (aromatic polymer consisting of 4-hydroxyphenyl propanoid building blocks), which is relatively inert and stable.

Many enzymes are capable of degrading and modifying the cell wall components. Cellulose is relatively stable due to crystalline structure, other cell wall components are more keen to participate in these dynamic processes. Numerous enzymes are involved in cell wall degradation and remodeling.

Modifications of xyloglucans is mediated by several enzymes such as endo-glucanase (breaking of β(1-4) bonds of xyloglucan), xyloglucan endotransglycosilase (remodellin of β(1-4) bonds network – breaking and re-synthesis of glycosyl bonds), expansin (breaks hydrogen bonds between cellulose fibrils and hemicellulose)

Pectin is another major component of cell walls in plants. The polymer has a linear backbone of α-galacturonic acid connected via α(1→4) glycosidic bonds. Pectin is heteropolysaccharide, and its structure, composition and degree of branching depends on a particular type and source. In some cases a number of other sugar residues can be connected to the linear backbone of α-galacturonic acid residues forming branches. These monosaccharides include D-xylose and D-apiose. In other cases the linear backbone itself can contain additional monosaccharide residues such as L-rhamnose alternating with α-galacturonic acids, and various sugars such as D-galactose, L-arabinose and D-xylose on the side chains. The carboxylic groups of α-galacturonic acid residues are usually esterified forming methyl esters but some residues stay in the form of acid or salt (Buchanan et al., 2000).

Modification of pectins involves the action of enzymes such as rectinesterase (deesterification - removes methyl group of carboxylic acid at C6 position), polygalacturonase (cleaves bond between two de-esterified galacturonic acids, which leads to depolymerisation and increase of pectin solubility) and galactanase (modification of the side chain).

Ripening of fruits is a good example of process that involves pectins degradation. The softening of fruits (which is important for their shelf life) caused by depolymerisation of pectin and loss of neutral sugars, galactose in particular (Tucker, 2013).

Stability of polysaccharide/ glycoconjugate vaccines

Encapsulated bacteria are pathogens with polysaccharide capsule. The capsule provides protection against the host's immune system.

Different capsular polysaccharides can be expressed by different strains of pathogenic bacteria. Structurally, these polysaccharides consist of either linear or branched repeated units. The length of unit can vary from one to up to eight sugar residues. Some rare monosaccharides found only in bacteria can be present. Also, phosphorylated and acetylated residues can be added. Presence and position of acetyl groups is the only source of heterogeneity.

Isolated or synthetic polysaccharides of the bacterial capsule can be used as antigens for eliciting a protective immune response (vaccination).

Any vaccine developed to enhance the immune response against these bacteria should usually contain multiple polysaccharides. First generation of polysaccharide based vaccines contain from one (the typhoid Vi antigen) to 23 (pneumococcal vaccine) serotype-specific polysaccharides.

Molecular size of polysaccharides in vaccine is important, since polysaccharides with low molecular weights are not immunogenic. Smallest polysaccharides for vaccine manufacturing have molecular weight around 100 kDa. The molecular weight can exceed 1 MDa for some vaccines (pneumococcal). Degradation of polysaccharides leads to decrease of molecular size and reduction of vaccine efficiency. Molecular sizing of polysaccharides is achieved by chromatographic (gel filtration) methods.

Glycoconjugate vaccines are more effective in younger children since they invoke immune response via different mechanism. These vaccines are prepared by chemical attachment of polysaccharides or oligosaccharides to suitable carrier proteins.

Degradation and depolymerization of glycan chains leads to the loss of immunogenity and is a major concern for vaccine developers. The first conjugate vaccine, against Hib and Group C meningococcal

infection, was unstable towards depolymerisation as a result of easy cleavage of phosphodiester bond under basic conditions. The molecular mechanism of degradation of these vaccines' components can be studied by NMR methods (Jones, 2013).

Stability of pectin-based drug delivery systems

Delivery of drugs to the target tissues and organs is a complex problem. When the drugs are administered orally, the problems of harsh acidic conditions in stomach and metabolizing in liver immediately upon adsorption in intestine must be addressed.

Pectins have a good chance to be used as drug carriers. Pectins are normal components of the diet and are therefore safe for human consumption. They are currently used in a number of drug delivery systems working through oral, nasal, vaginal and topical routes. Pectins are used as gels with incorporated drug substances. Drug release is controlled by diffusion and gel dissolution. A number of nasal sprays utilizing the pectin based gels are currently marketed.

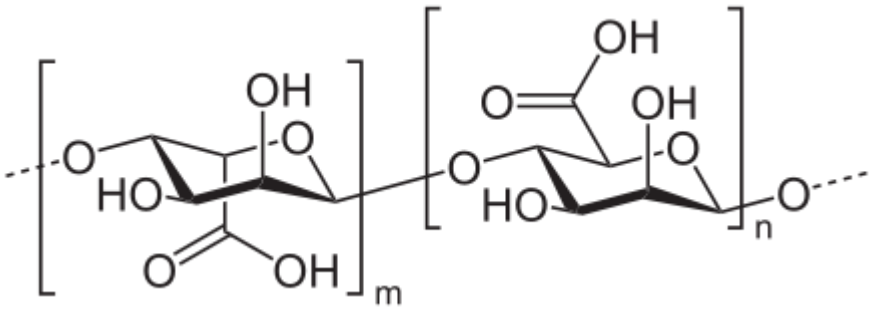
Pectins are subjects to both enzymatic digestion and thermal degradation due to de-esterification and depolymerization. Degradation rate depends on pH, water activity and temperature. Depolymerization increases with the rise of pH and temperature. Low methylated pectins are more stable. Increase of viscosity of pectin solutions over long storage (several months) reflects these chemical changes. These are important considerations in designing pecting-containing products with long shelf life.

Stability of chitosans and polysaccharides-based encapsulation systems

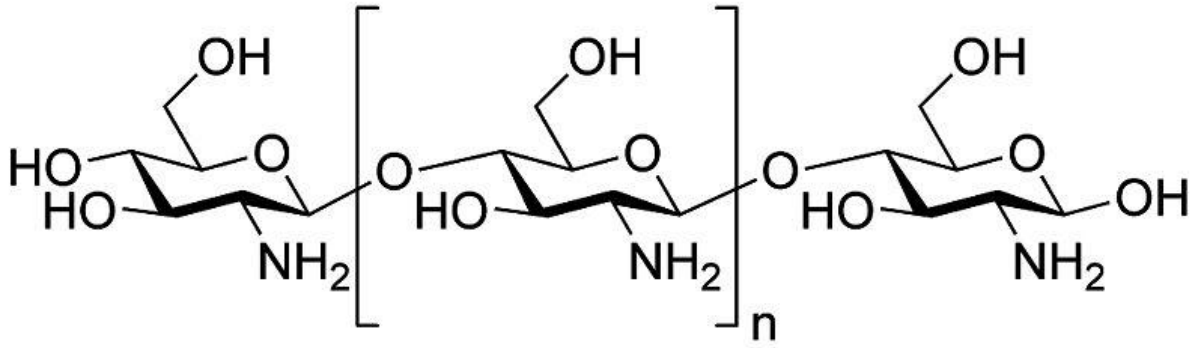
Nowadays, highly charged ionic polysaccharides attracted attention as potential vehicles for delivery of biological and pharmaceutical materials to various sites and cells in the body. These polymers are capable of forming complexes via electrostatic interactions, and therefore are capable of capturing and holding the compounds with opposite charge inside the micro- and nano-sized particles.

The structures of two polymers that seem to be particularly interesting as carriers are shown below. Alginate can have significant negative charge due to the presence of carboxylic acid residues, and chitosan is highly positively charged at physiological pH due to amino groups. Acetylation of functional groups in both polymers helps to modulate the charge of polysaccharides. Both polymers retain significant quantities of water and are classified as hydrogels.

Alginate:



Chitosan:



Cationic chitosan is of particular importance due to its ability to form complexes with negatively charged DNA and RNA. DNA-containing chitosan nanoparticles are under development as non-viral vectors for gene delivery and therapy.

Due to potential medicinal applications, stability of nanoparticles formed from on the basis of charged polysaccharides is very important. Chitosan based nanoparticles are not stable in normal *in vivo* conditions and quickly aggregate. However, coating them with alginate improves their stability – these nanogels remain stable in the presence of proteins. Nonetheless, even these particles tend to form complexes with proteins which results in the increase of their size (Schutz et al., 2013).

Techniques for assessing the polysaccharides molecular integrity

Hydrodynamic methods such as viscometry, analytical ultracentrifugation and light scattering provide arrange of useful information about the polysaccharide stability. They can assess heterogeneity of sample, molecular weight of polymer and its distribution, extent of aggregation and degradation, and conformation of polymer and its changes.

Viscometry provides information about polymer's conformation and its changes, state of aggregation or degradation, molecular weight. The method measures viscosity which depends on the above parameters. Viscometry on its own provides the average molecular mass.

Dynamic light scattering: estimates hydrodynamic radii of polymers and polymeric assemblies. Fluctuations in the intensity of scattered light caused by diffusion of particles allow to estimate the diffusion coefficient and its distribution. Hydrodynamic radius and diffusion coefficient are connected via Stokes-Einstein equation. Distribution of diffusion coefficients and hydrodynamic radii can be converted into distribution of molar mass.

SEC-MALC is size exclusion chromatography (SEC) coupled with multi-angle light scattering (MALC). SEC separates the sample according to molecular weight and angular scattered intensity is measured for each fraction separately. SEC-MALC allows easy measuring of the molecular mass distribution. Method is limited by measuring the molecular masses not exceeding 3,000,000 g/mol.

Analytical ultracentrifugation is another useful analytical technique. At lower rotor speed, sedimentation force and diffusion are comparable, and this sedimentation equilibrium experiment provides molar mass distribution.

At very high rotor speeds, sedimentation velocity provides information about conformation, flexibility and homogeneity of sample. Sedimentation coefficient depends on the molar mass, shape and volume of polymer's molecule.

Conclusion

Our understanding of the properties of polysaccharides improved dramatically in recent years. Now we can prepare huge variety of different practically important products that are based on these features. Clear understanding of polysaccharides degradation mechanisms helps not only to increase better shelf life of products but also to design specific preparations that exploit degradation mechanisms to achieve certain aims, such as controlled drug release.

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