Chapter 4

Polysaccharides in Solution: Experimental and Computational Studies

Bimali Jayawardena, Dinesh R. Pandithavidana and WMC Sameera

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.69863

Abstract

Carbohydrates can be found in many natural sources, and they play a central role in various biological processes. These versatile biopolymers are difficult to dissolve in solutions, and therefore converting them into functional forms is a significant challenge, whereby both experimental and computational studies become critical. This chapter discusses commonly used experimental approaches to increase solubility of carbohydrates in solutions and computational studies to rationalize their conformations and solvation effects. Advances of experimental and computational methods for the study of carbohydrates will guide the design approaches to use carbohydrates in industrially and academically relevant applications.

Keywords: solubility, computational studies, solvent effects, conformations

1. Introduction

Carbohydrates are one of the most abundant biomolecules on the earth, and they involve a number of biological processes, such as energy production and storage, structural maintenance, molecular recognition and cell growth. Polysaccharides are polymers of tens, hundreds or even many thousands of monosaccharides linked together through the glycosidic bonds. They are products of a natural carbon-capture process, photosynthesis, followed by further biosynthetic modifications. Some polysaccharides are produced on a very large scale in the nature, and some have industrial relevance, for example, materials and food applications, either in their native or in chemically modified forms.

Structures of the repeating units of some polysaccharides are shown in **Figure 1** [1]. The natural polysaccharides, cellulose and amylose, for instance, consist of a monosaccharide repeating unit with hydroxyl groups as the only functional group. Other related structures, such as curdlan and inulin, are based on furanosides. Amylopectin has a structure similar to amylose, and these two polysaccharides are the components of starch that consist of a branched structure with many amylose-like chains linked together by (α1–6)-branching points. Dextran is an (α1–6)-linked glucan and holds branching at the secondary hydroxyl groups. For example, in the regular comb dextran, every residue in the backbone is substituted by an (α1–3)-linked glucose unit. Xylans, a component of hemicelluloses, are made up of a (β1–4)-linked xylopyranose backbone, but it can also be branched. Xylose is a pentose, where the pyranose units in xylan do not have a

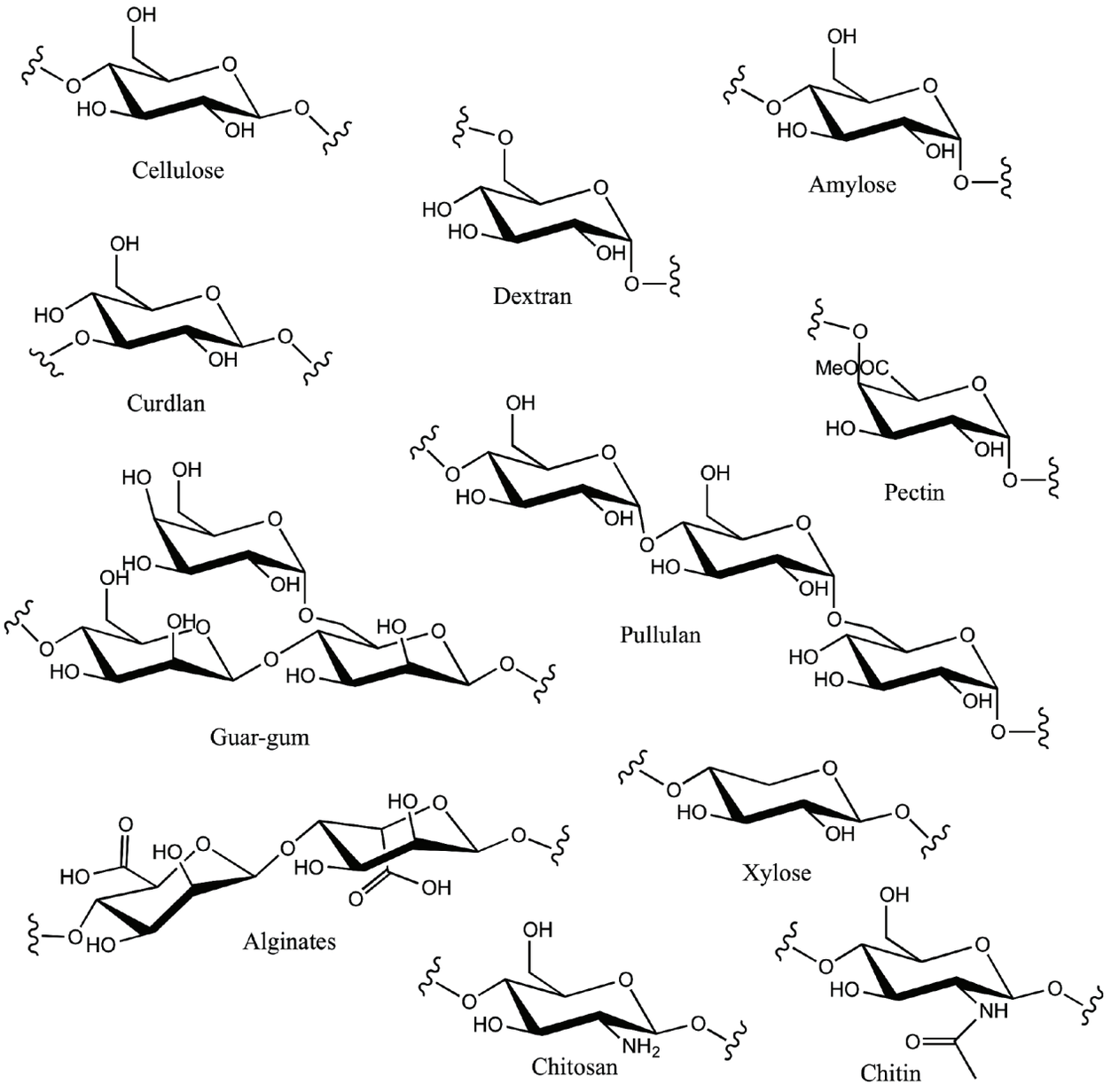

Figure 1. Structures of the repeating units of some of the polysaccharides [1].

primary hydroxyl group. Both guar gum and locust bean gum consist of (β1–4)-linked mannan backbones substituted by Gal(α1–6) units to some extent. In guar gum, approximately every other mannose residue is substituted with galactose, whereas in locust bean gum, long unsubstituted regions alternate with regions of heavy galactose branching [1, 2].

Some polysaccharides have other functional groups as well as the simple hydroxyl groups. Alginates and pectins are based on uronic acids, and their monosaccharide constituents are all oxidized at C-6 to the carboxylic acid level. Alginates consist of domains of (α1–4)-linked L-guluronic acid interspersed with domains of (β1–4)-linked mannuronic acid. Pectins are polysaccharides rich in galacturonic acid, although this acid can be found as its methyl ester. A simple backbone of (α1–4)-linked galacturonic acid methyl ester can also be substituted by other monosaccharide branches. A very common polysaccharide based on aminosugars is chitin/chitosan. The chitin/chitosan relationship can be regarded as a continuum, with polysaccharides containing more of the free base being called chitosan and those mostly N-acetylated being called chitin [3, 4].

The applications of polysaccharides are restricted due to their insolubility in most solvents. Polysaccharides are often insoluble in water or organic solvents. Non-aqueous solvent mixtures that dissolve in cellulose often consist of an organic liquid and an inorganic salt. Examples include DMA (dimethylacetamide)/LiCl, DMF/LiCl, DMI (1,3-dimethyl-2-imidazolinone)/LiCl and DMSO/TBAF (tetrabutylammonium fluoride). The DMSO/Et_3N/SO_2 mixture is a salt-free solvent for cellulose. Sometimes, it is necessary to heat to a high temperature (150°C) before cellulose dissolves in these solvents. Similar solvents or solvent mixtures are often used to dissolve neutral polysaccharides. In general, starch is more soluble than cellulose. Commonly used solvents for chitin include LiCl (5%)/DMA, LiCl/N-methyl-2-pyrrolidone, $CaCl_2$/MeOH and hexafluoroisopropyl alcohol [4–6].

Charged polysaccharides such as chitosan (which may be protonated on nitrogen) or polyuronates such as alginates (which can form carboxylate salts) show very different solubility. Further, chitosan is soluble in aqueous organic or mineral acids below pH 6.5 and also in DMSO [1, 3]. Ionic liquids (ILs) (at room temperature) are relatively new solvents that can be used to dissolve polysaccharides [7], including cellulose, hemicellulose and wood. Cellulose dissolves in ionic liquids, aided by conventional heating, microwave irradiation or sonication, with up to 25% (w/w) being obtained in [bmim]Cl [8]. Other ionic liquids gave 5–10% (w/w) solutions of cellulose. The properties of ionic liquids can be fine-tuned by modifying the structures of two ionic components. Increasing the length of the alkyl chains in the cation component resulted in a less efficient dissolution of cellulose. Amylose shows a very high solubility in ether-derived ionic liquids. Ionic liquids have been called 'green' solvents due to their recyclability and low vapor pressures (low volatility), but a low vapor pressure can limit the recyclability, as its purification becomes difficult. As a result, volatile and distillable ionic liquids have been designed for polysaccharide derivatization [9, 10].

Recently, numerous environmentally friendly methods have been used to dissolve polysaccharides. Subcritical water, microwave digestions and enzyme pretreatments facilitate the dissolution process. However, very little is known regarding the behavior of carbohydrates in solution. Hence, experimental and theoretical characterization of carbohydrate structures is

not only critical in terms of establishing the structure-function relationships of carbohydrates but also important in modifying their physical and chemical properties to improve their functional characteristics for numerous technological applications in the food, textile, paper and cosmetics industries. Therefore, both experimental and computational studies play a major role to establish their structural features, to improve their solubility and to design versatile catalysts to convert biomass into other forms such as fuels that are more conveniently used, transported and stored.

2. Carbohydrates in solution

Water is a very strongly hydrogen-bonded liquid, and breaking these bonds requires energy. Solutes that are unable to form hydrogen bonds or decrease the number of hydrogen bonds in water tend to have low solubility. Water-associating polymers and amphiphilic systems with polar units are soluble in water. Dissolution can be improved by modulating thickening, gelling and viscoelasticity through temperature, and in the presence of additives. The characters in the aqueous solutions of such polymers are mainly due to interchain associations between hydrophobic groups leading to physically cross-linked polymer chains.

Most standard techniques used to enhance the rate are by heating and stirring. These techniques speed up and increase the contact between solvents and solute. The kinetic control of dissolution is much more important for macromolecules than for low molecular weight solutes. It is known that multiplicity of inter-chain hydrogen bonds is the cause of aggregation and insolubility of polysaccharides in aqueous solutions. A number of research approaches have been initialized to increase the solubility of polysaccharides in water. The common technique involves the use of different solvents such as alkali or LiCl to break down intermolecular hydrogen bonds of polysaccharides, resulting in dissolution. However, such solvent systems are not applicable for bioactivity assays. Subcritical water (SCW) and super-heated water (SHW) are potential solvents that can be developed to dissolve polysaccharides. SCW has the necessary properties to dissolve water-insoluble polysaccharides as these behave like polar organic solvents and are environmental friendly.

The formation of a homogeneous solution by dissolution of a compound takes place only if the mixed state corresponds to a lower energy than two separate states thermodynamically. Parts of polysaccharide chain become fully solvated by kinetic action mainly by breaking more inter-polysaccharide bonds which are immediately solvated. With time, many sections become solvated and solubilized while fewer amounts of segments are still connected to the other parts of polysaccharide chain. This intermediate stage in the dissolution of the polymer molecule represents a transient gel stage. As the hydration process continues, polysaccharide molecule becomes highly surrounded by partially immobilized water. This layer represents the solvation layer, and molecules move into solution where they may remain monodispersed and preferred low-energy state conformations and shapes where they develop various degrees of gel structure. Polysaccharides dissolve in water by continuous hydration with the transfer of inter-polysaccharide binding to polysaccharide-water binding. The process is facilitated by entropy, as the molecules prefer lower-energy conformations. In all polysaccharides

contain sites, where molecules are in a disorganized manner with intermolecular forces and intermolecular hydrogen bonding partially filled because of random spatial arrangement. These amorphous regions therefore have numerous unsatisfied hydrogen-bonding positions that can hydrate [11].

When polymer is in the solvent, solvent molecules rapidly contact the polymer and penetrate into the amorphous region and to the surrounding available polymer sites, competing with and eventually reducing the number of intermolecular bonds, which results in a gel-like consistency. Polymer molecule diffusion is naturally much slower than solvent diffusion and in concentrated polymer solutions, and the diffusion is drastically retarded due to entanglement and aggregation. Entropy is the driving force for dissolution. For spontaneous dissolution, the change in free energy needs to be negative. The reason for difficulty of dissolving polymers is due to higher the molecular weight weaker the entropic-driving force of dissolution.

3. Methods for dissolving carbohydrates

The multiplicity of inter-chain hydrogen bonds is the origin of the aggregation and insolubility of polysaccharides in aqueous solution. Various methodologies have been embraced to decrease this impact [12]. A typical method includes the use of solvent such as alkali or LiCl/Me_2SO to break the intermolecular hydrogen bonds of the polysaccharides, resulting in dissolution. However, such solvent systems are not applicable for bioactivity assays [12]. Apart from that, various chemical modifications have been developed to enhance the solubility of polysaccharides (**Figure 2**). One such chemical modification is sulphation. According to the literature, it is prominent that water-insoluble polysaccharides indicate lower bioactivity. However, their sulphated derivatives show higher solubility that allows them to provide better functional attributes. So here, the sulphation has altered the bioactivity of polysaccharide by changing its chain conformation [13]. Molten salts with low melting points are known as ionic liquids (ILs). In recent years, ILs have been proposed as solvents for polysaccharides. According to Passos and Coimbra [14], 1-n-butyl-3-methylimadiazolium chloride is capable of dissolving cellulose. Due to the high melting temperatures and high viscosities of chloride salts, energy is needed in both the pretreatment and main processes. However, they suggest that carboxylic salt-type ILs are better solvents for a series of polysaccharides [15].

Physical methods (**Figure 2**) have been produced to disperse the supramolecular aggregates by enhancing the energy of the polymer chains. Through these methodologies, a homogeneous aqueous solution of polysaccharides can be prepared. The microwave heating strategy is a feasible method for solubility of the water-insoluble polysaccharides using only compressed water or dilute aqueous solutions. This strategy is also known as the 'microwave superheated water extraction' of polysaccharides and reported to permit milder response conditions, low production costs, arrangement of cleaner items with higher yields and minor wastes when contrasted to other methodologies [14]. When utilizing microwave-assisted extraction, temperature is one of the most important factors contributing to the recovery yield. The presence of higher temperature, higher recovery yield can be obtained. However, these high temperatures can cause degradation of products.

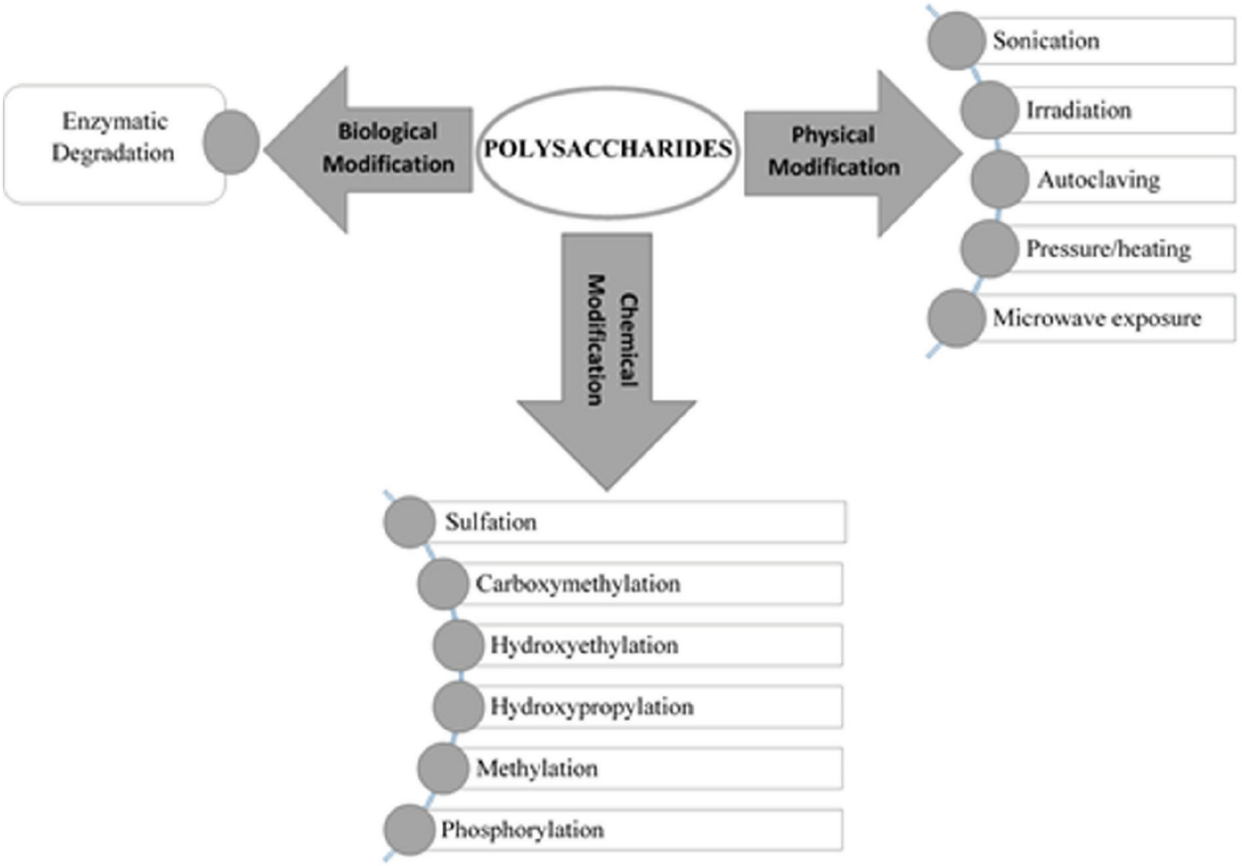

Figure 2. Methods for dissolving carbohydrates.

Ultrasonication is another physical approach used to dissolve polysaccharides. In this process, high molecular weight polysaccharide chains degrade to smaller sizes upon exposure to ultrasound radiation. Despite the fact that the exact mechanism is unclear, it has been attributed to cavitation, the formation and collapse of microscopic vapor bubbles produced by strong sound waves. The resultant stun waves give energy to the polysaccharide, which results in scission if polysaccharide is unable to disperse this energy.

4. Computational studies

In order to understand structure-function relationships of carbohydrates, the first step would be to understand conformations of their monomer units. A fundamental problem in the study of carbohydrates is the extent of conformational complexity, arising from the multitude of bonding possibilities for the primary and secondary hydroxyl groups, the ring-puckering modes and the rotational flexibility of the glycoside linkages. For example, a pyranoseanomer, there are five hydroxyl and one hydroxymethyl rotational dihedral angles, each of which has three staggered conformations. As a result, 729 different stereoisomers are possible. The number of conformations becomes 2916 when both two anomeric and the two main pyranose chair forms are taken into account. When we consider dimers or trimers, the total number of conformation becomes

enormous. At the same time, interactions between monomer units give rise to additional complexity. The aqueous phase investigation can be focused on deducing the interactions between polysaccharides and water with extending the chain length of the polymer, varying branching patterns and the substituent position of carbohydrate molecules [16, 17, 18].

The spectroscopic methods can be used for the study of carbohydrate conformations with some success. For example, X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy provide some insights about the carbohydrate conformationals. The electron microscopy, light or neutron diffraction, and infrared spectroscopy can also provide useful information [19]. Stability of a particular conformation can be determined by the balance between covalent and non-covalent interactions. These interactions determine the structure-function relationships of carbohydrates. This is indeed difficult to characterize in full from experimental studies, and therefore computational studies become critical.

Computational methods can be used to understand carbohydrate conformations. Commonly used semi-empirical methods, MNDO, AM1 and PM3 for instance, fail to describe weak interactions of carbohydrate conformations due to poor dispersion effects. On the other hand, force field methods (e.g., MM3, CHARMM, AMBER, GLYCAM, OPLS and GROMOS) performed well. Therefore, force field methods are commonly used molecular dynamics (MD) simulation of large carbohydrate structures.

Quantum chemical methods have been used to mono- and disaccharide conformations. However, quantum chemical methods are difficult to apply for very large carbohydrate structures due very high computational cost. The Hartree-Fock (HF) or standard density functional theory (DFT) describes hydrogen bonding, but these methods are failed to describe dispersion effects. According to recent studies, non-covalent interactions of biomolecules can be described accurately by using coupled cluster theory using single, double and perturbative triple excitations, CCSD(T), Møller-Plesset (MP) perturbation theory levels and variants, such as spin component-scaled (SCS) approach and the scaled opposite spin (SOS) approach. Also, local pair natural orbital (LPNO) techniques and coupled-electron pair approximation (CEPA) perform well for non-covalent interactions. These ab initio methods are, however, computationally demanding, and therefore some approximation methods, dispersion corrections for density functional and symmetry-adapted perturbation theory (DFT-SAPT) for instance, are viable alternatives. The quantum mechanics/molecular mechanics (QM/MM) methods can be applied for large systems, where non-covalent interactions are described in force field parameterization.

Over the past years, computational methods have been used for the study of monosaccharides and provided very useful information about the accuracy of modern computational approaches in describing the energies of small saccharides [20–26]. A recent study rationalized a comprehensive benchmark study of a test set of 58 structures [27]. In this work, coupled-cluster calculations extrapolated to the complete basis set limit (CCSD(T)/CBS) was used as reference energy and compared with the Møller-Plesset perturbation theory and its variants, the localized paired natural orbital-coupled electron pair approach (LPNO-CEPA) and 31 DFT methods (**Figure 3**). This study suggested that the LPNO-CEPA provides CCSD(T) quality results, and MP2 and SCS-MP2 also give good results. Among the density functional methods, the mPW2PLYP-D and M06-2X are the best choices.

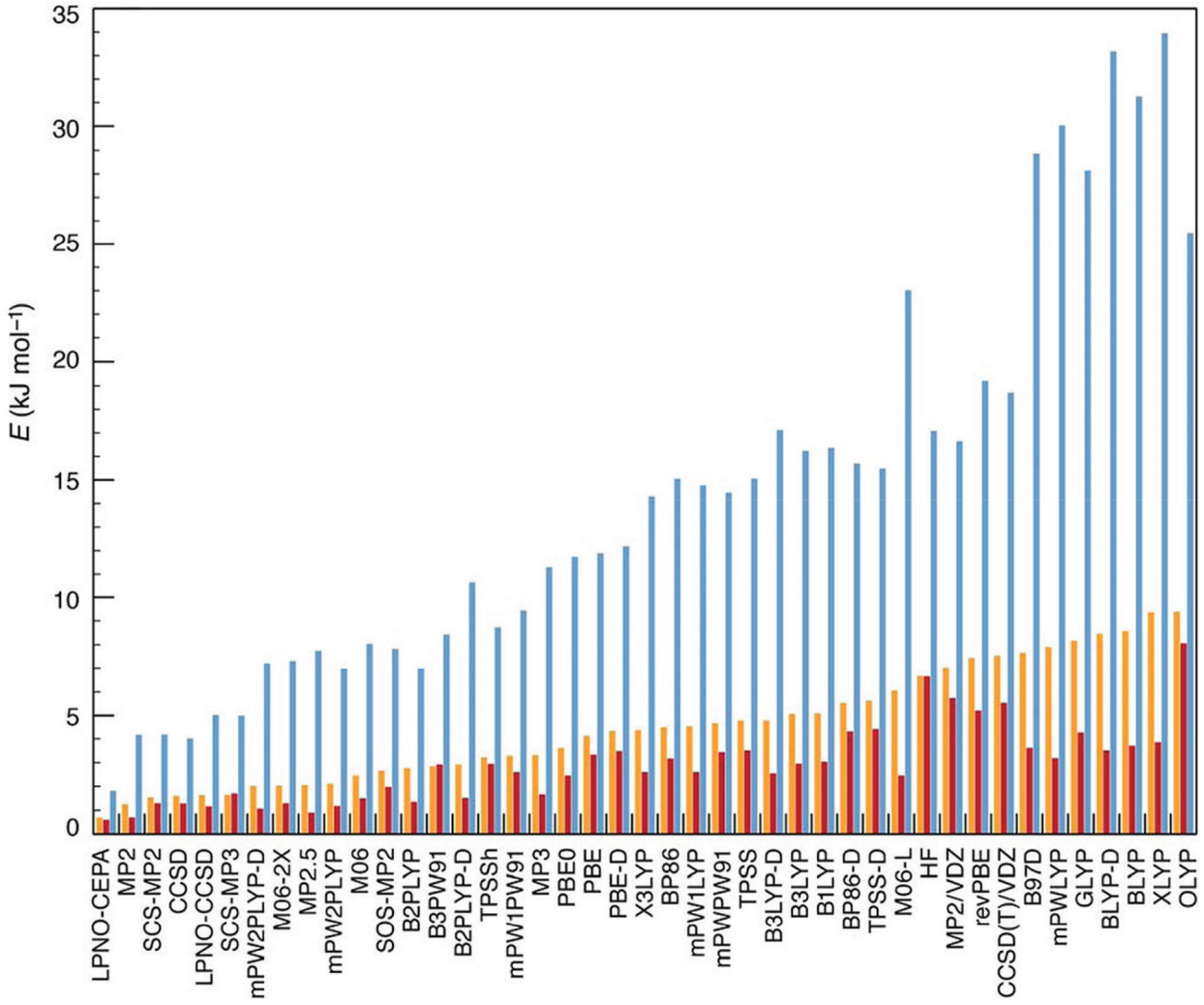

Figure 3. Calculated total average error, average errors excluding open-chain isomerism and maximum absolute errors. Reprinted with adaptation by permission from Ref. [27]. Copyright 2008 American Chemical Society.

Application to larger systems, such as plant or bacterial polysaccharides, will necessitate advances in not only computer performance but also theoretical approaches. Conformational sampling over a nanosecond time scale, which is the current limit of solvated molecular dynamics simulations, is still far too short to adequately sample the conformational space available to a polysaccharide in solution. MD simulations may achieve considerably more sampling, and the absence of explicit water is in some way compensated for within the force field, which may be applicable to polysaccharides [28]. Crystalline environments may be adequately sampled on much shorter time scales, and MD simulations of solid-state polysaccharides are useful in their structural and energetic interpretations [29]. Alternative techniques, such as Langevin dynamics, have been applied elegantly in macroscopic simulations of DNA super coiling, involving as many as 1000 base pairs, and may be applicable to polysaccharide simulations. However, preliminary Langevin dynamics on small molecules related to sugars indicate that it may be necessary to include at least a first shell of explicit waters [30]. Longer time-scale motions may also be approached by Brownian dynamics. In these simulations, the

level of detail has to be sacrificed; however, the benefits of obtaining a macroscopic model on the micro- or even millisecond time scale would be significant [30, 31].

Several different approaches have been suggested in literature to model solvent effects on these biomolecules [32, 33]. One possible way to overcome part of this problem is to include a limited number of solvent molecules in the calculation. In this case, the system of the solute and a small number of solvent molecules is treated as a supermolecule. The supermolecule approach may be useful in determining the specific solvation sites.However, for most processes in saccharide solution, the overall solvation energy rather than the optimal solvation sites is of interest. An alternative approach is to treat the solvent as a dielectric continuum (i.e. implicit solvation). This approach was based on the solvophobic model and was developed for saccharides [18].The continuum models are useful in estimating conformational energies of polar molecules. However, they are of limited validity for the quantitative treatment of ionic reactions and for studies of the balance of interactions between the solute-solute and solute-solvent.

A combination of supermolecule and continuum approaches has been reported recently. Probably, ultimate approach for studying solvent effects will be the brute force method of computer simulation of the given solution. This can be based on either Monte Carlo or molecular dynamics calculations including explicit solute and many solvent molecules. However, they are not suitable potential functions for calculating the potential energy of saccharides in different solvents. The method of free-energy calculation of possible conformers in dilute solutions involves creating a cavity of sufficient size to accommodate the solute molecule. The cavity formation requires a Gibbs free energy (ΔG_{cav}). Firstly, the solute molecule is introduced to the designed cavity, which then interacts with the surrounding solvent molecules. The calculation of the cavity term is based on an expression taken from the scaled particle theory, which has been successfully used for the study of thermodynamic properties of aqueous and non-aqueous solutions [34]. The electrostatic term is calculated according to theory of Onsager reciprocal relations of the reaction field that expresses the equality of certain ratios between flows and forces in thermodynamic systems out of equilibrium, but where a notion of local equilibrium exists as applied by Abraham and Bretschneider. The dispersion interactions take into account both attractive and repulsive non-bonded interaction, using a combination of the London dispersion equation and Born-type repulsions [35].

As a simple parameter for visualizing linkage dynamics and water interaction, intramolecular hydrogen bonds can be investigated computationally at regular intervals throughout the cellulose simulation. Their definition is based on bond angle and bond distance criteria. According to the literature, the number of observed hydrogen-bond pairs has been calculated as a function of time [36]. After a period as short as 500 ps, all of the persistent intramolecular hydrogen bonds can be observed, implying that these conformations are highly sampled during the simulation. Furthermore, few new interactions are seen after a period of 2 ns had elapsed, indicating that a large percentage of conformational space has been sampled during this period and confirming that simulations of length 5 ns are adequate to ensure statistical sampling of the major conformers. This is also evident from the observation that each of the internal linkages in the decasaccharide populated space in an identical way. If this is not

true, then inadequate conformational sampling would be suspected. Similar checks can made probably for all of the decasaccharide simulations, with similar results.

5. Conclusions

In this chapter, we discussed recent advances in experimental and theoretical studies of polysaccharides in solution. Solubility of polysaccharides in solution is very low, and therefore the development of suitable solvents for polysaccharides becomes critical in terms of converting them into other functional forms. To understand structure-function relationships of polysaccharides, computational studies are very important, and theory rationalizes the low-energy conformation of monomer, dimer or trimer units of polysaccharides. However, low-energy conformations of longer chains and interactions between solvent molecules are significant challenges for computational methods. MD simulations are well suited for such condensed phase modeling. Interactions between polysaccharides and other biological macromolecules are also amenable to computational approaches. Given the sophistication of these methods, it may be tempting to think of the simulation as being truly representative of the properties of the carbohydrate. But many aspects of the real system, such as proton exchange, pH effects, anomerization, induced polarization and other quantum effects, are absent. Despite these limitations, modern computational approaches can provide insight into physical properties, which may not be accessible experimentally.

Author details

Bimali Jayawardena[1], Dinesh R. Pandithavidana[1] and WMC Sameera[2]*

*Address all correspondence to: wmcsameera@sci.hokudai.ac.jp

1 Department of Chemistry, University of Kelaniya, Kelaniya, Sri Lanka

2 Department of Chemistry, Faculty of Science, Hokkaido University, Kita-ku, Sapporo, Hokkaido, Japan

References

[1] Cumpstey I. Chemical Modification of Polysaccharides. ISRN Organic Chemistry. 2013; **2013**:417672-417698. DOI: 10.1155/2013/417672

[2] Yalpani M. Tetrahedron. 1985;**41**:2957-3020. DOI: 10.1016/S0040-4020(01)96652-9

[3] Corma SI, Velty A. Chemical Reviews. 2007;**7**:2411-2502. DOI: 10.1021/cr050989d

[4] Vyver V, Geboers J, Jacobs PA, Sels BF. ChemCatChem. 2011;**3**:82-94. DOI: 10.1002/cctc.201000302

[5] Cunha AG, Gandini A. Cellulose. 2010;**17**:1045-1065. DOI: 10.1007/s10570-010-9435-5

[6] Vigo TL, Sachinvala N. Polymers for Advanced Technologies. 1999;**10**:311-320. DOI: 10.1002/(SICI)1099-1581(199906)10:6<311::AID-PAT880>3.0.CO;2-G

[7] Heinze T, Liebert T. Progress in Polymer Science. 2001;**26**:1689-1762. DOI: 10.1016/S0079-6700(01)00022-3

[8] Gericke M, Fardim P, Heinze T. Molecules. 2012;**17**:7458-7502. DOI: 10.3390/molecules-17067458

[9] Murugesana S, Linhardt RJ. Current Organic Synthesis. 2005;**2**:437-451. DOI: 10.2174/15 7017905774322640

[10] King AWT, Asikkala J, Mutikainen I, Järvi P, Kilpeläinen I. Angewandte Chemie International Edition. 2011;**50**:6301-6305. DOI: 10.1002/anie.201100274

[11] Lindman B, Karlston G, Stigsson L. Journal of Molecular liquids. 2010;**156**:76-79. DOI: 10.1016/j.ijbiomac.2006.03.030

[12] Tao Y, Xu W. Carbohydrate Research. 2008;**343**:3071-3078. DOI: 10.1016/j.carres.2008.09.009

[13] Nie X, Shi B, Ding Y, Tao W. International Journal of Biological Macromolecules. 2006; **39**:228-233. DOI: 10.1016/j.ijbiomac.2006.03.030

[14] Passos CP, Coimbra MA. Carbohydrate Polymers. 2013;**94**:626-627. DOI: 10.1016/j.carbpol.2013.01.088

[15] Abe M, Fukaya Y, Ohno H. Green Chemistry. 2010;**12**:1274-1275. DOI: 10.1039/C003976D

[16] Tayal A, Khan SA, Macromolecules. 2010;**33**:9488-9489. DOI: 10.1021/ma000736g

[17] Kirschner KN, Woods RJ. Proceedings of the National Academy of Science. 2001;**98**:10541-10545. DOI: 10.1073/pnas.191362798

[18] Cramer CJ, Truhlar DG. Journal of American Chemical Society. 1993;**115**:5745-5753. DOI: 10.1021/ja00066a046

[19] Davis AP, Wareham RS. Angewandte Chemie International Edition. 1999;**3**:2978-2996. DOI: 10.1002/(SICI)1521-3773(19991018)38:20<2978::AID-ANIE2978>3.0.CO;2-P

[20] Wladkowski BD, Chenoweth SA, Jones KE, Brown JW. Journal of Physical Chemistry A. 1998;**102**:5086-5092. DOI: 10.1021/jp980524+

[21] Momany FA, Willett JL. Journal of Computational Chemistry. 2000;**21**:1204-1219. DOI: 10.1002/1096-987X(200010)21:13<1204::AID-JCC9>3.0.CO;2-F

[22] Strati GL, Willett JL, Momany FA. Carbohydrate Research. 2002;**337**:1833-1849. DOI: 10.1529/biophysj.106.081539

[23] Momany FA, Schnupf U, Willett JL, Bosma WB. Structural Chemistry. 2007;**18**:611632. DOI: 10.1007/s11224-007-9191-9

[24] French AD, Johnson GP, Cramer CJ, Csonka GI. Carbohydrate Research. 2012;**350**:68-76. DOI: 10.1016/j.carres.2011.12.023

[25] Csonka GI, French AD, Johnson GP, Stortz CA. Journal of Chemical Theory and Computation. 2009;**5**:679-692. DOI: 10.1021/ct8004479

[26] Csonka GI, Kaminsky JJ. Journal of Chemical Theory and Computation. 2011;**7**:988-997. DOI: 10.1021/ct200007x

[27] Sameera WMC, Pantazis DA. Journal of Chemical Theory and Computation. 2012;**8**:2630-2645. DOI: 10.1021/ct3002305

[28] Stokke BT, Talashek TA, Brant DA. Macromolecules. 1994;**27**:1124-1135. DOI: 10.1021/ma00083a009

[29] Heiner AP, Sugiyama J, Teleman O. Carbohydrate Research. 1995;**273**:207-223. DOI: http://dx.doi.org/10.1016/0008-6215(95)00103-Z

[30] Widmalm G, Pastor RW. Journal of Chemical Society. 1992;**88**:1747-1754. DOI: 10.1039/FT9928801747

[31] Andrew SM, Thomasson KA, Northrup SA. Journal of American Chemical Society. 1993;**115**:5516-5521. DOI: 10.1021/ja00066a020

[32] Northrup SH, Thomasson KA, Miller CM. Biochemistry. 1993;**32**:6613-6623. DOI: 10.1016/S0006-3495(97)78838-6

[33] Jeffrey GA, Nanni R. Carbohydrate Research. 1980;**137**:21-30. DOI: 10.1016/0008-6215(85)85146-6

[34] Tvaroška I, Kožár T. Journal of Quantum Chemistry. 1983;**23**:765-778

[35] Varki A. Current Opinion in Cell Biology. 1992;**4**:257-266. DOI: 10.1016/0955-0674(92)90041-A

[36] Almond A, Sheehan JK. Glycobiology. 2003;**13**:255-264. DOI: 10.1093/glycob/cwg031