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Review

Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources

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ABSTRACT

Some polysaccharides isolated from natural sources show various important biological activities, such as antitumor, immunomodulatory, and anti-inflammatory effects, which are strongly affected by their chemical structures and chain conformations. This article attempts to review the current development on structural and conformational characterization of some importantly bioactive polysaccharides isolated from natural sources. The chemical structures were analyzed by FTIR, liquid-state NMR (one and two dimensions), solid-sate NMR, Raman spectroscopy, gas chromatography (GC), GC–Mass (GC–MS), and high-performance liquid chromatography (HPLC). The chain conformations of polysaccharides in solutions were investigated using static and dynamic light scattering, viscosity analysis based on the theory of dilute polymer solution, circular dichroism analysis, atomic force microscopy (AFM) including single molecular AFM and AFM-based single-molecule force spectroscopy, fluorescence correlation spectroscopy and NMR spectroscopy.

1. Introduction

Polysaccharides widely exist in the plants, microorganism (fungi and bacteria), algae, and animals. Together with proteins and polynucleotides, they are essential biomacromoleules in the life activities and play important roles in cell–cell communication, cell adhesion, and molecular recognition in the immune system (Dwek, 1996). In recent years, some bioactive polysaccharides isolated from natural sources have attracted much attention in the field of biochemistry and pharmacology. They exhibit various biological activities affected by different chemical structures (Table 1). Suarez et al. (2006) reported that the immunostimulatory activity of arabinogalactans extracted from *Chlorella pyrenoidosa* cells depended on their molecular weights. The higher molecular weight arabinogalactans exhibited immunostimulatory activity, but the lower molecular weight fractions did not. Further researches show that the activities of polysaccharides are not only dependent on their chemical structures, but also are related to their chain conformations (Tao, Zhang, Yan, & Wu, 2007). It is known that the antitumor activities may be related to the triple helical conformation of the $(1 \rightarrow 3)$-β-D-glucan backbone chain for some polysaccharides, such as lentinan from *Lentinus edodes* (Surenjav, Zhang, Xu, Zhang, & Zeng, 2006; Zhang, Li, Xu, & Zeng, 2005) and schphylizophyllan from *Schizophyllum commune* (Okamura et al., 1986; Tsuzuki, Ohno, Adachi, & Yadomae, 1999). Since Sakurai and Shinkai (2000) were the first to find that schizophyllan may form a helical complex with single-stranded homo-polynucleotides, many works about preparing a complex of schizophyllan and DNA or RNA for a non-toxic gene delivery system have been developed (Anada et al., 2005; Koumoto et al., 2004a; Koumoto et al., 2004b; Koumoto, Mizu, Sakurai, Kunitake, & Shinkai, 2004c; Mizu et al., 2004; Sakurai, Mizu, & Shinkai, 2001; Sakurai et al., 2002; Shimada et al., 2007; Takeda, Shimada, Kaneko, Shinkai, & Sakurai, 2007).

In general, it is interesting and important to elucidate the relation among chemical structures, chain conformations of polysaccharides and their biological activities. However, polysaccharides are usually composed of various monosaccharides linked with different glucosidic bonds. Some polysaccharides have hyperbranched structures. Moreover, polysaccharides often have high molecular weights, and tend to form aggregates in solution that can mask the behavior of individual macromolecules. In consequence, to characterize the chemical structures and chain conformations of polysaccharides is not an easy task. This article attempts to review the current structural and conformational characterization of some importantly bioactive polysaccharides isolated from natural sources. The chemical structures were analyzed by FTIR, liquid-state NMR (one and two dimensions), solid-sate NMR, Raman spectroscopy, gas chromatography (GC), GC–Mass (GC–MS), and high-performance liquid chromatography (HPLC). The chain conformations of polysaccharides in solutions were investigated using static and dynamic light scattering,

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Table 1
The chemical structures and activities of some polysaccharides isolated from natural resources.

Polysaccharides	Chemical structures	Activities	References
Lentinan	β-1,3-D glucan containing glycopyranosyl residues branched in position 6 of glucose unit	Antitumor, antiviral immunomodulatory and anticoagulatory	Kupfahl, Geginat, and Hof (2006), Lo, Tsao, Wang, and Chang (2007), Maeda, Takahama, Kohara, and Ynekawa (1997), Oka et al. (1996), Surenjav et al. (2006), Tomati et al. (2004), Vanea, Drage, and Snape (2006), Zhang et al. (2005)
Schizophyllan	β-1,3-D-glucan with one β-1,6-D-glucose side chain for every three main ch1‘ain residues	Antitumor	Bot, Smorenburg, Vreeker, Paques, and Clark (2001), Okamura et al. (1986), Tsuzuki et al. (1999)
Ganoderma lucidum polysaccharide	(1 → 4)-α-D-mannoxylan or (1 → 3)-β-D-glucan backbone branched with mono-, di- and oligosaccharide side chains, a fucose-containing glycoprotein, or acidic protein bound polysaccharide	Antitumor, immunomodulating activity, preventing oxidative damage, protecting liver, reducing serum glucose levels, antiherpetic activities, antiviral activity, antiulcerogenic effect, and inhibition of lipid peroxidation and oxidative DNA damage	Bao, Liu, Fang, and Li (2001), Bao, Wang, Dong, Fang, and Li (2002), Eo, Kim, Lee, and Han (2000), Kim, Eo, Oh, Lee, and Han (2000), Lai and Yang (2007), Lee et al. (2001), Peng, Zhang, Zhang, Xu, and Kennedy (2005), Shiao (2003), Sun, He, and Xie (2004); Wang et al. (2002); Zhu and Lin (2006), Zhu, Chen, and Lin (2007)
Auricularia auricular polysaccharide	Heteropolysaccharides consisted of a backbone chain of β-1,3-D-glucose residues with various branch residues, such as mannose, glucose, xylose and glucuronic acid	Antitumor, immunomodulatory, anticoagulant activity, anti-inflammatory and antioxidant acwtivity	Fan, Zhan, Yu, and Ma (2006), Wu, Ding, and Zhang (2006), Yoona et al. (2003), Zhang and Yang (1995), Zhang et al. (1995a, 1995b, 1999c)
Pachymaran (*Poria cocos*) polysaccharide	Linear and homogeneous β-1,3-D glucan	Antitumor and antimutagenic activity	Ding, Jiang, Zhang, and Wu (1998), Ding, Zhang, and Wu (1999), Xiao et al. (2007), Wu, Ng, and Lin (2004)
Coriolus versicolor polysaccharide	Protein-bound polysaccharide containing α-1,4 and β-1,3 glucosidic linkages in their polysasaccharide moieties	Antitumor, resistance against bacterial infection, immunopotentiation, immunomodulatory, immuno-stimulatory and analgesic effect	Chan and Yeung (2006a, 2006b, 2006c), Cui and Chisti (2003), Lee, Yang, and Wan (2006a), Ng (1998), Wang, Liu, Ng, Ooi, and Chang (1995), Wong et al. (2004), Yeung and Or (2007)
Heparin	A low-sulfated polysaccharide showing a specific decrease of the sulfatation in position 2 of the uronic acid units	Anticoagulant activity primarily from its binding to the serineprotease inhibitor (SERPIN) antithrombin III	Luppi et al. (2005)
α-D-Glucans isolated from the cell wall of *S. cerevisiae*	(1 → 3)-α-D-Glucans moderately branched with (1 → 6)-α-D-glucan chains	To Adsorb zearalenone, reduce its bioavailability in the digestive tract, and protect animals against its adverse ffects	Yiannikouris et al. (2006)
Tea polysaccharide	Protein-bound heteropolysaccharide containing L-arabinose, D-ribose, D-xylose, D-glucose, D-galatose, D-mammose and glucuronic acid in their polysaccharide moieties	Antioxidant activity, reducing the blood glucose and blood triglycerides, anti-hyperglycemia activity, anti-adhesive effects against pathogenic bacteria	Chen, Zhang, and Xie (2004, 2005), Chen, Zhang, Qu, and Xie (2007, 2008), Deng, Tao, Li, He, and Chen (1998); Lee et al. (2006b), Wang, Wang, Li, and Zhao (2001), Zhou et al. (2007)
Lycium barbarum polysaccharide	Protein-bound polysaccharide containing D-rhamnose, D-xylose, D-fructose, D-galactose, D-mannose, and D-glucose in their polysaccharide moieties and linked together by β-D-glycosidic linkages	Antioxidant activity, anti-irradiation activity, immunomodulatory, antitumor, hypoglycemic and hypolipidemic effects, protective effect DNA damage and beneficial effect on sexual behavior	Gan, Zhang, Yang, and Xu (2004), Li, Zhou, and Li (2007a, 2007b), Luo, Cai, Yan, Sun, and Corke (2004), Luo et al. (2006)
Litchi chinensis polysaccharide	Neutral heteropolysaccharide containing mannose, galactose and arabinose, and linked by 1-3-, 1-2- and 1-6-glycosidic linkages	Antioxidant activity	Yang et al. (2006)
Longan polysaccharide	Neutral heteropolysaccharide containing rhamnose, glucose and galactose	Antioxidant activity	Yang and Zhao et al. (2008); Yang and Jiang et al. (2008)
Ulvan from green seaweeds	Composed of variable proportions of different repeating sequences mostly based on disaccharide domains made of rhamnose, glucuronic acid, iduronic acid, xylose, and sulfate	Antitumor and immune modulation activities, strain-specific anti-influenza activities, and anticoagulant activities	Lahaye and Robic (2007)
Spirulina platensis polysaccharide	Sulfated polysaccharide consisted of two types of disaccharide repeating units, *O*-hexuronosylrhamnose (aldobiuronic acid) and *O*-rhamnosyl-3-*O*-methylrhamnos (acofriose) with sulfate groups	Anti-atherogenic and anti-thromobogenic activities	Kaji et al. (2002, 2004), Sekharam, Venkataraman, and Salimath (1987, 1989)

viscosity analysis based on the theory of dilute polymer solutions, circular dichroism analysis, and atomic force microscopy (AFM) including single molecular AFM and AFM-based single-molecule force spectroscopy, fluorescence correlation spectroscopy and NMR spectroscopy.

2. Isolation and purification of polysaccharides

The fungi, plant and algae are usually powdered, hydrolyzed by cellulase or undergo ultrasonication in order to remove the fat and oil soluble substances (Yang, Jiang, Zhao, & Wang, 2008). Polysaccharides are abstracted by water, aqueous NaCl solution, and dilute acidic or alkali aqueous solutions. H_2O_2 is normally used as a decoloring reagent. The free protein in the polysccharides is removed by Sevage method (Sevage, 1934). Protein became gels and precipitated in the Sevage reagent, which was composed of chloroform and *n*-butanol (4/1, v/v). Then the crude polysaccharides can be obtained by centrifugation, which are further purified and isolated according to their different solubilities in water and organic solvents, or based on their different ionic properties. The methods mainly include:

(1) The polyssachrides with different molecular weights and sizes are gradually separated using the precipitants like ethanol, methanol and acetone, or performing the gel chromatography. Sephadex, Sephacryl and Sepherose are commonly used gels.
(2) The acidic and neutral polysaccharides may be separated using cetyltrimethyl ammonium bromide (CTAB) or cetylpyridinium chloride (CPC), which may form a precipitated complex with the acidic polysaccharide. They can also be separated by the ion-exchange cellulose chromatography, e.g. DEAE–cellulose column (Dong, Yao, Fang, & Ding, 2007; Mazumder, Morvan, Thakur, & Ray, 2004) DEAE–Toyopearl column (Urai et al., 2007), DEAE–Sepharose fast-flow column (Yang et al., 2007).

3. Chemical structural characterization of polysaccharides

The chemical structures of polysaccharides, such as the sugar composition, type of glycosyl linkage and the branch structures, may be characterized by spectral analysis, chemical analysis and chromatography.

Table 2
The stretching vibration (ν) of some pyranoses (Zhang, 1994).

Type	Stretching vibration (cm^{-1})
α-D-Glucose	855–833
β-D-Glucose	905–876
α-D-Galactose	839–810
β-D-Galactose	914–866
α-D- or α-D-Mannose	843–818
β-D-Mannose	898–888
α-D-Xylose	760–740
β-D- or β-L-Arabinose	855–830

3.1. FTIR spectroscopy

FTIR spectroscopy is used to investigate the vibrations of molecules and polar bonds between the different atoms. Structures of polysaccharides, such as monosaccharide types, glucosidic bonds and functional groups, can be analyzed using FTIR spectroscopy (Mathlouthi & Koenig, 1986; Zhang, 1994). In the range of 1100–1010 cm^{-1}, three strong absorption peaks appear for pyranoside, and two peaks for furanoside. The characteristic absorptions of some pyranoses are listed in Table 2.

3.2. Raman spectroscopy

Compared with FTIR spectroscopy, Raman spectroscopy is sensitive to detect the vibrations of molecules and nonpolar bonds of the same atom. It is suitable to character the helical conformation and the plane fold of biomacromolecules (Zhu, Isaacs, Hecht, Tranter, & Barron, 2006). The Raman spectra of saccharides can be divided into four regions: the bands in the range of 350–600 cm^{-1} are assigned to skeletal modes of pyranose rings; the anomeric region is from 600 to 950 cm^{-1}; the glycosidic stretching modes appear in the region 950–1200 cm^{-1}; and the CH_2 and C–OH deformations region is from 1200 to 1500 cm^{-1} (Bell, Hecht, & Barron, 1994). Pereira, Sousa, Coelho, Amado, and Ribeiro-Claro (2003) reported that the FT-Raman spectra of carrageenan, alginic acid and agar exhibited a higher resolution than FTIR spectra in the region of 500–1500 cm^{-1}, which were sensitive to the type of polysaccharide and to the type of glycosidic linkage. The characteristic of Raman spectra of β-D-glucosides, such as cellobiose and cellulose, is the 377 cm^{-1} band; and for α-D-glucosidic polysaccharides, their Raman spectra are distinguished by the presence of strong bands at 479–483 cm^{-1} for amylose and amylopectin and 543 cm^{-1} for dextran, but these intense bands are absent in the case of pullulan (Zhbankov et al., 2000). For the deformation vibration (δ) of anomeric C–H of 1,4-D-saccharides, the absorption bands of α-D and β-D terminal isomers are 865–837 cm^{-1} and 905–887 cm^{-1}, respectively (Zhang, 1994). A strong Raman band of α-D helical amylose locates at 865 cm^{-1}; and three bands at 954, 941 and 926 cm^{-1} indicate six C—O—C bonds of glycosidic stretching modes (Lii, Stobinsk, Tomasik, & Liao, 2003; Zhang, 1994). The Raman bands of some saccharides are summarized in Table 3. Gierlinger, Schwanninger, Reinecke, and Burgert (2006) investigated molecular changes of cellulose and lignin during tensile deformation by Raman microscopy.

Table 3
The Raman bands of some saccharides.

Type	Band (cm^{-1})		References
	Skeletal modes of pyranose rings	Modes of anomeric carbons	
α-D-Glucose	440	842	Zhang (1994)
β-D-Glucose	440	900	Gelder, Gussem, Vandenabeele, and Moens (2007), Zhang (1994)
Trehalose	449	839	Gelder et al. (2007), Gussem, Vandenabeele, Verbeken, and Moens (2005)
α-D-Maltose		848	Bell et al. (1994)
β-D-Isomaltose		905	Bell et al. (1994), Zhang (1994)
Amylose	481	865	Fechner, Wartewig, Kleinebudde, and Neubert (2005), Gelder et al. (2007), Gussem et al. (2005), Kizil, Irudayaraj, and Seetharaman (2002), Schuster, Ehmoser, Gapes, and Lendl (2000), Zhang (1994), Zhbankov et al. (2000)
Amylopectin	477	850	Fechner et al. (2005), Gelder et al. (2007), Gussem et al. (2005), Kizil et al. (2002), Schuster et al. (2000), Zhang (1994), Zhbankov et al. (2000)
Dextran	543	844	Zhang (1994), Zhbankov et al. (2000)

3.3. NMR spectroscopy

3.3.1. Liquid-state NMR

NMR spectroscopy, especial liquid-state NMR, has become recognized as an important developing tool for chemical structural analysis of polysaccharides (Bubb, 2003). Most polysaccharides can be dissolved in water and dimethylsulfoxide (DMSO), thus deuterated water and DMSO (D_2O and DMSO-d6) are common solvents for polysaccharides in the liquid-state NMR experiments. The proton signals of polysaccharides overlap in the range of 3.5–5.5 ppm in the ^1H NMR spectrum, it is difficult to assign them. Leeuwen, Leeflang, Gerwig, and Kamerling (2008) investigated the ^1H NMR spectroscopy of the primary structural characterization of α-D-glucans in detail, in which chemical shift patterns for (α1 → 2)-, (α1 → 3)-, (α1 → 4)- and (α1 → 6)-linked D-glucose residues were analyzed. In contrast, the range of ^{13}C chemical shifts of polysaccharides is much wider than that of ^1H chemical shift, which come from 60 to 110 ppm. Table 4 lists the ^{13}C signals of glucan and their assignments (Zhang, 1994).

The fine chemical structures of polysaccharides such as sugar compositions, configurations and types of glycosidic linkages may be analyzed using two-dimensional (2D) NMR technologies. Correlated 2D NMR is by far the most common and easily used of the 2D technologies (Abraham, Fisher, & Loftus, 1998; Duus, Gotfredsen, Bock, & Seetharaman, 2000; Ning, 1998). It may be applied to both heteronuclear and homonuclear spin systems. Of the homonuclear correlation methods, the correlated spectroscopy technology is the most commonly used. The technology includes COSY (correlation spectroscopy), DQF-COSY (double quantum filtered-COSY), TOCSY (total correlation spectroscopy) and COSY-45. The heteronuclear correlated 2D experiment, particular for ^{13}C–^1H coupled systems, has also proved very useful, such as H,C-COSY, HMQC (heteronuclear multiple-quantum coherence) and HSQC (heteronuclear single-quantum coherence). In addition, HMBC (heteronuclear multiple-bond coherence) correlates long-distance coupled ^{13}C and ^1H nucleus, which is used equal to long-distance H, C-COSY. The nuclear Overhauser enhancement (NOE) phenomenon can produce an almost threefold increase in signal-to-noise over and above that produced by collapse of the fine structure, in which the distance between two nuclei is less than 5 Å. The NOESY (nuclear Overhauser enhancement spectroscopy) and ROESY (rotating frame Overhauser enhancement spectroscopy) are important 2D technologies to obtain the chemical structures and configurations of polysaccharides (Adeyeye et al., 2003; Niedziela et al., 2006; Yongye, Gonzalez-Outeiriño, Glushka, Schultheis, & Woods, 2008). It is interesting to note that diffusion ordered spectroscopy (DOSY) may be used to estimated the molecular weights and dispersion of molecular weights of neutral polysaccharides (Suarez et al. 2006).

The normal approaches to analyze the chemical structures of polysaccharides through 1D and 2D NMR spectroscopy are summarized as following (Castro, Fregolino, Gargiulo, Lanzetta, & Parrilli, 2008; Duus et al., 2000; Leeuwen et al., 2008):

(1) *Saccharide residues:* The anomeric carbon resonances of polysaccharides may be distinguished in the chemical shift ranges of 90–110 ppm, thus the saccharide residues is firstly determined in their ^{13}C NMR spectra. In consequence, the anomeric proton resonances in the chemical shift ranges of 4.4–5.5 ppm will be confirmed through the relations of protons and carbons from H, C-COSY, HMQC and HSQC.

(2) *Constituent monosaccahrides:* The cross peaks of H2, H3, H4, H5 and H6 of polysaccharides can be recognized step by step based on the cross peak of the anomeric proton in homonuclear DQF-COSY, TOCSY and COSY-45 spectra. In addition, the ^{13}C chemical shifts of C2, C3, C4, C5 and C6 can be found in heteronuclear H, C-COSY, HMQC and HSQC spectra based on the chemical shifts of such protons. Consequently, the assignments of individual residues of polysaccharides can be made according to the chemical shifts of most monosaccharides reported in the literature (Duus et al., 2000).

(3) *Glycosidic linkage and sequence:* The correlation between the amomeric protons on one monosaccharide and the carbons on another monosaccharide can be found in the HMBC spectrum. Then the glycosidic linkage and sequence of polysaccharides will be obtained. Such chemical structures can be confirmed through NOESY spectroscopy (Liu et al., 2008).

(4) *Anomeric configuration:* The β-anomeric ^{13}C resonances locate in the downfield compared to those of α-anomer types for most saccharides (Zhang, 1994). The chemical shifts of β-anomeric ^{13}C locate in the range of 103–105 ppm, while the chemical shifts of α-anomeric ^{13}C are from 97 to 101 ppm (Bubb, 2003). The coupling constant (J), like the vicinal coupling constant (^3J), and the ^{13}C and ^1H coupling constants ($^1J_{CH}$ and $^4J_{CH}$) may help to recognize the anomeric conformations of saccharides (Duus et al., 2000). For example, the $^1J_{C1-H1}$ values about 170 and 160 Hz indicate an α-anomeric and a β-anomeric configuration for 1-4-D polysaccharide, respectively.

(5) *Position of substituted groups:* The chemical shifts normally downfield shift about 0.2–0.5 ppm for protons and 6–7 ppm for ^{13}C after the hydroxyl groups of polysaccharides are substituted by methyl, acetyl, sulfate and phosphate groups (Duus et al., 2000). Cui et al. (2008) reported that the chemical shifts of sulfated C3 of a (1 → 6)-α-D-glucan isolated from the plant's root are 83.4 and 80.2 ppm, which downfield shifted ∼2–5 ppm compared with the chemical shift of unsubstituted C3 at 78.4 ppm. The chemical shifts of some substituted groups are listed in Table 5.

Recently, 1D and 2D homo- or hetero-nuclear experiments, such as DQF-COSY, HSQC, HMBC and NOESY are widely used to analyze the chemical structures and configurations of various bioactive polysaccharides, e.g. an antimutagenic polysaccharide isolated from *Sepiella maindroni* ink (Liu et al., 2008), an antinociceptive and anti-inflammatory *Lentinus edodes* heterogalactan (Carbonero et al., 2008), a heteroglycan isolated from edible mushroom *Volvariella bombycina* (Das et al., 2008), an exopolysac-

Table 4
Assignments of ^{13}C chemical shifts of glucan (Zhang, 1994).

Glucan	C-1	C-2	C-3	C-4	C-5	C-6	Solvent
[α-Glc(1–3)-]$_n$	101.3	72.2	83.2	71.7	73.7	62.2	D_2O
[α-Glc(1–4)-]$_n$	99.86	71.93	73.07	78.77	71.48	60.50	DMSO-d_6
[β-Glc(1–2)-]$_n$	102.7	83.1	77.0	69.3	76.1	61.4	D_2O
[β-Glc(1–3)-]$_n$	104.7	74.9	88.0	69.9	77.8	62.5	D_2O
[β-Glc(1–4)-]$_n$	103.4	74.3	76.1	79.9	75.4	61.5	D_2O
[β-Glc(1–6)-]$_n$	104.2	74.2	75.7	69.6	76.1	70.0	D_2O

Table 5
Chemical shifts of substituted groups of polyasaccharides.

Substituted groups	Chemical shift			References
	^1H	^{13}C	^{31}P	
Methyl	1.28	17.8	–	Liu et al. (2008)
Acetyl	2.02	24.8	–	Liu et al. (2008)
Carboxyl	–	176.7, 177.3	–	Liu et al. (2008)
Phosphate	–	–	−0.78, −1.67	Ganeshapillai, Vinogradov, Rousseau, Weese, and Monteiro (2008)

charides produced by *Leuconostoc citreum* and *Weissella colysac* (Maina, Tenkanen, Maaheimo, Juvonen, & Virkki, 2008), a polysaccharide isolated from edible mushroom *Pleurotus sajor-caju* (Roy, Maiti, Mondal, Das, & Islam, 2008), a polysaccharide (Fr. I) isolated from edible mushroom *Volvariella diplasia* (Ghosh et al., 2008), a β-(1 → 3)-D-glucan isolate from cultured fruit bodies of *Sparassis crispa* (Tada et al., 2007), an antioxidant (1 → 6)-a-D-glucan from the root of *Pueraria lobata* (Willd.) Ohwi (Cui et al., 2008), a sulfated polysaccharide from red seaweed *Gracilaria birdiae* (Maciel et al., 2008), and a bacterial polysaccharide named as Fucogel (Guetta, Mazeau, Auzely, Milas, & Rinaudo, 2003).

3D or higher multiple-dimensional NMR experiments can be carried out by the combination of 2D experiments with multiple evolution times (Duus et al., 2000). They correlate the interactions of different nuclei along the *x*, *y*, and *z* dimensions. The *x* and *y* dimensions usually consist of proton–proton coupling, e.g. TOCSY and NOESY, that are correlated to another nucleus in the *z* plane, e.g. HSQC and HMBC (Mopper, Stubbins, Ritchie, Bialk, & Hatcher, 2007). The main advantage of using high-dimension NMR experiments is to increase the separation of resonances in the ^{13}C dimension, resulting in improving the resolution of the spectra (Allerdings et al., 2005; Xu & Bush, 1996). It was reported that the structure of a lipopolysaccharide (LPS) from *Vibrio cholerae* O139 was analyzed through 1D, 2D and 3D NOESY-TOCSY and 3D TOCSY-NOESY experiments (Cox, Brisson, Varma, & Perry, 1996).

3.3.2. Solid-state NMR

Liquid-state NMR gives high-resolution spectra since the usually dominant dipolar interactions are averaged to zero. In contrast, the linewidths of solid-state NMR become broader mainly due to the anisotropic character and dipolar interaction (Xue, 1993). The anisotropic parts of the interactions from the molecules can be removed when the solid sample rotate at 54.7°. This is the basis of the technology called magic-angle-spinning (MAS) which is essential to achieve high-resolution ^{13}C solid-state NMR spectra (Mcbrierty & Packer, 1993). The intensity of the solid ^{13}C signals can be enhanced using cross polarization (CP) technology, in which the polarization transfer from ^{1}H to ^{13}C (Mcbrierty & Packer, 1993). In recent years, solid-state NMR is used to analyze the chemical structures of polysaccharide in order to overcome the solubility problem, since the samples can be measured in a solid and dehydrated form (Spevacek & Brus, 2008). Pizzoferrato et al. (2000) reported the ratio between proteins and polysaccharides was directly determined through solid ^{13}C CP/MAS spectroscopy. The chemical structures of an extracellular glucans from the fungi *Acremonium persicinum* C38 polysaccharides was analyzed by solid state ^{13}C NMR, which composed of a backbone of (1 → 3)-β-linked glucosyl residues with single (1 → 6)-β-linked glucosyl side branches (Schmid et al., 2007).

3.4. Chemical analysis and chromatography

The monosaccharide compositions, types of glycosidic linkages and branching of polysaccharides may be also analyzed by chemical analysis and chromatography. GC, GC–MS and HPLC methods are employed after polysaccahirdes are hydrolyzed by trifluoroacetic acid (TFA) (Urai et al., 2007), or are derived by the methylation (Ciucanu & Kerek, 1984), periodic acid oxidation (Abdel-Akher, Hamilton, Montgomery, & Smith, 1952) and Smith degradation (Datta, Basu, & Roy, 1999). The oligosaccharides of heparin were separated by SAX-HPLC and quantified at 232 nm using a 5-μm Spherisorb SAX column (Luppi, Cesaretti, & Volpi, 2005). The monosaccharide linkages of three immunomodulating polysaccharides of the pectic arabinogalactan type, isolated from the roots of the medicinal plants *Vernonia kotschyana* (Vk2a) and *Cochlospermum tinctorium* (Ct50A1 and Ct50A2), were analyzed by GC–MS after reducing uronic acid groups, methyaltion and methyl esterification (Nergard et al., 2006).

Some polysaccharides compose of main chain and branch chains. Tao and Zhang (2006) and Tao et al. (2007) reported a water-insoluble hyperbranched polysaccharide from fungus sclerotia of *Pleurotus tuber-regium*, of which the branching degree (BD) was determined as to be 65.5% according to Eq. (1):

$$BD = \frac{(N_T + N_B)}{(N_T + N_B + N_L)} \tag{1}$$

where N_T, N_B and N_L are the total number of the terminal residues, branched residues and linear residues, respectively.

The uronic acids in the acidic polysacchairdes are importantly bioactive and reactive groups. Their contents are commonly estimated using the spectrophotometric methods, such as carbazole/sulfuric acid (Radhakrishnamurthy & Berenson, 1963) and sulfamate/*m*-hydroxydiphenyl (Blumenkrantz & Asboe, 1973). Recently, Li, Kisara, Danielesson, Lindstrom, and Gellerstedt (2007) reported an improved methodology for the accurate quantification of uronic acid in xylans and other polysaccharides, which combined the carbazole/sulfuric acid method and GC after methanolysis and subsequent acetylation. Chen, Zhang, and Xie (2004) reported that the uronic acids in tea polysaccharides were quantitatively analyzed using HPLC. This method showed that the minimum detectable limit concentration of uronic acid was 10 μg/ml, and the analysis of a standard range of galacturonic acid concentrations (100–4000 μg/ml) yielded linear results.

4. Chain conformational analysis of polysaccharides in solution

4.1. Analysis of polysaccharide conformations based on the theory of dilute polymer solutions

The conformation of polysaccharides in solutions, especially in aqueous solutions, can be investigated according to the theory of dilute polymer solutions. The intrinsic viscosity $[\eta]$ is a characteristic property of polysaccharide solution. Huggins and Kraemer equations are used to estimate the $[\eta]$ value by extrapolating to an infinite dilution (Goh, Haisman, & Singh, 2006a; Goh, Hemar, & Singh, 2005; Goh, Pinder, Hall, & Hemar, 2006b):

$$\eta_{sp}/C = [\eta] + K'[\eta]^2C \tag{2}$$

$$(\ln \eta_r)/C = [\eta] + K''[\eta]^2C \tag{3}$$

where K' is the Huggins' constant and K'' is the Kraemer' constant, η_{sp}/C is the reduced specific viscosity, and $(\ln \eta_r)/C$ is the inherent viscosity. The K' value is usually roughly between 0.3 and 0.8 with 0.3–0.4 for a polymer in good solvents and 0.5–0.8 for polymers in theta solvents; and above 0.8, aggregation of the polymers is likely to occur. For a random coil polymer, $K' - K''$ = 0.5.

The rheological properties of the polysaccharides containing acidic groups are generally affected by the polyelectrolyte effect. The intrinsic viscosity decreases with increasing ionic strengths of the solution according to the relation (Goh et al., 2006b; McConaughy, Stroud, Boudreaux, Hester, & McCormick, 2008; Tinland & Rinaudo 1989):

$$[\eta] = [\eta]_\infty + SI^{-1/2} \tag{4}$$

where $[\eta]_\infty$ is the intrinsic viscosity at infinite ionic strength, I is the ionic strength of the solution, and S is a constant. The $[\eta]_\infty$ and S values can be obtained from the intercept and the slope of the linear functions by the measurement of the $[\eta]$ of a polymer at different ionic strength.

Smidsrod and Haug (1971) proposed an empirical relation to compare the flexibility of polymers:

$$S = B([\eta]_{0.1})^{\nu} \tag{5}$$

where $[\eta]_{0.1}$ is the intrinsic viscosity of a polymer at 0.1 mol/l ionic strength, the average value exponent ν of 1.3 is used, B is an empirical parameter which relates to chain flexibility. A low value of B represents the stiff polymer backbones. Xanthan adopts a stiff conformation and has a B value equal to 5.25×10^{-3} (Tinland & Rinaudo 1989), whereas the B value of flexible carboxymethylcellulose is 0.045–0.065 (Smidsrod & Haug, 1971). Flaxseed polysaccharide exhibits a semi-flexible conformation and has a B value about 0.018 (Goh et al., 2006b).

The weight averaged molecular weight (M_w), the second virial coefficient (A_2) and the radius of gyration (R_g) in dilute polymer solution are calculated by the Zimm method of static light scattering (SLS) based on the Rayleigh–Gans–Debye theory for light scattering through Eq. (6).

$$\frac{Kc}{R_\theta} = \frac{1}{M_W}\left[1 + \frac{16\pi^2 n^2}{3\lambda^2} R_g^2 \sin^2\left(\frac{\theta}{2}\right)\right] + 2A_2 c \tag{6}$$

where the optical constant $K = [4\pi^2 n^2 (dn/dc)^2]/N_A\lambda^4$; n is the refraction index of solvent and solution; θ is the observation angle; λ is the wavelength in vacuum of the laser, N_A is Avogadro's number and c is the concentration of the polymer solution. The Rayleigh ratio, $R_\theta = (I_\theta r^2/I_0)$, I_0 and I_θ are the intensity of the incident light and the scattered light, r is the distance from the light source to the measuring point. A plot of (Kc/R_θ) against $[\sin^2(\theta/2) + kc]$ can be used to determine the molecular parameters (where k is an arbitrary constant). By extrapolating the data to zero angles and concentrations, R_g and A_2 can be obtained from the slopes, respectively. The intercept of both extrapolated lines to zero angles and zero concentrations yields M_w.

The size of polysaccharides in solution is measured by dynamic light scattering (DLS) method (Goh et al., 2006b; Li, Cui, & Wang, 2006; Ma, Wang, & Zhang, 2008). Hydrodynamic radius (R_h) is calculated using the Einstein–Stokes equation:

$$R_h = \frac{k_B T}{6\pi\eta D} \tag{7}$$

where D is the diffusion coefficient, k_B, T, and η are the Boltzmann constant, the absolute temperature, and the solvent viscosity, respectively.

The following equations reveal the relations between $[\eta]$, M_w, A_2, R_g and R_h; and the conformation of polysaccharide in solution can be elucidated from the obtained parameters.

$$\rho = \frac{R_g}{R_h} \tag{8}$$

For extended chains (cylinders) $\rho \geqslant 2$, for flexible random coils ρ is 1.5–1.8 in a good solvent and 1.3 in a θ solvent, and for compact spheres ρ is about 0.775 (Nichifor, Lopes, Carpov, & Melo, 1999).

$$[\eta] = KM_w^{\alpha} \tag{9}$$

Both parameters of K and α are constants for a given polymer at a given temperature in a given solvent. For flexible random coils in a good solvent α is 0.5–0.8, about 0.5 for compact coils in a θ solvent, above 0.8 and even beyond 1.0 for rigid polymer chains (Huang & Zhang, 2005; Ma et al., 2008).

$$R_g = kM_w^{\alpha'} \tag{10}$$

Both k and α' are constants for a given polymer at a given temperature in a given solvent. Usually, α' of about 0.7 suggests a relatively stiff rod-like conformation, α' values are about 0.6 and 0.5 for flexible random coils in a good solvent and a theta solvent, respectively, and about 0.33 for compact coils in a θ solvent (Huang & Zhang, 2005; Tao & Zhang, 2006; Tao & Zhang, 2008; Goh et al., 2006b).

The A_2 value directly reflects the degree of interactions between polymers and solvents (Zhang, Xue, Mo, & Jin, 2003). For polymers in a good solvent the A_2 is positive; and $A_2 \leqslant 0$ for polymers in a θ solvent or aggregation. The A_2 value usually decreases with increasing of molecular weights of polymers and obeys the following relation:

$$A_2 = KM_w^{-b} \tag{11}$$

Both K and b are constants for a given polymer at a given temperature in a given solvent. The b value is normally 0.2–0.3 at a good solvent limit; and a higher value (>0.5) is a characteristic of branched structures (Tao et al., 2007).

Tao et al. (2007) investigated the chain conformation of water-insoluble hyperbranched polysaccharide from fungus using static laser light scattering, dynamic light scattering, and viscometry. The various scaling laws and molecular parameters of polysaccharide were calculated from the data of M_w, R_g, A_2, and $[\eta]$ according to the theory of polymer solutions. The result showed that the polysaccharide exhibited a sphere-like conformation in LiCl/dimethylsulfoxide solution.

Lentinan is a β-(1 → 3)-D-glucan containing glycopyranosyl residues branched in position 6 of glucose unit (Fig. 1). The repeating unit consists of five β-(1 → 3)-glucose residues and two β-(1 → 6)-glucose side branches. The conformation of lentian transformed from triple-helical chains to single random-coil chains with the changes of solvents and temperatures. The values of M_w, R_g, R_h, $[\eta]$ and specific optical rotation at 589 nm $[\alpha]_{589}$ of lentian varied with the concentrations of aqueous NaOH solution (w_{NaOH}), indicating the induced helix-coil transition (Zhang, Zhang, & Xu, 2004). Lentinan existed as linear, circular, and branched triple-helical chains at w_{NaOH} lower than 0.05 M, and as single random-coil chains at w_{NaOH} higher than 0.08 M. The helix-coil transition was irreversible. But the denatured lentian in 0.15 M NaOH aqueous solution could be renatured to the triple-helical conformation by dialyzing. Xu, Zhang, Zhang, and Wu (2004) found that the triple helical chains of lentinan in water were denatured in dimethlysulf-

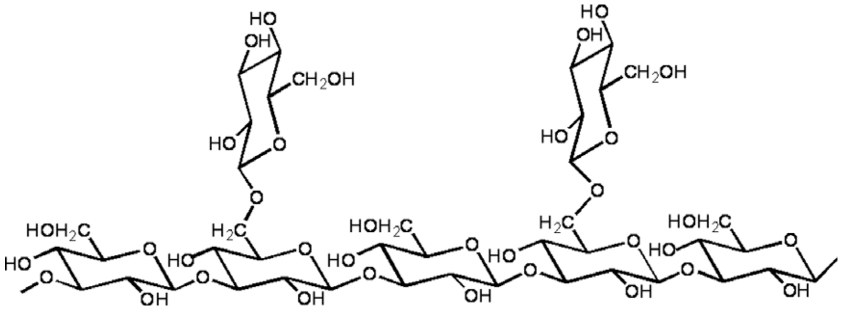

Fig. 1. Chemical structure of lentinan (Zhang et al., 2008).

oxide (DMSO) into single random coils, through comparison of the values of their $[\alpha]_D$, $(\ln\eta_r)/c$ and R_h in ware, DMSO and the mixture solvents with different w_H (the weight fraction of water in the mixed solvent) values. Wang, Xu, and Zhang (2008) further studied thermally induced conformation transition of triple-helical lentinan. They reported that some solution parameters of lentinan, such as M_w, R_g, R_h, $[\eta]$ and specific optical rotation, depended on temperature in 0.9% NaCl aqueous solution. The conformation transitions of lentinan from triple strand to single chain and from extended chains to winding chains were completed rapidly at 130–145 °C (Fig. 2). This was induced by destruction of the intra- and intermolecular hydrogen bonds in lentinan. The thermally induced conformational transition was irreversible. Zhang, Xu, and Zhang (2008) found a well-organized triple-helical structure (triplex I) formed through hydrogen-bonding with the surrounding water molecules at a temperature lower than 9 °C, and it was broken with increasing temperature to become a free triple-helical structure (triplex II). This conformation transition was proved to be thermally reversible.

For polysaccharides (GLFP and GLMP) extracted from *Ganoderma lucidum* (Lai & Yang, 2007), when water was used as the solvent, the Huggins' constants were in 0.59−0.93, implying that molecular aggregations were likely occurred to some extent. However, dimethylsulfoxide (DMSO) was a better solvent due to the low Huggins' constants were in 0.39−0.48. Both GLFP and GLMP exhibited as a less compact and extended conformation in DMSO as indicated by the higher Mark–Houwink exponents (1.32−1.66) and lower power dependence of viscosity on concentration.

The relationship of R_g and M_w of a (1 → 6)-α-D-glucan from the plant's root was represented by $R_g = 1.12 \times 10^{-2}\, M_w^{0.56\pm0.008}$, indicating that the polysaccharide exhibited as a random coil conformation in aqueous solution (Cui et al., 2008).

Polysaccharides may exhibit some conformations in solutions, such as coils, semi-flexible chains, stiff chains, and helical chains including single, double and triple helical chains. The theory of wormlike chain model is famous for describing the flexibility of polymer chains in solutions. The relation of the model was proposed by Bohdanecky (1983) and Bushi, Tsvetkov, and Lysenko (1981) based on the Yamakawa–Fujii–Yoshizaki theory (Huang & Zhang, 2005; Yamakawa & Fujii, 1974; Yamakawa & Yoshizaki, 1980):

$$(M^2/[\eta])^{1/3} = A_\eta + B_\eta M^{1/2} \tag{12}$$

$$A_\eta = A_0 M_L \Phi_{0,\infty}^{-1/3} \tag{13}$$

$$B_\eta = B_0 \Phi_{0,\infty}^{-1/3} (2q/M_\mathrm{L})^{-1/2} \tag{14}$$

where, q is the persistence length, M_L is the molecular weight per unit contour length, A_0 and B_0 are functions of $d/2q$, d is the cylinder diameter. The value of B_0 is in the range of 1.05–1.08, and the value of A_0 is determined by following equations:

$$d = d_r \cdot 2q \tag{15}$$

$$d_r^2/A_0 = (4\Phi_{0,\infty}/1.215\pi N_A)(v/A_\eta)B_\eta^4 \tag{16}$$

$$\log(d_r^2/A_0) = 0.173 + 2.158 \log d_r \quad (d_r \le 0.1) \tag{17}$$

where, the value of $\Phi_{0,\infty}$ is 2.86×10^{23} mol^{-1} for $d_\mathrm{r} \leqslant 0.1$, N_A is the Avogadro constant, v is the partial special volume of a polymer chain, which is normally 0.68 cm^3/g for polysaccharides. The values of A_η and B_η can be obtained from the slope and intercept of the Bohdanecky plot according to Eq. (12). Then the value of d_r^2/A_0

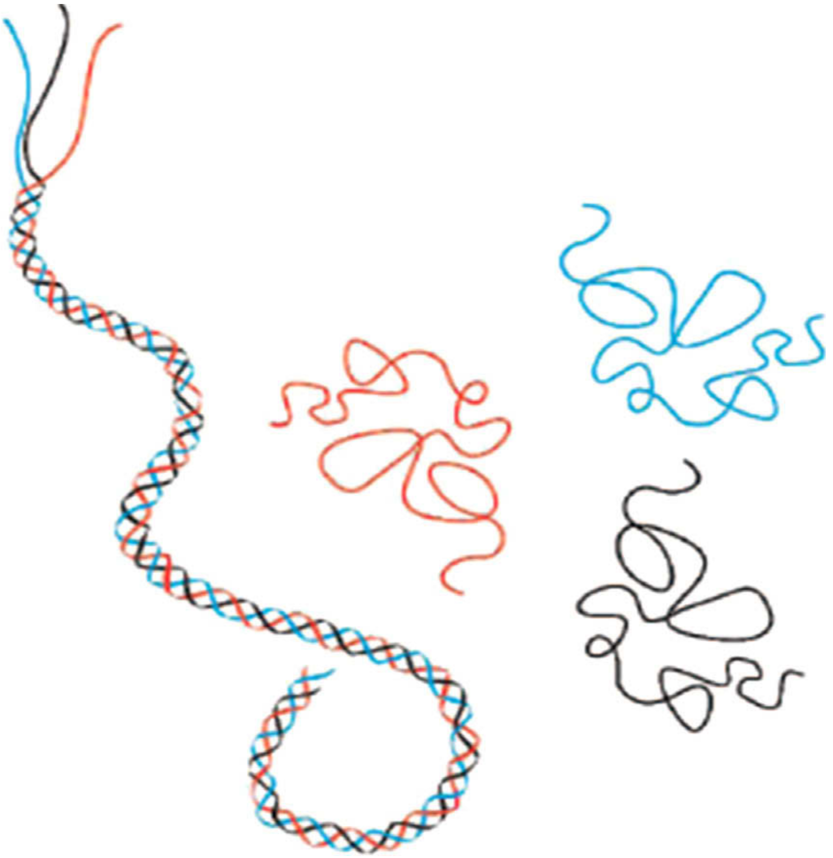

Fig. 2. Schematic representation of the thermally-induced conformation transition of lentinan solution at 137 °C (Wang et al., 2008).

can be evaluated from Eq. (16), and the d_r and A_0 can be further evaluated via the dependence of d_r^2/A_0 on d_r from Eq. (17).

The above theory does not take into account the polydispersity effect on the measured [η]. Although such an effect is generally small, the deduced wormlike chain parameters may be somewhat misestimated (Takahashi et al., 2004). The polydispersity effects may use the Schulz–Zimm distribution function defined as:

$$f_w(M)dM = \left[\frac{(m+1)^{m+1}}{m!}\right]\left(\frac{M}{M_w}\right)^m \times \exp\left[-(m+1)\frac{M}{M_w}\right]d\left(\frac{M}{M_w}\right) \tag{18}$$

where m is the distribution parameter related to number-average molar mass M_n, M_w, and M_z by $M_w/M_n = 1 + 1/m$ or $M_z/M_w = (m+2)/(m+1)$. [η] of a polydisperse wormlike cylinder is then expressed by:

$$[\eta] = \frac{\Gamma(m+1+a)}{(m+1)^a\Gamma(m+1)}[\eta]_0 \tag{19}$$

where α is the exponent value of [η] and $\Gamma(x)$ is the gamma function. The wormlike chain parameters of q, M_L and d are thus determined based on the compensated [η].

According to the unperturbed wormlike chain model, the relation of the unperturbed R_g of a monodisperse wormlike chain was proposed by Benoit and Doty (Benoit & Dotv, 1953; Zhang et al., 2005):

$$R_g = (qM_w/3M_L) - q^2 + (2q^3M_L/M_w)[1 - (qM_L/M_w)(1 - e^{-\frac{M_w}{q}M_L})] \tag{20}$$

Murakami, Norisuye, and Fujita (1980); Norisuye, 1993 further proposed that, for the semi-flexible chains, stiff chains, and helical polymer chains, the q and M_L parameters could be estimated from a plot of $(M_w/R_g)^{1/2}$ against $1/M_w$ according to Eq. (21):

$$(M_w/R_g)^{1/2} = (3M_L/q)^{1/2}[1 + 3qM_L/(2M_w)] \tag{21}$$

According to these theories, the conformations of some active polysaccharides were analyzed. It is shown in Table 6 that the q and M_L values of the stiff-chain polysaccharides are much larger than those of the flexible random polysaccharides.

The pitch h of the polysaccharide helix is related to M_L (Yanaki, Norisuye, & Fujita, 1980; Kashiwagi, Norisuye, & Fujita, 1981):

$$h = \frac{\frac{M_0}{x}}{\frac{M_0}{n_s}} \tag{22}$$

where M_0 is the molar mass of the polysaccharide repeat unit, x is the number of glucose on a repeated reside, and n_s is the helix number.

The characteristic ratio C_∞ reflects the flexibility of the polymer that is affected by the bond angle and steric hindrance, which is expressed by (Huang & Zhang, 2005; Zhang et al., 2001):

$$C_\infty = \frac{M_0}{\lambda M_L l^2} \tag{23}$$

where M_0 is the average molar mass of a glucose residue in repeat unit, λ^{-1} is the Kuhn's segment length ($\lambda^{-1} = 2q$), and l is the average length of glucose.

The value of C_∞ for wheat β-glucan was estimated to be 16.88, which was considered to be rigid (Li et al., 2006). Zhang et al. (2001) reported that C_∞ value of a water-insoluble glucan isolated from sclerotium of *Pleurotus tuberregium* was 16.8 in DMSO. However, it existed as a dense random-coil chain analyzed from other parameters. The higher C_∞ was attributed to a larger hindrance caused by side chains of glucan. It was found that the C_∞ values for a water-insoluble (1 → 3)-α-D-glucan isolated from *Poria cocos* mycelia and its sulfated derivative were calculated to be 14.9 ± 0.2 and 31.8 ± 1, respectively (Huang & Zhang, 2005). The results indicated that the glucan existed as an extended flexible chain in 0.25 mol/l LiCl/Me_2SO, and its sulfated derivative existed as a semi-stiff chain in 0.15 mol/l aqueous NaCl solution.

4.2. Circular dichroism

Some polysaccharides are substituted by specific chromophores with optical activity, such as uronic acids, acetate groups, and amide. The conformational changes of these polysaccharides may affect the polarizability, static field contributions, and orientation of the chromophores, resulting in the change of the optical activity (Buffington, Pysh, Chakrabart, & Balazs, 1977). The optically active absorption bands of the substituted chromophores arising from the

Table 6
The chain parameters of some polysaccharides.

Polysaccharides	q (nm)	M_L (nm^{-1})	Flexibility	Solvent	References
Schizophyllan	170–230	2150	Triple helix	H_2O	Yanaki et al. (1980)
Lentinan	100	2240	Triple helix	0.9% Aqueous NaCl solution	Zhang et al. (2005)
Xanthan	120	1940	Double helix	H_2O	Sato, Norisuye, and Fujita (1984)
Succinoglycan	50	1500	Double helix or aggregate composed of paired single helices	0.1 mol/l Aqueous NaCl solution	Kido et al. (2001), Nakanishi and Norisuye (2003)
β-(1 → 3) Glucan isolated from Auricularia auricular	90	1030	Single helix	H_2O	Zhang and Yang (1995)
Curdlan	6.8	890	Semi-flexible chains	0.3 mol/l NaOH	Nakata, Kawaguchi, Kodama, and Konno (1998)
β-(1 → 4) Glucan isolated from Auricularia auricular	12.3	820	Semi-stiff chains	0.1 mol/l aqueous NaCl solution	Ma et al. (2008)
Water-insoluble glucan isolated from Poria cocos mycelia	6.3	760	Extended flexible chains	0.25 mol/l LiCl/Me_2SO	Huang and Zhang (2005)
Sulfated derivative of above glucan	13.1	1060	Semi-stiff chains	0.15 mol/l aqueous NaCl solution	Huang and Zhang (2005)
Polyguluronate	5.2	359	Semi-flexible chains	H_2O	Lee, Bouhadir, and Mooney (2002)
Wheat β-glucan	4.4	–	Extended random coils	0.5 mol/l NaOH	Li et al. (2006)
Mannoxylan isolated from Ganoderma tsugae	4.2	832	flexible random coils	0.25 mol/l LiCl/DMSO	Peng et al. (2005)
Water-insoluble glucan isolated from sclerotium of Pleurotus tuberregium	3.1	408	Dense random-coil chains	DMSO	Zhang et al. (2001)

n–π^* transitions located in the range 200–250 nm (Buffington et al., 1977; Listowsky, Avigad, & Englard, 1970; Southwick, McDonnell, Jamieson, & Blackwell, 1979). Therefore Circular Dichroism (CD) may provide a convenient method of investigating the conformational change of polysaccharides substituent by chromophores.

The backbone of xanthan is a linear 1,4-β-D-glucan, and every second backbone residue has a β(1 → 3) substituted trisaccharide side chain (β-D-mannopyranosyl-(1 → 4)-α-D-glucuropyranosyl-(1 → 2)-β-D-mannopyranoside 6-*O*-acetate). The terminal D-mannose residue of the side chain may have a pyruvicacid residue linked to the 4- and 6-positions (Southwick et al., 1979). The CD analysis showed that xanthan transferred from the ordered conformation (single or double helix) to the random coil varied with the polymer concentration, temperature, pH value, and ionic strength (Bresolin, Milas, Rinaudo, & Ganter, 1998; Brigand & Muller, 1997; Dentini, Crescenzi, & Blasi, 1984; Milas & Rinaudo, 1979; Paradossi, Chiessi, Barbiroli, & Fessas, 2002; Southwick et al., 1979). The spectra of the ordered conformation showed that the maximum wavelength ($\lambda_{\max}$) was 210 nm and the minimum wavelength ($\lambda_{\min}$) was 225 nm, and the random coil conformation with $\lambda_{\max} = 205$ and $\lambda_{\min} = 218$ nm.

Gellan gum is a helix-forming polysaccharide, which consists of tetraccharide repeating units, 1.3-β-D-glucose, 1,4-β-D-glucuronic acid, 1,4-β-D-glucose, and 1,4-α-L-rhamnose. The CD spectra of gellan showed a single peak around 200 nm, which was attributed to a carboxyl group in a repeating unit (Nitta et al., 2003). The ellipticity at 202 nm decreased on cooling and increased on heating. This change indicated the helix-coil transition of the conformational change of gellan.

4.3. AFM

Single molecular AFM is a powerful technique for directly observing the conformation of individual macromolecules. The persistence length can be determined from statistical analysis of the changed in the tangen direction of polymer chain as a function of segement separation of the chain trajectory (Sletmoen, Maurstad, Sikorski, Paulsen, & Stokke, 2003). Camesano and Wilkinson (2001) reported that the conformations of xanthan at different salt concentrations were quantitatively analyzed using the single molecular AFM technology. Xanthan existed as a single helix in pure water, and changed to a double helix in 0.5 mol/l aqueous KCl solution. The persistence length and number-average contour lenght decreased from 1651 and 417 to 450 nm and ~150 nm, respectively. The persistence length of schizophyllan in aqeuous solution was determined to be 150 ± 20 nm and 149 ± 21 nm by quantitative AFM analysis (Balnois & Wilkinson, 2002; Meunier & Wilkinson, 2002), which were in agreement with the measurements performed by TEM (Stokke, Elgaseter, Hara, Kitamura, & Takeo, 1993) and based on the theory of dilute polymer solution (Yanaki et al., 1980).

The AFM-based single-molecule force spectroscopy (AFM–SMFS) technology is a powerful tool to characterize the force-induced conformational transitions, the dynamics, and supramolecular structures of polysaccharides at the molecular level (Abu-lail & Camesano, 2003; Giannotti et al., 2007; Liu, Wang, & Zhang, 2006; Zhang & Marszalek, 2006a; Zhang & Marszalek, 2006b; Zhang et al., 2003). It is not limited to using water as a medium. The conformation of polymers is thus detected under various environmentally controlled conditions, such as solvent, temperature, salt, and electrochemical potential (Giannotti & Vancso, 2007). The change in free energy per monomer associated with the conformation transition is analyzed from the force spectra. Such techniques can also be used to provide intra- and intermolecular interactions of polysaccharides at the single-chain level directly (Giannotti & Vancso, 2007; Sletmoen et al., 2003). The conformational behavior of hyaluronan (HA) chains in aqueous NaCl solution was characterized using AFM-SMFS technology (Giannotti et al., 2007). The changed from a nonrandom coil to a random coil behavior with increasing temperature from 29 ± 1 to 46 ± 1 °C was observed, and the superstructure of HA single chains in aqueous NaCl solution was a hydrogen-bonded network between the polar groups of HA and possible water. AFM-SMFS was used to investigate the mechanism and dynamics of conformational transition of curdlan in aqueous NaOH solutions with different concentrations, and enhanced hydrophobic interaction by thermal treatment (Zhang, Wang, Wang, & Zhang, 2003). AFM-SMFS of xanthan showed the difference between ordered and random coil structures (Li, Rief, Oesterhelt, & Gaub, 1998). Zhang and Marszalek (2006a, 2006b) reported that the glycosidic linkage-type of polysaccharides and elasticity of polysaccharide molecules were conveniently studied using AFM-SMFS technology.

4.4. Other methods

Fluorescence correlation spectroscopy (FCS) is interesting to determine the conformations and sizes of polysaccharides at lower concentration of about 10^{-8} mol/l (Meunier & Wilkinson, 2002). Schizophyllan and dextran were labeled by the fluorophore rhodamine green X-succinimidyl ester hydrochloride (R6113) on the reducing end groups of the polysaccharides. The average value of the diffusion coefficient for schizophyllan was determined to be $(7.6 \pm 0.4) \times 10^{-8}$ cm^2 s^{-1}, which was lower than that of dextran. This was in agreement with their molecular compactness, i.e., schizophyllan formed a triple helical rod while dextran exhibited as a coil conformation in water. The values of contour length were between 162 and 188 nm for the schizophyllan/R6113 conjugate dissolved in 0.1 M borate (pH 9.3). These values were significantly smaller than lengths determined in water, because borate made the conjugated dissociate into small aggregates.

NMR spectroscopy is a powerful technique to study the conformational dynamics of polymers in dilute solutions. In particular, ^{13}C NMR relaxation can reflect motion at several carbon sites in a repeat unit of the polymer chain (Dais & Vlachou, 2001). Lycknert and Widmalm (2004) reported that the flexibility and dynamics of the *Escherichia coli* O91 O-antigen polysaccharide were studied by ^{13}C NMR relaxation at two magnetic field strengths, 9.4 and 14.1 T. The result revealed a complex dynamical behavior of the polysaccharide from several dynamical models, in which the sugar residues could be described by a global correlation time and different correlation times for internal motions related to their position in the repeating unit along the polysaccharide. The conformation of the trisaccharide branches on bacterial polysaccharide was investigated by one-dimension ^{1}H, ^{1}H T-ROESY spectroscopy and measurement of trans-glycosidic $^3J_{\mathrm{CH}}$ coupling constants (Eklund, Lycknert, Soderman, & Widmalm, 2005). The results showed that the trisaccharide exhibited a flexible conformation along the ψ glycosidic torsion angles.

5. Conclusions

To make clear the chemical structures and chain conformations of polysaccharides is important to understand their biological activities. Combination of FTIR, NMR, Raman spectroscopy, GC, GC–MS, and HPLC methods is useful to characterize the chemical structures of bioactive polysaccharides. Static and dynamic light scattering, viscosity analysis based on the theory of polymer dilute solution may disclose the chain conformations of polysaccharides in solutions in detail. Furthermore, the chain conformations of polysaccharides substituted by specific chromophores with optical activity may be investigated by CD analysis. Single molecular AFM and AFM-SMFS technologies can directly detect the conformations of individual polysaccharide molecules. Fluorescence correlation spectroscopy and NMR spectroscopy are also helpful to understand the conformations of polysaccharides.

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