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Review

Structural diversity of fungal glucans

Andriy Synytsya*, Miroslav Novák

Department of Carbohydrates and Cereals, Institute of Chemical Technology in Prague, Technická 5, 166 28 Prague 6 Dejvice, Czech Republic

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ABSTRACT

Fungal glucans represent various structurally different D-glucose polymers with a large diversity of molecular mass and configuration. According to glucose anomeric structure, it is possible to distinguish α-D-glucans, β-D-glucans and mixed α,β-D-glucans. Further discrimination could be made on the basis of glycosidic bond position in a pyranoid ring, distribution of specific glycosidic bonds along a chain, branching and molecular mass. Fungal glucans can be chemically modified to obtain various derivatives of potential industrial or medicinal importance. NMR spectroscopy is a powerful tool in structural analysis of fungal glucans. Together with chemolytic methods like methylation analysis and periodate oxidation, NMR is able to determine exact structure of these polysaccharides. Fungal glucans or their derivatives exert various biological activities, which are usually linked to structure, molecular mass and substitution degree.

Contents

* Corresponding author at: Tel.: +420 220 443 116; fax: +420 220 445 130.
E-mail address: sinicaa@vscht.cz (A. Synytsya).

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1. Introduction

Fungal glucans are structirally variable polymers of D-glucopyranose (D-Glc*p*). They constitute an obligatory part of cell walls in mycelia, fruiting bodies or other parts of different micro- and macromycetes. Some exopolysaccharides of microscopic fungi were also defined as glucans. Basic structure of common fungal glucans is demonstrated in Fig. 1. Despite of simplicity of their monosaccharide composition (according to definition they should contain only glucose), large diversity can be found concerning number and anomeric configuration of D-Glc*p* units, position and sequence of glycosidic bonds along a chain, branching degree and chain conformation. Branched glucans may contain various side chains, with one or more monosaccharide units, attached to the backbone at different positions.

According to anomeric structure of D-Glc*p* units, it is possible to distinguish three main groups of these fungal polysaccharides: α-D-glucans, β-D-glucans and mixed α,β-D-glucans. Further discrimination can be made on the basis of glycoside bond positions or molecular mass, *i.e.*, linear or branched glucans, (1→3)-, (1→4)- and/or (1→6)-linked glucans, high-, medium- or low-M_w glucans, *etc.* Besides (1→3)-β-D-glucans known for their immunomodulation and antitumour activities (Stone & Clarke, 1992), a wide range of fungal glucans of different structure have been described (Chakraborty, Mondal, Pramanik, Rout, & Islam, 2004; Singh, Saini, & Kennedy, 2008; Synytsya et al., 2009; Wasser, 2002; Wiater et al., 2011; and many others). Some fungal glucans were chemically modified to obtain water-soluble derivatives of potential industrial or medicinal importance.

To determine exact structure of fungal glucans many analytical methods have been applied. First of all it is chemolytic methods including methylation anylysis, oxidation methods (periodate, lead(IV) acetate), Smith's degradation, and other. NMR spectroscopy is the most effective up-to-date non-destructive method of structural analysis. It has been widely used in characterisation of fungal glucans. Chemical shifts and coupling constants of ^{1}H and ^{13}C identify anomeric forms of D-Glc*p* residues and indicate positions of glycosidic linkages or substituents (Mulloy, 1996). The H-1 and C-1 signals at δ 4.9–5.1 (4.3–4.6) and δ 98–100 (103–104), respectively, indicate α- (β-) anomeric form of D-Glc*p* (effect of anomeric configuration). Furthermore, a downfield shift of carbon signal (δ ~4.5–8) confirms that this carbon participate in glycosidic bond formation (α-glycosylation effect), while a less pronounced upfield shift takes place for the neighbour carbon signals (β-glycosylation effect) (Goffin et al., 2009). Correlation homo- and heteronuclear NMR experiments are commonly used to help in the signal assignment (Kim et al., 2000; Lukondeh, Ashbolt, Rogers, & Hook, 2003; Mulloy, 1996; Yalin, Cuirong, & Yuanjiang, 2006). Homonuclear ^{1}H, ^{1}H correlation spectroscopy (COSY) detects interaction between neighbour protons in a D-Glc*p* unit, while total correlation spectroscopy (TOCSY) may assign all the protons based on their interaction with one of them (commonly H-1). Heteronuclear single/multiple-quantum correlation spectroscopy (HSQC, HMQC) is used to assign C–H signals. Finally, both heteronuclear single- or multiple-bond correlation spectroscopy (HSBC, HMBC) and nuclear or rotating-frame Overhauser effect spectroscopy (NOESY, ROESY) are able to determine inter-unit connections in the glucan macromolecule.

2. Fungal α-D-glucans

2.1. Linear α-D-glucans

Linear α-D-glucans were found in many yeasts and higher fungi. Chemical structure of these polysaccharides is variable,

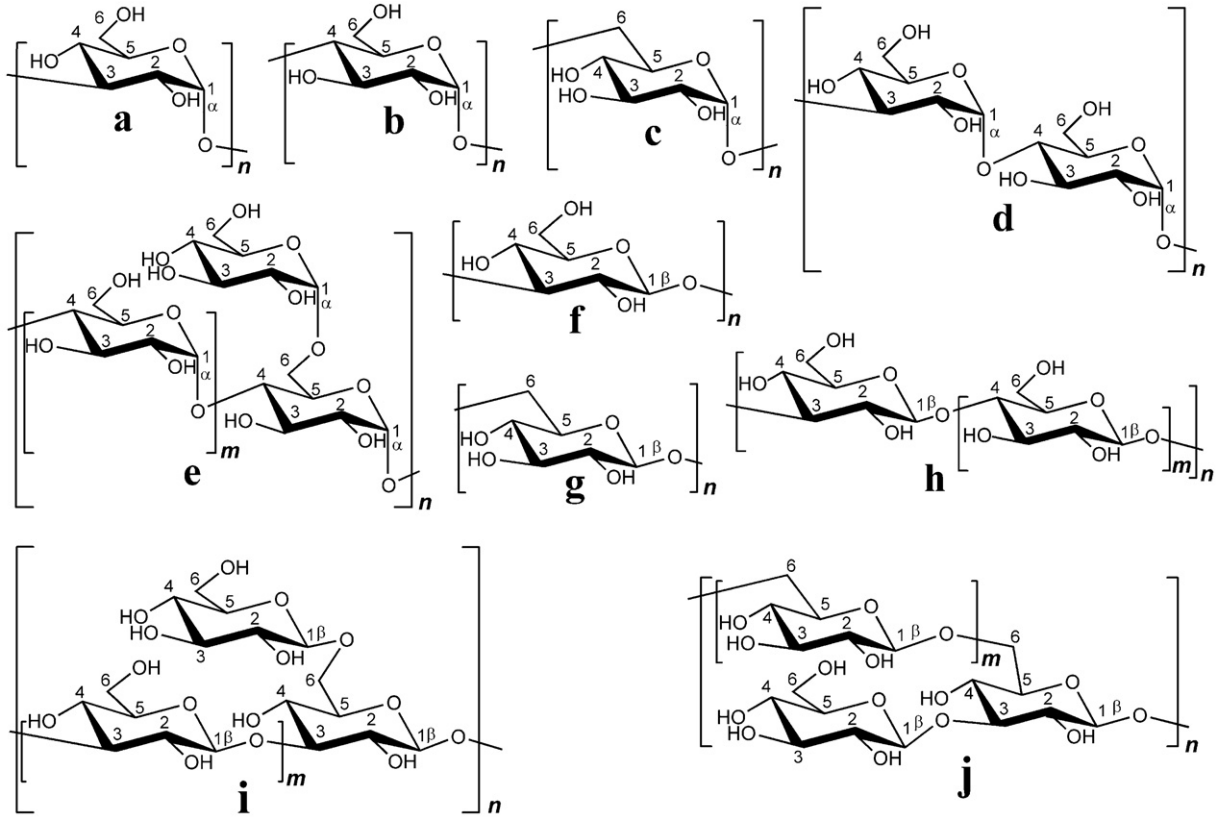


Fig. 1. Structure of fungal glucans: (a) (1→3)-α-D-glucan; (b) (1→4)-α-D-glucan; (c) (1→6)-α-D-glucan; (d) mixed-linkage (1→3),(1→4)-α-D-glucan; (e) branched (1→4),(1→6)-α-D-glucan; (f) (1→3)-β-D-glucan; (g) (1→6)-β-D-glucan; (h) mixed-linkage (1→3),(1→4)-β-D-glucan; (i) branched (1→3),(1→6)-β-D-glucan; (j) branched (1→6),(1→3)-β-D-glucan.

Table 1
Chemical shifts of the ^{13}C resonance signals for linear fungal α-D-glucans.

Source (name)	Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6	Reference
Aspergillus niger	→4)-α-D-Glc*p*-(1→	100.0	72.7	73.9	**79.8**	71.4	60.9	Bock et al. (1983)
(nigeran)	→3)-α-D-Glc*p*-(1→	101.0	71.4	**83.2**	70.3	73.5	61.3	
(pseudonigeran)	→3)-α-D-Glc*p*-(1→	100.9	71.3	**83.8**	70.8	73.1	61.2	Carbonero, Sassaki, Gorin et al., 2002
Agaricus blazei	→4)-α-D-Glc*p*-(1→	99.8	71.5	75.3	78.7	71.2	60.1	Gonzaga et al. (2005)
Armillariella tabescens (HCP)	→6)-α-D-Glc*p*-(1→	97.6	73.3	73.4	69.5	70.1	**65.5**	Han et al. (2011)
Sarcodon aspratus (IPS-B2)	→6)-α-D-Glc*p*-(1→	100.2	72.0	72.7	73.9	75.9	**68.1**	Luo et al., 2008
Termitomyces eurhizus	→3)-α-D-Glc*p*-(1→	97.0	70.3	**80.1**	69.8	73.4	61.8	Mondal et al. (2004)
(PS-I)	→6)-α-D-Glc*p*-(1→	99.2	72.9	74.9	71.0	71.7	**67.1**	
(PS-II)	→6)-α-D-Glc*p*-(1→	98.8	71.3	74.6	70.8	72.5	**66.7**	
Aureobasidium pullulans	→6)-α-D-Glc*p*-(1→	101.1	72.1	74.0	70.6	71.3	**67.6**	McIntyre and Vogel (1993)
(pullulan) G6-**G4**-G4	→4)-α-D-Glc*p*-(1→	98.8	72.1	74.2	**78.7**	72.1	61.8	
G4-**G4**-G6	→4)-α-D-Glc*p*-(1→	100.7	72.1	74.2	**78.2**	72.1	61.5	
G4-**G4**-G4	→4)-α-D-Glc*p*-(1→	100.7	72.1	74.2	77.7	72.1	61.5	
Teloschistes flavicans	→6)-α-D-Glc*p*-(1→	98.1	70.6	72.1	71.3	73.3	**66.8**	Reis et al. (2002)
(pullulan) G6-**G4**-G6	→4)-α-D-Glc*p*-(1→	100.5	71.5	71.4	**79.5**	73.6	60.7	

depending on a fungal source. Some of linear α-D-glucans are composed of (1→3)-, (1→4)- or (1→6)-linked α-D-Glc*p* units, while others contain a combination of the mentioned glycosidic bonds (Grün, 2003).

2.1.1. Linear α-D-glucans having one type of linkage

Linear (1→3)-α-D-glucans (1, Fig. 1a) are common cell wall polysaccharides of various fungi. For example, this structure was established for **pseudonigeran** isolated from mycelium of *Aspergillus niger* (Horisberger, Lewis, & Smith, 1972). Kiho, Yoshida, Nagai, Ukai, and Hara (1989) described (1→3)-α-D-glucan (M_w = 560 kDa) obtained from *Agrocybe cylinducea* by alkali extraction. James and Cherniak (1990) extracted very pure linear (1→3)-α-D-glucan (M_w ~ 9 kDa) from trama of *Piptoporus betulinus* using a 4-methylmorpholine-*N*-oxide–water (3:2) mixture and Me_2SO.

[3)-α-d-Glc*p*-(1 →]$_n$ (1)

Similar α-D-glucans were isolated from mycelia of *Penicillium chrysogenum* (Wang, Deng, Li, & Tan, 2007) and *Poria cocos* (Jin et al., 2004), fruiting bodies (basidiocarps) and mycelia of *Ganoderma tsugae* (Chen, Zhou, Zhang, Nakamura, & Norisuye, 1998; Peng, Zhang, Zhang, Xu, & Kennedy, 2005) and *Ganoderma lucidum* (Chen, Zhang, Nakamura, & Norisuye, 1998), basidiocarps of *Pleurotus ostreatus* (Synytsya et al., 2009; Wiater et al., 2011), *Pleurotus eryngii* (Synytsya et al., 2009), *Lentinula edodes* (Unursaikhan, Xu, Zeng, & Zhang, 2006; Wiater et al., 2011; Zhang, Zhang, & Cheng, 2000, 2002), *Laetiporus sulphurous* (Wiater et al., 2011), *Piptoporus betulinus* (Wiater et al., 2011) and many other sources. ^{13}C NMR spectrum of pseudonigeran from lichenised basidiomycete *Dictyonema glabratum* (Carbonero, Sassaki, Gorin & Iacomini, 2002) showed the signals at δ 100.9 (C-1) and 83.8 (C-3) indicated (1→3)-α-glycosidic linkages (Table 1).

Palacios, García-Lafuente, Guillamón, and Villares (2012) described α-D-glucan **PH** isolated by hot water extraction from basidiocarps of *Pleurotus ostreatus*. This polysaccharide was almost totally hydrolysed by α-glucosidase. Methylation analysis and ^1H NMR confirmed that it is amylose-like linear (1→4)-α-D-glucan (2, Fig. 1b). Gonzaga, Ricardo, Heatley, and Soares (2005) isolated water soluble polysaccharides from basidiocarps of *Agaricus blazei*. Authors reported the presence of (1→4)-α-D-glucan together with (1→6)-β-D-glucan in the fractions that was confirmed by ^{13}C NMR.

[4)-α-d-Glc*p*-(1 →]$_n$ (2)

Luo, Xu, Yu, Yang, and Zheng (2008) isolated biologically active α-D-glucan **IPS-B2** (M_w = 49.5 kDa) from a hot aqueous extract of *Armillariella tabescens* mycelia. Han et al. (2011) described a water-soluble α-D-glucan **HCP** (M_w = 670 kDa) isolated from basidiocarps of *Sarcodon aspratus*. Mondal, Chakraborty, Pramanik, Rout, and Islam (2004) reported a structure of α-D-glucan, named **PS-II** (M_w ~ 6 kDa), isolated from a hot aqueous extract of *Termitomyces eurhizus* basidiocarps. All these polysaccharides were characterised as simple linear (1→6)-α-D-glucans (3, Fig. 1c).

[6)-α-d-Glc*p*-(1 →]$_n$ (3)

This configuration was confirmed by NMR analysis (Table 1). Single α-anomeric proton and carbon signals were observed at δ 4.82–4.92 and 97.6–100.2, respectively. These signals showed strong correlation in HSQC experiment. The downfield chemical shift at δ 65.5–68.1 (C-6) indicated substitution at O-6. The glycosidic (1→6)-linkage was also confirmed by key HMBC correlation from H-1 (δ 4.82) to C-6 (δ 65.5) (Luo et al., 2008).

2.1.2. Linear mixed-linkage α-D-glucans

Nigeran (4, Fig. 1d), a cold-water insoluble polysaccharide originated from mycelia of *Aspergillus niger* and some other species of *Aspergillus* and *Penicillium* genera, consists of alternating (1→3)- and (1→4)-linked α-D-Glc*p* residues (Bobbitt & Nordin, 1978; Bock, Gagnaire, Vignon, & Vincendon, 1983). Its ^{13}C NMR spectrum showed carbon resonances of both types of the units (Table 1). The low-field signals at δ 79.8 (C-4) and 83.2 (C-3) indicated substitution of α-D-Glc*p* units at the corresponding positions, *i.e.*, respective (1→4)- and (1→3)-α-glycosidic linkages. However, Painter (1990) reported that only 78% of the (1→4)-linked α-D-Glc*p* residues of the *Aspergillus niger* nigeran were present as isolated singlets having (1→3)-linked neighbour residues, while the rest 22% were present as isolated doublets (2), and sequences of more than two contiguous (1→4)-linked units were not found. Tsumuraya, Misaki, Takaya, and Torii (1978) described α-D-glucan, named **elsinan** isolated from culture filtrates of *Elsinoe leucospila* grown on a potato extract-sucrose medium. This glucan is an essentially linear polymer containing both (1→4)-α- and (1→3)-α-linkages and is mainly composed of (1→3)-α-linked maltotriose residues.

{3)-α-d-Glc*p*-(1 → [4)-α-d-Glc*p*-(1 →]$_m$}$_n$ m = 1or2 (4)

Isolichenan (5) is a cold-water soluble α-D-glucan (M_w vary from ~6–8 to 2000 kD) of the lichen *Cetraria islandica* consisting of (l→3)- and (1→4)-linked residues in the ratio of 3:1, 3:2 or 2:1 (Olafsdottir et al., 1999).

{[3)-α-d-Glc*p*-(1 →]$_m$4)-α-d-Glc*p*-(1 →}$_n$ m = 1, 2or3 (5)

Nigeran- or isolichenan-type α-D-glucans with alternating (1→3)- and (1→4)-linkages in various ratios were isolated from

Table 2
Chemical shifts of the ^{13}C resonance signals for branched fungal α-D-glucans.

Source (name)	Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6	Reference
Agaricus bisporus	→4)-α-D-Glc*p*-(1→	100.3			**78.9**		60.3	Smiderle et al. (2010)
	→4,6)-α-D-Glc*p*-(1→	100.1					**66.2**	
	α-D-Glc*p*-(1→	99.7	71.8	72.8	70.0	71.3	60.8	
Flammulina velutipes (FVP2)	→4)-α-D-Glc*p*-(1→	102.8	74.0	76.1	**79.8**	73.2	63.4	Pang et al. (2007)
	→4,6)-α-D-Glc*p*-(1→	102.4	74.2	76.1	**79.8**	73.2	**72.2**	
	α-D-Glc*p*-(1→	101.6	75.7	76.1	74.4	75.6	63.4	
Cordyceps sinensis	→4)-α-D-Glc*p*-(1→	99.9	71.8	73.3	**78.6**	70.8	61.0	Yalin et al. (2006)
	→4,6)-α-D-Glc*p*-(1→				**78.6**		**71.3**	
	α-D-Glc*p*-(1→	98.7	72.1	74.0	69.7	72.7		

lichenised fungi of *Cladina*, *Cladonia*, *Parmotrema*, *Ramalina*, *Rimelia* and other genera (Baron, Gorin, & Iacomini, 1988; Carbonero et al., 2001; Carbonero, Montai, Woranovicz-Barreira, Gorin, & Iacomini, 2002, 2005; Cordeiro, Stocker-Wörgötter, Gorin, & Iacomini, 2003, 2004; Stuelp, Carneiro-Leão, Gorin, & Iacomini, 1999; Woranovicz-Barreira et al., 1999). Both these linear mixed linkage (1→3),(1→4)-α-D-glucans were found in the thalli of lichen *Ramalina peruviana* (Cordeiro et al., 2004), but only nigeran was also found in free mycobiont. Authors suggested fungal origin for both polysaccharides, but isolichenan should be produced by the mycobiont in the presence of *Trebouxia* photobiont only.

Water insoluble α-D-glucan (M_w ~850 kDa) was isolated from *Aspergillus wentii* (Choma et al., 2012). It was a linear polymer composed almost exclusively of (1→3)-linked α-D-Glc*p*, but the chain was divided into blocks of 200 units separated by a short spacers (1-3 units) of (1→4)-linked α-D-Glc*p*.

Grün (2003) and Grün et al. (2005) described cell wall α-D-glucans isolated from wild-type and mutant yeasts *Schizosaccharomyces pombe*. The wild-type polysaccharide was a polymer (*DP* = 260) with two interconnected linear chains, each consisting of about 120 (1→3)-linked α-D-Glc*p* and some (1→4)-linked α-D-Glc*p* residues at the reducing end. By contrast, α-D-glucan of the mutant yeast consisted of a single chain only. Authors proposed that coupling of two α-D-glucan chains is necessary for creation of the mature polysaccharide essential for yeast morphogenesis. An alkali-soluble α-D-glucan was isolated from basidiocarps of *Lentinula edodes* (Shida, Uchida, & Matsuda, 1978). This glucan was partially degraded by amylolytic enzymes. It was shown to have a slightly branched structure composed of (1→3)- and (1→4)-linked α-D-Glc*p* units in the ratio 5.3:1. The latter residues are present in the regions near non-reducing ends.

Mondal et al. (2004) reported a structure of α-D-glucan **PS-I** (M_w ~9 kDa) isolated from the hot aqueous extract of *Termitomyces eurhizus* basidiocarps. The polysaccharide was identified as a linear mixed linkage (1→3),(1→6)-α-D-glucan with blocking distribution of these two types of glycosidic bonds (6).

$$[6)\text{-}\alpha\text{-d-Glc}p\text{-}(1\rightarrow]_m[3)\text{-}\alpha\text{-d-Glc}p\text{-}(1\rightarrow]_n \quad (6)$$

The ^1H NMR spectrum of **PS-I** had two H-1 signals at δ 5.33 and 5.08 assigned to (1→3)- and (1→6)-linked α-D-Glc*p* units, respectively, in a molar ratio of ~1: 2.5 (Mondal et al., 2004). Corresponding C-1 signals were found at δ 97.01 and 99.18. The C-3 and C-6 signals showed characteristic downfield shifts due to the α-glycosylation effect (Table 1). The sequence of glycosidic linkages in **PS-I** was confirmed by the specific inter-unit NOE contacts observed in NOESY experiment.

Pullulan (7) is water-soluble α-D-glucan originated from *Aureobasidium pullulans* (Singh et al., 2008), *Tremella mesenterica* (Jennings & Smith, 1973) or *Cyttaria harioti* (Waksman, de Lederkremer, & Cerezo, 1977). This linear polysaccharide contains both (1→4)-α- and (1→6)-α-linkages in a ~2:1 molar ratio. It is a copolymer of regularly repeating maltotriose fragments or, in some strains of *Aureobasidium pullulans*, 5–7% of maltotetraose fragments connected by (1→6)-α-glycosidic bonds (Catley & Whelan, 1971). Reis, Tischer, Gorin, and Iacomini (2002) described pullulan-like α-D-glucan from the lichenised ascomycete *Teloschistes flavicans*. It consisted of equal amount of alternating (1→4)- and (1→6)-linked α-D-Glc*p* units.

$$\{6)\text{-}\alpha\text{-d-Glc}p\text{-}(1\rightarrow[4)\text{-}\alpha\text{-d-Glc}p\text{-}(1\rightarrow]_m\}_n \quad m = 1, 2 \text{or} 3 \quad (7)$$

^{13}C NMR data of pullulans (Table 1) suggest that the C-4 carbon resonance signal of (1→4)-linked α-D-Glc*p* residues shifted downfield when one or two neighbour units are (1→6)-linked (McIntyre & Vogel, 1993; Reis et al., 2002). Thus this signal can be used for evaluation of glycosidic bond distribution in pullulan. Linear (1→4),(1→6)-α-D-glucan, a polysaccharidic part of biologically active proteoglycan, was found as the main polysaccharide component in a hot water extract obtained from the fruiting bodies of *Agaricus blazei* (Mizuno, Morimoto, Minato, & Tsuchida, 1998).

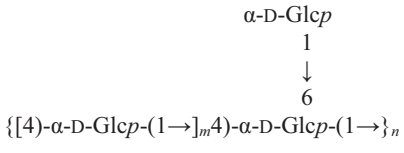
2.2. Branched α-D-glucans

Two fractions of branched (1→4),(1→6)-α-D-glucan (**glycogen**), soluble and insoluble, were obtained from baker's yeast *Saccharomyces cerevisiae* (Gunja-Smith & Smith, 1974). The water soluble fraction is intracellular, while the insoluble one is integrated with cell wall β-D-glucan and can be solubilised by acetic acid extraction or β-D-glucanase (Arvindekar & Patil, 2002). Yeast glycogen consists of linear fragments of 10–14 (1→4)-linked α-D-Glc*p* units; these fragments are joined by (1→6)-linkages (Aklujkar, Sankh, & Arvindekar, 2008; Kwiatkowski, Thielen, Glenney, & Moran, 2009). Qiu, Tang, Tong, Ding, and Zuo (2007) deduced structures of two glycogen-like polysaccharides isolated from *Gastrodia elata* Bl. as (1→4)-α-D-glucans with (1→4)-linked α-D-Glc*p* side chains attached to O-6 with different branching degrees (8).

$$\begin{array}{c}[4)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow]_m\,4)\text{-}\alpha\text{-D-Glc}p \\ \qquad\qquad\qquad\qquad\qquad\quad 1 \\ \qquad\qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad\qquad\quad 6 \\ \{[4)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow]_m4)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow\}_n\end{array} \quad (8)$$

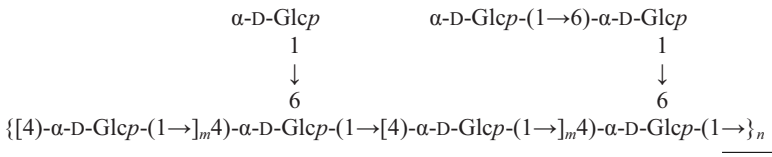
Branched (1→4),(1→6)-α-D-glucans with single α-D-Glc*p* in side chains (9, Fig. 1e) have been described for various fungi. Smiderle et al. (2010) isolated branched α-D-glucan from a hot water extract of *Agaricus bisporus* basidiocarps. This polysaccharide was completely degraded by α-amylase. Similar polysaccharide has been isolated from mycelium of *Flammulina velutipes* (Pang et al., 2007). Yalin et al. (2006) reported that mycelium of a Chinese edible fungus *Cordyceps sinensis* contained similar branched α-D-glucan, which gave with iodine a faint blue colour complex (λ_{max} = 564 nm) and thus indicated (1→4)-α-D-glucan with short side chains. NMR analysis of these polysaccharides confirmed the α-configuration of the D-Glc*p* residues by the position of H-1 (δ 4.74–5.47) and C-1 (δ 98.7–102.8) resonances (Table 2). The C-4 carbon signal at δ

78.9–79.8 indicated the (1→4)-linkage as the major one. Methylene carbon signals were found at δ 60.3–63.4 (non-substituted) and 66.2–72.2 (O-substituted). Therefore, these three polysaccharides consisted of (1→4)-α-D-glucan backbone substituted at O-6 by single α-D-Glc*p* units in the ratio 1:7 (*Flammulina velutipes*), 1:8 (*Agaricus bisporus*) or 1:10 (*Cordyceps sinensis*).



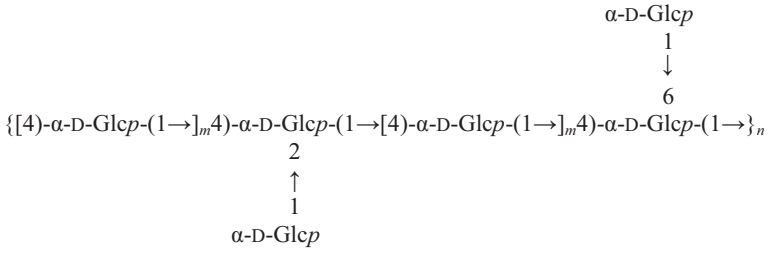
(9)

Li, Dobruchowska, Gerwig, Dijkhuizen, and Kamerling (2013) isolated water-soluble α-D-glucan (M_w = 1267 kDa) from fruiting bodies of *Coprinus comatus*. This polysaccharide had a (1→4)-linked α-D-Glc*p* backbone with ~10% branching at O-6 by terminal α-D-Glc*p* or α-D-Glc*p*-(1→6)-α-D-Glc*p* disaccharide (7:3) (10).



(10)

Hoshi et al. (2005) isolated a bioactive α-D-glucan-protein complex from mycelia of *Tricholoma matsutake*. ^{1}H and ^{13}C NMR spectra confirmed presence of α-anomeric sugars (H-1 at δ ~5.4 and C-1 at δ ~100), and methylation analysis detected O-4 linked and much less O-2 and O-6 linked D-Glc*p* units. Thus, it was suggested that the polysaccharide part of this complex consisted of (1→4)-linked α-D-Glc*p* backbone with small amount of α-D-Glc*p* side chains attached to the O-2 and O-6 positions of some backbone units (11).



(11)

3. Fungal β-D-glucans

3.1. Linear β-D-glucans

Linear β-D-glucans were found in many fungal sources including lichens. These polysaccharides are composed of (1→3)- or (1→6)-linked β-D-Glc*p* units. Cellulose-like (1→4)-β-D-glucans were not found in fungal sources. However, combination of (1→4)- and (1→3)-linkages is possible for linear mixed linkage β-D-glucans (lichenans) of some lichenised fungi.

3.1.1. Linear β-D-glucans having one type of linkage

Pachyman (12, Fig. 1f) is simple linear (1→3)-β-D-glucan derived from sclerotia of *Poria cocos*. This polysaccharide is insoluble in water at room temperature and can be hydrolysed by endo-(1→3)-β-D-glucanase (Hoffmann, Simson, & Timell, 1971). Similar β-D-glucans were isolated from baker's yeast *Saccharomyces cerevisiae* (Freimund, Sauter, Käppeli, & Dutler, 2003; Medeiros et al., 2012) and from basidiocarps of *Termitomyces eurhizus* (Chakraborty, Mondal, Rout, & Islam, 2006) and *Ganoderma lucidum* (Wang & Zhang, 2009) by alkali extraction. Lichenised and some free-living fungi contain linear (1→3)-β-D-glucan, often defined as **laminaran** (Alquini, Carbonero, Rosado, Cosentino, & Iacomini, 2004; Baron et al., 1988). Previously algal origin of this polysaccharide was assumed in some reports (Carbonero et al., 2001; Carbonero, Montai, Woranovicz-Barreira et al., 2002; Baron et al., 1988; Stuelp et al., 1999) because of its low content in lichen thalli and its structure similarity with algal laminarans. However, isolation of (1→3)-β-D-glucan from the aposymbiotically cultured mycobiont finally confirmed its fungal origin (Cordeiro et al., 2003, 2004).

[3)-β-d-Glc*p*-(1 →]$_n$ (12)

A high-field H-1 signal at δ 4.55 and low-field C-1 signal at δ 102.7 indicated β-configuration of water-insoluble D-glucan from basidiocarps of *Laetiporus sulphureus* (Alquini et al., 2004). The ^{13}C spectrum of this β-D-glucan contained six signals, as expected for a hexose homopolysaccharide (Table 3). Low field signal at δ 86.0 indicated glycosylation at O-3, while an inverted DEPT signal at δ 60.9 is typical for unsubstituted C-6 (CH_2OH) carbons. The other signals at δ 76.3, 72.7, and 68.3 corresponded to unsubstituted C-5, C-2 and C-4, respectively. These results confirmed that this polysaccharide is linear (1→3)-β-D-glucan (laminaran). Similarly, ^{13}C NMR spectra of the mentioned β-D-glucans from baker's yeast and *Termitomyces eurhizus* confirmed their structure (Table 3).

Pustulan (13, Fig. 1f) is water-soluble linear (1→6)-β-D-glucan isolated from the lichen *Lasallia pustulata* (Pereyra, Prieto, Bernabé, & Leal, 2003) and reported to be a taxonomic marker for lichens and lichenised fungi of the family *Umbilicariaceae* (Narui, Sawada, Culberson, Culberson, & Shibata, 1999). In the native state this polysaccharide is partially O-acetylated (*DS* ~10%).

[6)-β-d-Glc*p*-(1 →]$_n$ (13)

In addition to alkali-soluble yeast (1→3)-β-D-glucan mentioned above, *Saccharomyces cerevisiae* also contains acid-soluble (1→6)-β-D-glucan (Fleet & Manners, 1976; Manners, Masson, Patterson, Bjørndal, & Lindberg, 1973). Two linear β-D-glucans, *i.e.*, (1→3)-β-D-glucan (laminaran) and slightly O-acetylated (1→6)-β-D-glucan (pustulan), were isolated from the lichenised fungus *Umbilicaria mammulata* (Carbonero, Smiderle, Gracher et al., 2006). ^{13}C NMR spectra confirmed the structure of these polysaccharides (Table 3). Minor carbon signals of pustulan at δ 20.8 (CH_3), 175.0 (C=O), 77.7 and 71.2 (not shown) arose from O-acetyls and O-acetylated carbons. Sassaki et al. (2002) described a pustulan-like exopolysaccharide (M_r = 200 kD) produced by phytopathogenic fungus *Guignardia citricarpa*. Kruppa et al. (2009) reported that linear (1→6)-β-D-glucan is the major polysaccharide isolated from *Malassezia sympodialis* cell wall.

Similar β-D-glucans were isolated from basidiocarps of *Agaricus bitorquis* (Nandan et al., 2008), *Agaricus blazei* (Kawagishi, Inagaki, Kanao, & Mizuno, 1989; Kawagishi et al., 1990), *Bulgaria inquinans*

Table 3
Chemical shifts of the ^{13}C resonance signals for linear fungal β-D-glucans.

Source	Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6	Reference
Termitomyces eurhizus	→3)-β-D-Glc*p*-(1→	103.3	73.9	**86.3**	68.7	76.2	61.1	Chakraborty et al. (2006)
Laetiporus sulphureus	→3)-β-D-Glc*p*-(1→	102.7	72.7	**86.0**	68.3	76.3	60.9	Alquini et al. (2004)
Saccharomyces cerevisiae	→3)-β-D-Glc*p*-(1→	102.9	72.9	**86.0**	68.4	76.3	60.9	Medeiros et al. (2012)
	→3)-β-D-Glc*p*-(1→	102.5	72.5	**85.8**	68.2	76.0	60.7	Freimund et al. (2003)
Umbilicaria mammulata (two linear β-D-glucans)	→3)-β-D-Glc*p*-(1→	102.8	72.7	**86.0**	68.3	76.2	60.7	Carbonero, Smiderle, Gracher, et al., 2006
	→6)-β-D-Glc*p*-(1→	103.1	73.3	76.4	69.9	75.4	**68.4**	
Agaricus bitorquis	→6)-β-D-Glc*p*-(1→	103.4	73.5	76.0	69.9	75.3	**69.2**	Nandan et al. (2008)
Agaricus blazei	→6)-β-D-Glc*p*-(1→	103.0	73.1	75.6	69.5	74.9	**68.8**	Gonzaga et al. (2005)
Guignardia citricarpa	→6)-β-D-Glc*p*-(1→	103.3	73.3	76.6	69.9	75.4	**67.2**	Sassaki et al. (2002)
Bulgaria inquinans	→6)-β-D-Glc*p*-(1→	104.7	74.7	76.6	71.1	77.3	**70.5**	Bi et al. (2009)
Parmotrema austrosinense	→3)-β-D-Glc*p*-(1→	103.5		**87.0**	68.4	76.3	60.8	Carbonero et al. (2005)
(lichenan)	→4)-β-D-Glc*p*-(1→	102.6			80.1		60.4	

(Bi et al., 2009) and somatic hybrid Pflo Vv5 FB, obtained through protoplast fusion between *Pleurotus florida* and *Volvariella volvacea* strains (Das et al., 2010). Glucans of the somatic hybrid and mushrooms *Agaricus bitorquis* were water-soluble and were isolated from hot water extracts, while the polysaccharide of *Agaricus blazei* formed a water-insoluble complex with protein.

NMR spectra of polysaccharide **ABPS** from *Agaricus bitorquis* showed anomeric CH signals (H-1 at δ 4.50, C-1 at δ 103.4) and coupling constants ($J_{H\text{-}1,H\text{-}2}$ ~8.5 Hz, $J_{H\text{-}1,C\text{-}1}$ ~160 Hz) typical for β-anomeric form of D-Glc*p*. The C-6 signal is shifted downfield to δ 69.2 due to glycosylation at O-6 position (Table 3). Inter-unit ^{13}C–^{1}H correlations obtained from the HMBC experiment also confirmed (1→6)-connection between β-D-Glc*p* units. Cross peaks were found between H-1 (δ 4.50) and C-6 (δ 69.2), and between C-1 (δ 103.4) and H-6 (δ 4.20 and 3.84) of neighbour residues (Nandan et al., 2008).

Ukawa, Ito, and Hisamatsu (2000) described three polysaccharide fractions isolated from a hot-water extract of *Lyophyllum decastes* basidiocarps. The highest M_w (305 kDa) and lowest M_w (14 kDa) fractions were identified as (1→3)-β-D-glucan and (1→6)-β-D-glucan, respectively; the fraction of M_w = 130 kDa was defined as a mixture of these two polysaccharides or branched (1→3),(1→6)-β-D-glucan.

3.1.2. Linear mixed-linkage β-D-glucans

Lichenan (14, Fig. 1h) is a linear mixed-linkage (1→3),(1→4)-β-D-glucan of the lichen *Cetraria islandica*, having a closely related structure to storage cereal β-D-glucans. Despite this similarity, Honegger and Haisch (2001) suggested based on SEM and immunocytochemical analyses that it is primary a structural element of the fungal cell wall rather than a storage component of lichenised fungi. Lichenan consists of cellotriosyl (78%), cellotetraosyl (4%) and longer cellulose-like (18%) segments (Wood, Weisz, & Blackwell, 1994). Following lichenase digestion of lichenan, the molar ratio of tri- to tetrasaccharides (*DP3*/*DP4*) was found to be much higher (24.5) than the corresponding values of cereal β-D-glucans (3.7–2.1) (Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004).

$$\{3)\text{-β-d-Glc}p\text{-}(1 \rightarrow [4)\text{-}\beta\text{-d-Glc}p\text{-}(1 \rightarrow]_m\}_n \quad (14)$$

Lichenan-type polysaccharides have been also described for lichenised fungi of genera *Parmotrema* and *Rimelia* (Carbonero et al., 2005). The β-configuration was of these glucans was confirmed by high-field resonances at of δ 4.38 and 4.28 (H-1) assigned to (1→3)- and (1→4)-linked β-D-Glc*p* units, respectively. The ratio of glycosidic linkages (1:3.1) determined by comparing the areas of these peaks was identical to the value obtained by methylation analys is. The low-field carbon signals at δ 87.0 (C-3) and 80.0–80.1 (C-4) confirmed glycosylation at O-3 and O-4, while the signal at δ 60.4 (C-6) arised from non-substituted CH_2OH groups. HPLC analysis of Smith degraded products confirm that all the (1→3)-linked units are interspersed between the (1→4)-linked ones.

Table 4
Degree of branching (*DB*) of fungal β-D-glucans.

Name	Source	*DB* (mol mol^{-1})
Pachymaran (pachyman)	*Poria cocos*	0.015–0.02
Yeast glucan	*Saccharomyces cerevisiae*	0.03–0.2
Yeast glucan	*Candida albicans*	0.14
Lentinan	*Lentinula edodes*	0.23–0.42
Pleuran	*Pleurotus ostreatus*	0.25
Scleroglucan	*Sclerotium glucanicum*	0.30–0.33
Grifolan	*Grifola frondosa*	0.31–0.36
Schizophyllan	*Schizophyllum commune*	0.33
GLG	*Ganoderma lucidum*	0.35
SSG	*Sclerotinia sclerotiorum*	0.50
Pestalotan	*Pestalotia* sp. 815	0.60
Epiglucan	*Epicoccum nigrum*	0.67

3.2. Branched β-D-glucans

Branched β-D-glucans containing (1→3)- and (1→6)-glycosidic linkages are the main common constituents of fungal cell walls. There are many reports about isolation, structure and biological effects of these polysaccharides (Chen & Seviour, 2007; Wasser, 2002; Stone & Clarke, 1992). Most of branched β-D-glucans have a (1→3)-linked β-D-Glc*p* backbone and side-chains of (1→6)-linked β-D-Glc*p* units (Chen & Seviour, 2007), while in some cases the arrangement is opposite, *i.e.*, the main chain is formed by (1→6)-β-linked units with (1→3)-β-linked branches (Fig. 1h) (Dong, Yao, Yang, & Fang, 2002; Ge, Zhang, & Sun, 2010; Sun et al., 2012). A degree of branching (*DB*) as well as possible branching at O-4 and even at O-2 depends on a fungal source and a way of isolation and purification. The *DB* values of some fungal β-D-glucans are given in Table 4 (Chen & Seviour, 2007; Novak & Vetvicka, 2008).

3.2.1. Branched β-D-glucans with (1→3)-β-D-glucan backbone

As it was mentioned above, many branched β-D-glucans from various sources have (1→3)-linked β-D-Glc*p* backbone with single β-D-Glc*p* side chains attached at O-6 position (15, Fig. 1i). Great variability in *DB* and branching distribution as well as complexation with other cell wall polysaccharides, mainly with chitin, are common for these polysaccharides.

$$\begin{array}{c} \text{β-D-Glc}p \\ 1 \\ \downarrow \\ 6 \\ \{[3)\text{-β-D-Glc}p\text{-}(1\rightarrow]_m 3)\text{-β-D-Glc}p\text{-}(1\rightarrow\}_n \end{array} \quad (15)$$

Table 5
Chemical shifts of the ^{13}C resonance signals for branched fungal β-D-glucans.

Source (name)	Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6	Reference
Grifola frondosa (Grifolan LE)	→3)-β-D-Glc*p*-(1→	103.0	72.8	**86.6**	68.6	76.2	60.9	Tada et al. (2009)
	→3)-β-D-Glc*p*-(1→	103.0	73.1	**86.1**	68.6	76.4	61.0	
	→3,6)-β-D-Glc*p*-(1→	103.0	72.9	**85.8**	68.7	75.0	**68.6**	
	β-D-Glc*p*-(1→	103.1	73.7	76.3	70.2	76.6	61.2	
Ganoderma lucidum (GLG)	→3)-β-D-Glc*p*-(1→	103.1	73.2	**86.1**, **86.7**	68.7	76.5	61.2	Chang and Lu (2004)
	→3,6)-β-D-Glc*p*-(1→	103.0	73.0	**85.8**	68.7	75.0	**68.7**	
	β-D-Glc*p*-(1→	103.1	73.8	76.5	70.3	76.3	61.0	
Lentinula squarrosulus	→3)-β-D-Glc*p*-(1→	102.9	73.5	**84.8**, **85.0**	69.9	75.9	61.1	Bhunia et al. (2010)
	→6)-β-D-Glc*p*-(1→	103.2	73.5	75.9	70.0	75.3	69.3	
	→3,6)-β-D-Glc*p*-(1→	103.4	73.3	**84.4**	69.9	75.1	**69.0**	
	β-D-Glc*p*-(1→	102.7	74.4	76.2	70.0	75.9	61.1	
Botryosphaeria sp. (botryosphaeran)	→3)-β-D-Glc*p*-(1→	103.1	73.0	**86.3**	68.7	76.7	61.1, 60.9, 60.7	Barbosa et al. (2003)
	→3)-β-D-Glc*p*-(1→[a]	103.1	73.0	86.3	68.7	76.7	61.1	
	→6)-β-D-Glc*p*-(1→	102.9	73.0	74.8	68.7	76.3	**70.1**	
	→3,6)-β-D-Glc*p*-(1→	102.9	73.0	**86.0**, **85.5**	68.7	76.3	**70.1**	
	β-D-Glc*p*-(1→	103.3	73.8	75.5	68.8	76.7	61.1	
Calocybe indica (calocyban)	→3)-β-D-Glc*p*-(1→	103.9	73.8, 73.6	**87.3**, **87.7**	69.5	77.4	61.9	Mandal et al. (2010)
	→3,4)-β-D-Glc*p*-(1→	103.9	73.6	**87.0**	**79.9**	75.7	61.9	
	β-D-Glc*p*-(1→	103.9	74.7	77.6	71.2	77.1	62.1	
Pfle1r (*Pleurotus florida* × *Lentinula edodes*) (PS-I)	→6)-β-D-Glc*p*-(1→	103.0	73.0	74.9	69.6	75.5	**68.8**; **69.0**	Maji et al. (2012)
	→3,6)-β-D-Glc*p*-(1→	102.7	72.8	**84.2**	69.5	75.5	**68.7**	
	β-D-Glc*p*-(1→	102.9	73.0	75.9	69.6	75.9	60.7	
Agaricus brasiliensis (Ab2-2N)	→6)-β-D-Glc*p*-(1→	105.1	75.2	77.8	71.8	77.1	**71.0**	Dong et al. (2002)
	→3)-β-D-Glc*p*-(1→	105.1	75.2	**86.5**	71.8	78.1	62.9	
	→3,6)-β-D-Glc*p*-(1→	104.7	75.2	**86.5**	71.8	77.1	**70.4**	
	β-D-Glc*p*-(1→	104.4	75.2	77.8	71.8	78.1	62.9	
Phellinus ribis (PRP)	→4)-β-D-Glc*p*-(1→	105.6	75.8	78.3	**78.6**	74.7	65.2	Liu and Wang (2007)
	→6)-β-D-Glc*p*-(1→	105.3	76.0	77.6	72.3	72 7	**71.4**	
	→3,6)-β-D-Glc*p*-(1→			**87.2**				
	β-D-Glc*p*-(1→	107.0	74.1	78.2	70.9	74.7	63.4	
Ganoderma resinaceum	→3)-β-D-Glc*p*-(1→	102.8		**84.8**				Amaral et al. (2008)
	→3)-β-D-Glc*p*-(1→[a]	102.9	72.8	**86.2**	68.5	76.4	60.9	
	→4)-β-D-Glc*p*-(1→	102.4			**79.1**			
	→3,6)-β-D-Glc*p*-(1→	102.6					**69.0**	
	β-D-Glc*p*-(1→	103.0						

[a] Product of Smith degradation.

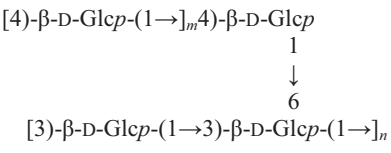
Some of these (1→3),(1→6)-β-D-glucans have own names derived from the fungal source. **Lentinan** (*DB* ~0.5–0.33), a polysaccharide from *Lentinula (Lentinus) edodes*, is one of the best known among them (Saitô, Ohki, & Sasaki, 1979; Saitô, Ohki, Takasuka, & Sasaki, 1977; Sasaki & Takasuka, 1976; Zhang, Li, Wang, Zhang, & Cheung, 2011). Similar highly branched β-D-glucan was isolated from *Aureobasidium pullulans* (Tada et al., 2008). Its molecule was shown to comprise a mixture of a (1→3)-β-D-glucan backbone with (1→6)-linked β-D-Glc*p* in side chains attached to the every second (major structure) or the every third (minor structure) residue. Following three polysaccharides having trivial names were also defined as (1→3),(1→6)-β-D-glucans but something less branched (*DB* ~0.33–0.25). These are **grifolan** extracted from fungus *Grifola frondosa* (Ohno et al., 1986; Tada, Adachi, Ishibashi, & Ohno, 2009) and two extracellular polysaccharides **schizophyllan** and **scleroglucan** produced by fungi *Schizophyllan commune* (Tabata, Ito, Kojima, Kawabata, & Misaki, 1981) and *Sclerotium* sp. (Coviello et al., 2005), respectively. NMR data of **grifolan LE** were presented by Tada et al. (2009). The H-1 (δ 4.2–4.7) and C-1 (δ 103.0–103.1) resonance signals indicated β-anomeric configuration. These signals were assigned to four types of β-D-Glc*p* units (Table 5). Observed four inter-unite ^{1}H, ^{13}C HMBC cross-peaks indicated specific glycosidic linkages between these units. The ratio of the main chain to the side chain units calculated from the areas of anomeric protons was approximately 3:1.

Many other (1→3),(1→6)-β-D-glucans of similar structure were isolated from basidiocarps of *Boletus erythropus* (Chauveau, Talaga, Wieruszeski, Strecker, & Chavant, 1996), *Dictyophora indusiata* (Wang et al., 2009), *Hericium erinaceum* (Dong, Jia, & Fang, 2006), *Pleurotus florida* (Rout, Mondal, Chakraborty, & Islam, 2008; Rout, Mondal, Chakraborty, Pramanik, & Islam, 2005), *Pleurotus pulmonarius* (Carbonero, Gracher, Smiderle et al., 2006; Smiderle et al., 2006, 2008, 2010), *Pleurotus eryngii* (Synytsya et al., 2009), *Pleurotus ostreatoroseus* (Carbonero, Gracher, Smiderle et al., 2006), *Pleurotus ostreatus* (Palacios et al., 2012; Synytsya et al., 2009; Yoshioka, Tabeta, Saitô, Uehara, & Fukuoka, 1985), *Pleurotus tuber-regium* (Chenghua et al., 2000; Zhang, Zhang, Dong, Guo, Song, & Cheung, 2001), Pleurotus sajor-chaju (Carbonero et al., 2012), *Sparassis crispa* (Tada et al., 2007) and many other sources. Branched (1→3),(1→6)-β-D-glucans were isolated from basidiocarps (stems) of *Pleurotus ostreatus* and *Pleurotus eryngii* (Synytsya et al., 2009). These polysaccharides, partially complexed with proteins, predominated in the hot water extracts of these mushrooms. In the alkali extracts β-D-glucans were found in lesser amounts together with more pronounced (1→3)-α-D-glucans, and the alkali-insoluble solids were defined as chitin-glucan complexes with the prevalence of (1→3),(1→6)-β-D-glucans. Šandula, Kogan, Kačuráková, and Machová (1999) described two water-insoluble (1→3),(1→6)-β-D-glucans isolated from *S. cerevisiae* and *A. niger*. The former glucan has a low-branched structure (*DB* = 0.125), while the latter one was complexed with chitin. Lukondeh et al. (2003) isolated low branched alkali-insoluble β-D-glucan (M_w ~300 kDa) containing mostly (1→3)-β-linkages from yeast cells of *Kluyveromyces marxianus*.

Various branched (1→3),(1→6)-β-D-glucans were isolated from fruiting bodies (Bao, Wang, Dong, Fang, & Li, 2002; Chang & Lu, 2004; Hung, Wang, Chen, & Yang, 2008) and submerged culture mycelia (Sone, Okuda, Wada, Kishida, & Misaki, 1985) of medicinal mushroom *Ganoderma lucidum*, as well as from fruiting bodies of other *Ganoderma* species, *i.e.*, *G. appalantum* (Usui, Iwasaki, & Mizuno, 1983), *G. japonicum* (Ukai, Yokoyama, Hara, & Kiho, 1982) and *G. resinaceum* (Amaral et al., 2008). Structure of these polysaccharides depended on a source and a way of isolation.

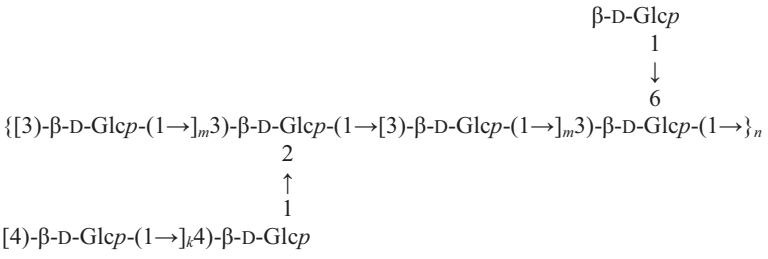
Polysaccharide from *Ganoderma japonicum* (Ukai et al., 1982) was defined as extremely low-branched water-insoluble (1→3),(1→6)-β-D-glucan (M_w = 82 kDa, *DB* ~0.03). By contrast, glucan **GLG** from fruiting bodies of *Ganoderma lucidum* (Chang & Lu, 2004) is highly branched polysaccharide. The ^{13}C NMR resonance signals (Table 5) at δ 86.1 (C-3) and 103.1 (C-1) indicated that it has the (1→3)-β-D-glucan backbone, but evident splitting of C-3 (δ 85.8–86.7) and C-2 (δ 73.0–73.8) regions into three signals of each kind confirmed pronounced difference between the units. Two peaks at δ 70.3 (C-4) and 75.0 (C-5) arose from branching →3,6)-β-D-Glc*p*-(1→ and side chain terminal β-D-Glc*p*-(1→ units, respectively. The *DB* of **GLG** (0.35) was obtained from the ratio between integrated peak areas of proton resonance signals H-1 at δ 4.24 (side chains) and 4.54 (internal units); for comparison, the *DB* of more branched lentinan was 0.42 (Chang & Lu, 2004).

Amaral et al. (2008) described water-soluble β-D-glucan from basidiocarps of *Ganoderma resinaceum* further purified by alkaline treatment. Structure of this polysaccharide was analysed by ^{13}C NMR (Table 5). The downfield shifted carbon signals at δ 84.8 (C-3), 79.1 (C-4) and 69.0 (C-6) arose from O-3, O-4 and O-6 glycosylated β-D-Glc*p* units, respectively; signals of non-substituted C-6 were found at δ 61.0 and 60.4. The backbone structure was identified by a controlled Smith degradation, which gave linear (1→3)-β-D-glucan. Thus the native polysaccharide was defined as a highly branched glucan (*DB* ~0.5) containing a (1→3)-linked β-D-Glc*p* backbone partially substituted at O-6 by side chains of (1→4)-linked β-D-Glc*p* on the every second backbone residue (16).



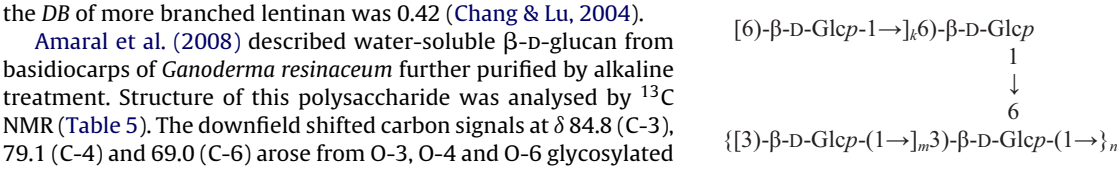
(16)

Several branched (1→3)-β-D-glucan obtained from basidiocarps and submerged culture mycelia of *Ganoderma lucidum* (Sone et al., 1985) had different *DB* values (1/3–1/23) and two types of side chains, *i.e.*, mainly single β-D-Glc*p* attached at O-6 and a few short (1→4)-β-D-glucan residues at the O-2 positions (17).



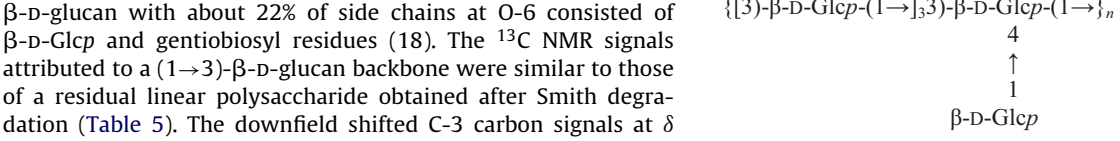
(17)

Barbosa, Steluti, Dekker, Cardoso, and Corradi da Silva (2003) described **botryosphaeran**, an exopolysaccharide produced by lignolytic fungus *Botryosphaeria* sp. It was suggested to be (1→3)-β-D-glucan with about 22% of side chains at O-6 consisted of β-D-Glc*p* and gentiobiosyl residues (18). The ^{13}C NMR signals attributed to a (1→3)-β-D-glucan backbone were similar to those of a residual linear polysaccharide obtained after Smith degradation (Table 5). The downfield shifted C-3 carbon signals at δ 85.5–86.3 confirmed (1→3)-linkages. Among them, the signal of non-substituted backbone units at δ 86.3 was more intense than the others; lesser C-3 signals at δ 86.0 and 85.5 were attributed to branching point units carrying O-6 linked β-D-Glc*p* and gentiobiosyl residues, respectively. Resonance signals at δ 70.1 arose from the O-substituted C-6 carbons and disappeared after Smith degradation. Non-substituted C-6 carbons showed signals at δ 60.7–61.1, and the main signal at δ 61.1 was comparable to that obtained after Smith degradation and thus attributed to internal units inside linear fragments of the backbone. Other signals at δ 60.9 and 60.7 arose from non-substituted backbone units attached to the branching point units. Authors proposed random distribution of branching along a chain based on relative intensities of C-3 substituted and free C-6 regions. Methacanona, Madla, Kirtikara, & Prasitsil (2005) described the structure of several fungal exopolysaccharides isolated from three strains of fungi, *Akanthomyces pistillariiformis* BCC2694, *Cordyceps dipterigena* BCC2073 and *Phytocordyceps* sp. BCC2744. These polysaccharides were irregularly branched (1→3),(1→6)-β-D-glucan having a (1→3)-β-D-glucan backbone substituted at O-6 with single or (1→6)-liked two or three β-D-Glc*p* units in side chains (18). The highest *DB* was observed for the former strain polysaccharide, followed subsequently by the second and third ones.



(18)

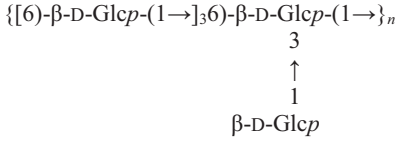
Mandal et al. (2010) described **calocyban** (M_w ~200 kDa), a new water-insoluble (1→3),(1→4)-β-glucan isolated from an alkaline extract of *Calocybe indica* basidiocarps. A ^{13}C NMR spectrum of this polysaccharide confirmed β-anomeric structure, presence of terminal β-D-Glc*p* and the lack of O-6 linkage (Table 5). The downfield shifted signals at δ 87.0 (C-3) and 79.9 (C-4) indicated (1→3,4)-linked β-D-Glc*p* as a branching point; two other signals of C-3 at δ 87.3 and 87.7 belonged to backbone (1→3)-linked β-D-Glc*p* units. The former signal was upfield shifted due to neighboring effect of rigid branching point units, whereas the latter appearing in downfield region indicated backbone units aside from branching. Therefore, calocyban consists of a (1→3)-β-D-glucan backbone with (1→4)-linked β-D-Glc*p* units as side chains bound to the every fourth unit (19). Wu, Sun, and Pan (2005) described an extracellular polysaccharide isolated from a hot water extract of *Cordyceps sinensis* mycelia. It also consisted of a (1→3)-β-D-glucan backbone carried individual (1→4)-linked β-D-Glc*p* residues.



(19)

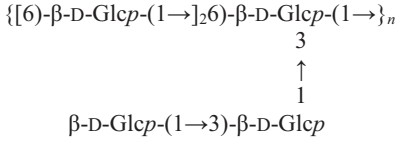
3.2.2. Branched β-D-glucans with β-(1→6)-D-glucan backbone

Maji et al. (2012) described a water soluble β-D-glucan **PS-I** isolated from a hot aqueous extract of basidiocarps of an edible hybrid mushroom Pfle1r of *Pleurotus florida* and *Lentinula edodes*. A ^{13}C NMR spectrum of **PS-I** showed three anomeric carbon signals at δ 103.0, 102.9 and 102.7 (Table 5); the former one was almost three times more intense than each of the latter ones. The downfield shifts of C-3 at δ 84.2 and C-6 at δ 68.7 with respect to the standard values of methyl glycosides indicated the presence of a branching unit →3,6)-β-D-Glc*p*(1→. This residue is the most rigid part of the backbone of this glucan, so it's C-6 signal appeared at the upfield region in comparison to that of the other (1→6)-β-linked residues. Among the three latter residues, one was linked to the rigid branching point unit, hence, its C-6 signal (δ 69) showed δ 0.2 downfield shift with respect to that of another two residues (δ 68.8) due to the neighbouring effect. Finally, one residue showed resonance signals similar to the standard values of methyl glycoside of β-D-Glc*p* and was established as a terminal unit. Therefore, this polysaccharide consists of a (1→6)-β-D-glucan backbone with single β-D-Glc*p* side chains at the O-3 position of the every four residue of the main chain (20, Fig. 1j).



(20)

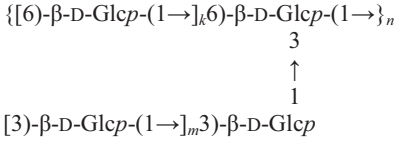
Medicinal mushroom *Agaricus brasiliensis* (=*Agaricus blazei*) is a source of various α- and β-D-glucans forming complexes with proteins (Gonzaga et al., 2005). Mizuno et al. (1990) identified water soluble (1→6),(1→3)-β-D-glucan from this mushroom. According to Dong et al. (2002), this polysaccharide, named **Ab2-2N**, has a (1→6)-β-D-glucan backbone with β-D-Glc*p*-(1→3)-β-D-Glc*p*-1→ disaccharide side chains attached at O-3 of every third backbone residue (21). Three C-1 resonance signals at δ 105.1 (internal O-6 or O-3 substituted units), 104.7 (branching O-6 and O-3 disubstituted units) and 104.4 (terminal units) indicated β-anomeric configuration (Table 5). The signals of substituted C-6 and C-3 were downfield shifted, respectively, to δ 71.0 and 86.5 in comparison with those of non-substituted carbons found at δ 62.9 (C-6) and 78.1 (C-3). Comparing of ^{13}C NMR signal intensities led authors to the conclusion that glycosylation at O-3 (side chains) is less pronounced than at O-6 (backbone). Similar structure was reported for water-soluble β-D-glucans originated from *Pleurotus citrinopileatus* (**PCP-W1**, M_w = 45 kDa) (Sun et al., 2012) and *Phellinus baumii* (M_w = 1920 kDa) (Ge, Zhang, & Sun, 2009).



(21)

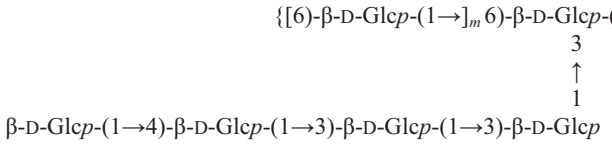
As it was mentioned above, Kawagishi et al. (1989, 1990) described alkali soluble linear (1→6)-β-D-glucan of *A. blazei* isolated as a part of a protein-glucan complex. Contrary to this, Ohno et al. (2001) detected a small but sufficient amount of (1→3)-linkages in such β-D-glucan. According to Camelini et al. (2005), β-D-glucan preparations from *Agaricus brasiliensis* basidiocarps in different stages of maturity contained greater amounts of (1→6)-β-D-glucans and the (1→3)-β-D-glucan content increased with the fruiting bodies maturation. As a rule, highly branched structures predominated in hot water extracts of fungal glucans, while alkali-soluble fractions mostly were linear polysaccharides with no or small branching.

Insoluble cell wall glucan isolated from fungi *Candida albicans* (Iorio et al., 2008) consisted of (1→6)-β-D-glucan backbones carrying O-3 linked shorter (1→3)-β-D-glucan side chains (22). The ^{1}H and ^{13}C resonances typical of linear (1→6)-β-D-glucan and (1→3)-β-D-glucan fragments were observed for the water soluble derivatives of this polysaccharide obtained by partial enzymatic hydrolysis with endo-(1→3)-β-D-glucanase. A high molecular fraction (46.3%) consisted of long (1→6)-β-D-glucan fragments with short (1→3)-β-D-glucan side chains (*DB* = 0.14), whereas a low molecular fraction contained glucose and short linear (1→3)-β-D-glucan fragments. Further digestion of the high molecular fraction by (1→6)-β-D-glucan ase yielded glucose and short linear or O-3 branched (1→6)-β-D-glucan fragments.



(22)

Han, Chai, Jia, Han, and Tu (2010) described water-soluble polysaccharide **HBP** (M_w = 430 kDa) isolated from basidiocarps of *Sarcodon aspratus*. Five H-1 resonance signals were found in the region of δ 4.52–4.78, while only one C-1 signal was observed around δ 103.7; all of them confirmed β-anomeric configuration. The downfield shifted carbon signals at δ 85.0 (C-3) and 81.0 (C-4) confirmed glycosylation at these positions (Table 5). Similarly, downshifted C-6 signals of at δ 69.6 and 68.8 confirmed O-6 substitution. The sequence of glycosyl residues was determined from NOESY and HMBC experiments. Authors concluded that **HBP** is a glucan with a (1→6)-linked β-D-Glc*p* backbone randomly substituted at O-3 position by tetrasaccharide side chains composed of two (1→3)-linked β-D-Glc*p* residues and a terminal (1→4)-β-D-Glc*p* residue (23).



(23)

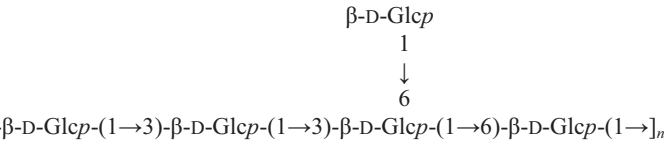
3.2.3. Branched β-D-glucans with mixed-linkage backbone

Karácsonyi and Kuniak (1994) described **pleuran**, an alkali-insoluble cell wall β-D-glucan isolated from basidiocarps of *Pleurotus ostreatus*. This polysaccharide having a (1→3)-β-D-glucan backbone substituted at O-6 of the every fourth unit with single β-D-Glc*p* also contained a small proportion (7%) of (1→6)- and (1→4)-linked residues in the backbone chain. Bhunia et al. (2010) described unusual branched (1→3),(1→6)-β-D-glucan (M_w ~198 kDa) from basidiocarps of *Lentinula (Lentinus) squarrosulus* using 2D NMR. HMQC data confirmed correlations between anomeric proton signal at δ 4.52 and two anomeric carbon signals at δ 103.4 and 102.9, and, similarly, between H-1 signal at δ 4.50 and two C-1 signals at δ 103.2 and 102.7 (Table 5). The signal at δ 102.9 was almost double intense than the other C-1 signals. The HMBC

Table 6
Chemical shifts of the ^{13}C resonance signals for linear fungal α,β-D-glucans.

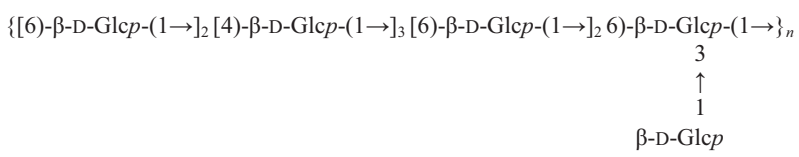
Source (name)	Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6	Reference
Astraeus hygrometricus (AQS-I)	→4)-α-D-Glc*p*-(1→	98.4	71.8	70.6	**75.3**	69.3	61.2	Chakraborty et al. (2004)
	→6)-β-D-Glc*p*-(1→	103.4	73.5	76.0	69.9	73.8	**66.0**	
Termitomyces microcarpus (PS-I)	→4)-α-D-Glc*p*-(1→	98.0	71.4	71.7	**75.7**	69.6	60.6	Chandra et al. (2007)
	→3)-β-D-Glc*p*-(1→	102.4	72.3	**85.1**	68.9	75.9	60.7	

and NOESY data supported that (1→6)-linkages were present both in the backbone and side chains of this polysaccharide (24).



(24)

Liu and Wang (2007) described a water-soluble β-D-glucan **PRP** isolated from a hot water extract of fruiting bodies of *Phellinus ribis*. A ^{13}C NMR spectrum contained three anomeric signals at δ 107.0, 105.6 and 105.3, assigned to terminal, (1→4)- and (1→6)-linked β-D-Glc*p* residues, respectively (Table 5). The C-6 signals (inverted in DEPT spectrum) were found at δ 63.4, 65.2 and 71.4; the latter indicated O-6 substitution. The C-3 signal of branching (1 →3),(1→6)-β-D-Glc*p* residues showed a downfield shift to δ 87.2 because of O-3 substitution. Resonance signals of branching and terminal units disappeared after mild hydrolysis of **PRP**, yielding linear (1→4),(1→6)-β-D-glucan. Authors concluded that this polysaccharide consists of a mixed (1→4),(1→6)-linked β-D-glucan backbone with single β-D-Glc*p* side chains at the O-3 position of the (1→6)-linked backbone units the every eights residue of the main chain (25).



(25)

Krestin (**PSK**, polysaccharopeptide Krestin) prepared from medicinal mushroom *Trametes (Coriolus) versicolor* is a mixture of polysaccharide–peptide complexes (M_w ~100 kD) consisting of β-D-glucans covalently linked to various peptides (25–38%) (Tsukagoshi et al., 1984). Proposed structure of the polysaccharide part of **PSK** is branched β-D-glucan having a (1→4)-β-D-glucan backbone with (1→6)-β- and (1→3)-β-linked side chains attached to approximately the every fourth backbone unit (Ooi & Liu, 2000; Tsukagoshi et al., 1984). Jeong et al. (2004). **Polysaccharopeptide (PSP)**, another biologically active glucan–peptide complex from the same source, probably is (1→3)-β-D-glucan branched at O-4 and O-6 positions and also covalently linked to peptide moieties (Chu, Ho, & Chow, 2002). However, other sources reported more complex character of the **PSK** and **PSP** polysaccharides (Cui & Chisti, 2003). According to Ng (1998), both preparations contain (1→4)-α- and (1→3)-β-linked D-Glc*p*. While D-glucose is the major monosaccharide unit, other sugars are also present in significant amounts (Cheng, Wu, Zhou, & Cheng, 1998; Wang, Ng, Liu, Ooi, & Chang, 1996).

4. Fungal mixed-linkage α, β-D-glucans

4.1. Linear mixed-linkage α, β-D-glucans

There are two reports about fungal glucans having alternating α- and β-glycosidic linkages along the chain. Chakraborty et al. (2004) described such linear α,β-D-glucan **AQS-I** from fruiting bodies of *Astraeus hygrometricus*. ^1H NMR spectrum of this polysaccharide showed two H-1 signals at δ 4.83 ($J_{H\text{-}1,H\text{-}2}$ 3.9 Hz) and 4.39 ($J_{H\text{-}1,H\text{-}2}$ 8.5 Hz) assigned to (1→4)-α- and (1→6)-β-linked D-Glc*p* units, respectively (1:1 mol mol^{-1}). The corresponding C-1 signals were found at δ 98.4 and 103.4 as evident from HSQC experiments (Table 6). The downfield shifted carbon signals at δ 66.0 (C-6) and 75.3 (C-4) arose from O-6 and O-4 substituted units, respectively. The alternating sequence of the units along a chain was confirmed by NOESY experiment. Therefore, in **AQS-I** the D-Glc*p* units are linked by repeating (1→4)-α- and (1→6)-β-glycosidic bonds (26).

[4)-α-d-Glc*p*-(1 → 6)-*β*-d-Glc*p*-(1 →]$_n$ (26)

Chandra et al. (2007) described another linear α,β-D-glucan **PS-I** isolated from an edible mushroom *Termitomyces microcarpus*. A ^1H NMR spectrum of this polysaccharide showed two H-1 signals at δ 5.14 ($J_{H\text{-}1,H\text{-}2}$ ~3.5 Hz) and 4.55 ppm ($J_{H\text{-}1,H\text{-}2}$ ~6.5 Hz) assigned to α- and β-D-Glc*p* units (1:1 mol mol^{-1}), respectively. Corresponding C-1 signals were observed at δ 98 and 102.4 (Table 6). The downfield shifted carbon signals at δ 75.7 (C-4) and 85.1 (C-3) confirmed the presence of (1→4)-α- and (1→3)-β-linkages (*α*-glycosylation effect). In addition, in both cases the signals of neighbour carbons showed an upfield shift due to the β-glycosylation effect. NOESY experiment confirmed the alternating sequence of these glycosidic bonds in **PS-I** (27).

[4)-α-d-Glc*p*-(1 → 3)-*β*-d-Glc*p*-(1 →]$_n$ (27)

Both these linear mixed-linkage α,β-D-glucans are well soluble in water.

4.2. Branched mixed-linkage α, β-D-glucans

Several reports are devoted to mixed-linkage α,β-D-branched fungal glucans, *i.e.*, those containing both α- and β-glycosidic linkages of various positions and configurations depending on a fungal source and a way of isolation. Following structural variants of such polysaccharides are defined.

4.2.1. α-D-Glucan backbone with β-linked side chains

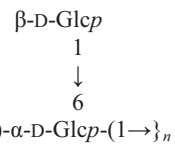
Olennikov, Agafonova, Rokhin, Penzina, and Borovskii (2012) described branched *α*,*β*-D-glucan, named **piptoporan I** (M_w ~270 kDa), isolated from the fruiting bodies of wood-decaying

Table 7
Chemical shifts of the ^{13}C resonance signals for branched fungal α,β-D-glucans.

Source (name)	Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6	Reference
Piptoporus betulinus(Piptoporan I)	→3)-α-D-Glc*p*-(1→	101.5	72.6	**85.3**	70.1	73.8	61.2	Olennikov et al. (2012)
	→3)-α-D-Glc*p*-(1→[a]	100.1	71.4	**84.9**	69.8	73.4	60.9	
	→3,6)-α-D-Glc*p*-(1→	101.8	72.6	**86.3**	70.1	73.5	**68.1**	
	β-D-Glc*p*-(1→	106.3	73.1	76.1	71.3	75.8	61.8	
Pleurotus florida (MRFS–HW)	→3)-α-D-Glc*p*-(1→	99.4	69.9				60.7	Santos-Neves et al. (2008)
	→3)-α-D-Glc*p*-(1→[a]	99.6	70.9	**83.0**	69.7	72.1	60.7	
	→3,6)-α-D-Glc*p*-(1→	99.5					**69.0**	
	→3)-β-D-Glc*p*-(1→	102.9	73.2					
	β-D-Glc*p*-(1→	102.8	73.3					
Calocybe indica (PS-I)	→6)-β-D-Glc*p*-(1→	103.4	73.3	76.0	69.9	75.3	**69.3**	Mandal et al. (2010)
	→4,6)-β-D-Glc*p*-(1→	103.2	73.4	75.3	**76.4**	73.7	**69.2**	
	α-D-Glc*p*-(1→	100.0	71.6	73.4	70.0	71.9	61.1	
Pleurotus florida	→3)-α-D-Glc*p*-(1→	100.1	70.0	**80.4**	69.3	72.5	61.1	Rout et al. (2005)
	α-D-Glc*p*-(1→	99.8	72.1	74.0	71.0	72.5	60.6	
	→3)-α-D-Glc*p*-(1→[a]	100.2	71.3	**83.2**	69.8	72.3	60.5	
	→3,6)-β-D-Glc*p*-(1→	103.1	73.0	**85.2**	68.8	76.4	**67.0**	
	→3)-β-D-Glc*p*-(1→[a]	103.4	73.2	**86.6**	68.8	76.7	61.2	
Somatic hybrid PCH9FB of *Pleurotus florida* and *Calocybe indica* var. APK2	→4)-α-D-Glc*p*-(1→	98.3	71.9	73.5	**76.4**	70.0	61.6	Maity et al. (2011)
	α-D-Glc*p*-(1→	100.2	71.9	73.5	69.9	71.5	61.6	
	→6)-β-D-Glc*p*-(1→	103.4	73.4	75.9	69.9	70.4	**69.2**	
	→4,6)-β-D-Glc*p*-(1→	103.3	73.4	75.3	**76.4**	69.9	**69.2**	
Pleurotus florida, cultivar Assam Florida	→3)-α-D-Glc*p*-(1→	99.4	69.8	**80.1**	67.2	71.6	61.1	Roy et al. (2009)
	α-D-Glc*p*-(1→	98.5	71.6	73.4	70.0	71.8	61.4	
	→3)-β-D-Glc*p*-(1→	103.1	73.4	**85.2**	69.8	76.6	61.1	
	→6)-β-D-Glc*p*-(1→	102.0	73.5	76.4	70.0	75.2	**69.0**	
	→3,6)-β-D-Glc*p*-(1→	103.3	73.2	**85.2**	69.8	75.9	**68.8**	
Calocybe indica	→4)-α-D-Glc*p*-(1→	100.0	71.9	73.2	**76.0**	72.2	60.8	Mandal et al. (2012)
	α-D-Glc*p*-(1→	98.3	71.7	73.5	69.9	72.9	61.1	
	→6)-β-D-Glc*p*-(1→	103.4	74.4	76.0	69.9	75.3	**69.3**	
Pleurotus sajor-caju	→4,6)-β-D-Glc*p*-(1→	103.2	74.5	75.3	**76.0**	75.0	**69.2**	Pramanik et al. (2007)
	→6)-α-D-Glc*p*-(1→	98.4	69.4	66.7	70.3	69.7	**68.6**	
	→2,6)-α-D-Glc*p*-(1→	98.8	**77.0**	73.3	70.3	69.7	**68.6**	
	→3)-β-D-Glc*p*-(1→	102.7	73.0	**83.8**	76.1	73.3	60.6	
	β-D-Glc*p*-(1→	102.4	73.3	74.8	76.1	76.1	60.6	
Volvariella diplasia	→4)-α-D-Glc*p*-(1→	99.0	72.4	73.1	**76.6**	70.8	61.4	Ghosh et al. (2008)
	→4,6)-α-D-Glc*p*-(1→	98.5	72.4	73.1	**77.4**	69.9	**68.7**	
	→6)-β-D-Glc*p*-(1→	103.4	73.8	76.0	70.8	75.1	**69.2**	
	β-D-Glc*p*-(1→	103.4	73.4	76.6	70.8	76.0	61.2	

[a] Product of Smith degradation.

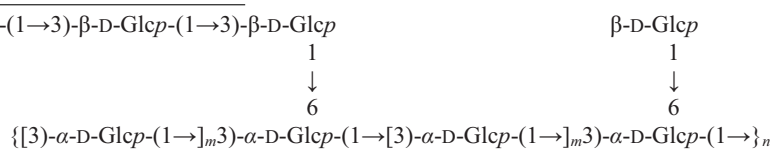
fungus *Piptoporus betulinus*. It was branched polysaccharide with a (1→3)-α-D-glucan backbone substituted at the *O*-6 position by single β-D-Glc*p* residues (*DB* = 17.3%) (28).



(28)

Three C-1 carbon signals at δ 101.5, 101.8 and 106.3 indicated unsubstituted and substituted backbone α-D-Glc*p* and side chain β-D-Glc*p* units, respectively (Table 7). The C-3 carbon signals of the unsubstituted and substituted backbone units at δ 85.3 and 86.3, respectively, and the C-6 signal of the substituted moieties at δ 68.1 confirmed the substitution at these positions. By contrast, the C-5 signal of the substituted backbone units was upfield shifted to δ 73.5, which also supported substitution at *O*-6. The carbon signals of side chain units were close to those of free β-D-Glc*p*. Smith-degraded piptoporan I (M_w = 225 kDa) was identified by ^{13}C NMR as linear (1→3)-α-D-glucan.

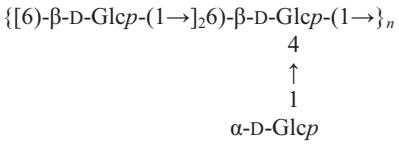
Santos-Neves et al. (2008) identified branched α,β-D-glucan **MRFS–HW** (M_w ~1100 kDa) isolated from fruiting bodies of *P. florida*. ^{13}C NMR spectra of this polysaccharide showed several C-1 signals at δ 102.8 (terminal β-D-Glc*p*), 102.9 (O-3 substituted β-D-Glc*p*), 99.4 (O-3 substituted α-D-Glc*p*) and 99.5 (3,6-substituted α-D-Glc*p*) (Table 7). In addition, high and low field H-1 signals at δ 5.43 and 4.59 confirmed the presence of α- and β-D-Glc*p*. The (1→3)- and (1→6)-glycosidic linkages were indicated by the down-field shifted C-3 (81.4, 80.9 and 80.7) and C-6 (δ 69.0, 68.6 and 68.5) signals, respectively. The backbone structure of **MRFS–HW** was confirmed by controlled triple Smith degradations, which gave linear (1→3)-α-D-glucan as the final product (Table 7). Authors concluded that this polysaccharide contained a (1→3)-α-D-glucan backbone partially substituted at O-6 by single β-D-Glc*p*, and a smaller amount of trisaccharide side chains with (1→3)-linked *β*-D-Glc*p* units (29).



(29)

4.2.2. β-D-Glucan backbone with α-linked side chains

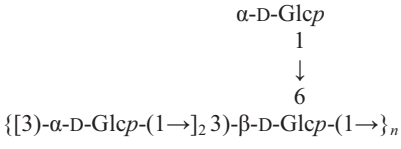
Mandal et al. (2010) described water-soluble branched α,β-D-glucan **PS-I** (M_w ~187 kDa) isolated from an alkaline extract of *Calocybe indica* basidiocarps. A ^{1}H NMR spectrum contained three H-1 signals at δ 5.38, 4.52 and 4.50 (1:1:2); corresponding C-1 signals appeared at δ 100.0, 103.2 and 103.4. The downfield shifts of C-4 (δ 76.4) and C-6 (δ 69.2 and 69.3) indicated O-4 and O-6 substitution (Table 7). According to NOESY and HMBC experiments, this polysaccharide consisted of a (1→6)-β-D-glucan backbone with a side chain of (1→4)-linked α-D-Glc*p* at the every third backbone unit (30).



(30)

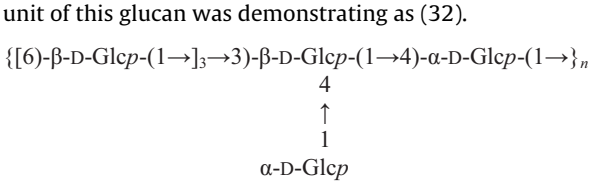
4.2.3. Mixed-linkage α,β-D-glucan backbone with α-linked side chains

Rout et al. (2005) described the structure of water soluble branched α,β-D-glucan from an aqueous extract of the fruiting bodies of *Pleurotus florida*. Three H-1 signals were found at δ 5.09, 4.95 and 4.44 (2:1:1); corresponding C-1 resonances appear at δ 100.1, 99.8 and 103.1. Thus this glucan was composed of a tetrasaccharide repeating fragment consisting of three α-D-Glc*p* and one β-D-Glc*p* units. Smith degradation led to insoluble product giving twelve ^{13}C NMR signals; two of them at δ 103.4 and 100.2 (1:2) arose from (1→3)-β- and (1→3)-α-linked D-Glc*p* units, respectively (Table 7). This was confirmed by downfield shifted C-3 signals of these units at δ 86.6 and 83.2 ppm, respectively, as a result of the α-glycosylation effect. Therefore, the backbone of the native polysaccharide was mixed (1→3)-α,β-D-glucan, and α-D-Glcp side chains were attached at O-6 to all the β-anomeric backbone units (31).



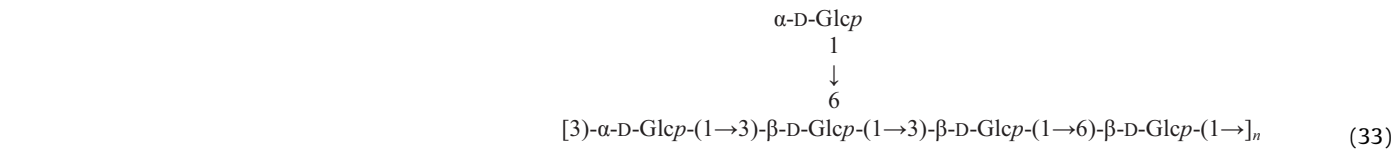
(31)

Maity et al. (2011) isolated water-soluble branched α,β-D-glucan (~198 kDa) from an alkaline extract of the fruiting bodies of a somatic hybrid PCH9FB of *Pleurotus florida* and *Calocybe indica* var. APK2 strains. The ^{1}H NMR spectrum showed two H-1α signals at δ 5.38 and 4.95, and two H-1β signals at δ 4.51 and 4.50 (1:1:1:3). Corresponding C-1 signals were found in the anomeric region at δ 98.3, 100.2, 103.3 and 103.4 (Table 7). The downfield shift of C-4 (δ 76.4) and C-6 (δ 69.2) confirmed O-4 and/or O-6 glycosylation of some units. The unit sequence was determined from ROESY, NOESY and HMBC experiments. Structure of the repeating unit of this glucan was demonstrating as (32).



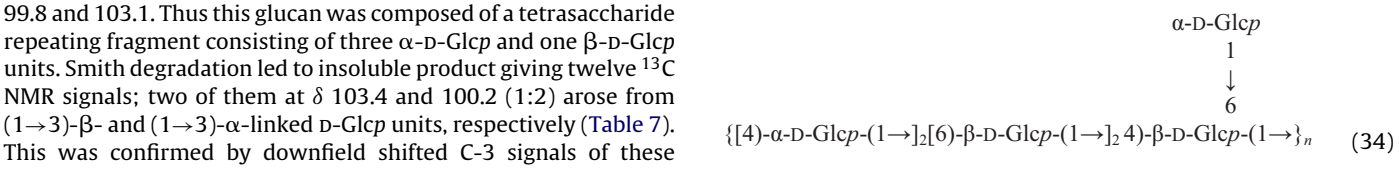
(32)

Roy et al. (2009) described another mixed α,β-D-glucan isolated from a hot water extract of the fruiting bodies of *Pleurotus florida*, cultivar Assam Florida. NMR spectra had five H-1 signals at δ 5.11, 4.97, 4.50, 4.49 and 4.48 and corresponding five C-1 signals at δ 99.4, 98.5, 103.3, 103.1 and 102.0 (1:1:1:1:1). All these five residues were identified by correlation NMR experiments (Table 7). Smith degradation led to formation of oligosaccharide containing (1→3)-α- and (1→3)-β-glycosidic bonds. Thus the native polysaccharide consisted of a (1→3)-α,β-(1→6)-β-D-glucan backbone with O-6 linked α-D-Glc*p* side chains at the every fourth (1→3)-β-linked backbone unit (33).



(33)

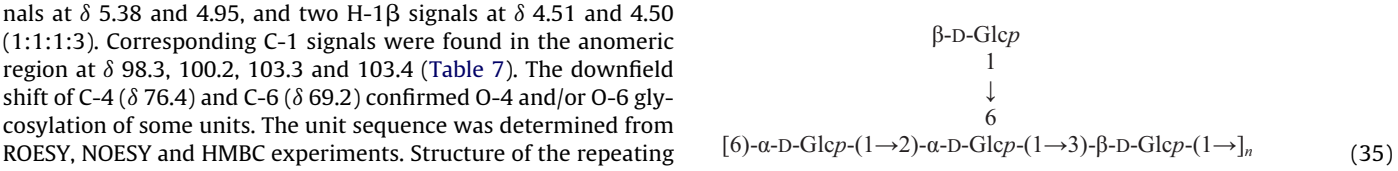
Mandal et al. (2012) described water-soluble glucan isolated from a hot aqueous extract of the fruiting bodies of *Calocybe indica*. A ^{1}H NMR spectrum of this polysaccharide showed four anomeric proton signals at δ 5.37, 4.93, 4.51 and 4.50 in a ratio of nearly 2:1:1:2; corresponding carbon signals were found at δ 100.0, 98.3, 103.2, and 103.4 (Table 7). First two residues were assigned as α-D-Glc*p*, the second two as β-D-Glc*p*. The downfield shifted C-4 signal at δ 76.0 indicated presence of (1→4)-linked α-D-Glc*p*; another α-anomeric unit is a terminal one. Similarly, the downfield shifts of C-4 (δ 76.0) and C-6 (δ 69.2 and 69.3) signals indicated (1→4,6)- and (1→6)-linked β-D-Glc*p* units. Two dimensional correlation NMR experiments confirmed following structure of this polysaccharide (34).



(34)

4.2.4. Mixed-linkage α,β-D-glucan backbone with β-linked side chains

Pramanik, Chakraborty, Mondal, and Islam (2007) obtained water-soluble mixed α,β-D-glucan from the fruiting bodies of *Pleurotus sajor-caju*. Four H-1 signals of equile intensity were found at δ 5.08, 4.94, 4.47, and 4.46; corresponding C-1 signals arose at δ 98.8, 98.4, 102.7 and 102.4 (Table 7). Free and linking C-6 carbon signals appeared at δ 60.6 and 68.6, respectively. The downfield shift of C-2 to δ 77.0 indicated (1→2)-glycosidic bonds, unusual for fungal glucans. According to correlation NMR analysis, the structure of the tetrasaccharide repeating fragment of this glucan was determined as (35).



(35)

Branched α,β-D-glucan (~70 kDa) described by Ghosh, Chandra, Ojha, and Islam (2008) was isolated from a hot aqueous extract of the fruiting bodies of *Volvariella diplasia*. Four H-1 signals were found at δ 5.05, 4.91, 4.42, and 4.40 (1:1:1:1), while three corresponding C-1 signals appeared at δ 99.0, 98.5 and 103.4 (1:1:2) (Table 7). The sequences of glycosyl residues were confirmed by HMBC experiment. The structure of this polysaccharide was described as (36).

β-D-Glc*p*
1
↓
6
[6)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→]$_n$ (36)

5. Structure–activity relationship

Many of fungal glucans exert biological activity, which is usually linked to their structure and molecular weight, in particular. Among the most studied fungal glucans showing notable physiological effects belong linear and branched β-D-glucans described above. These effects are their most important quality and the reason why so much attention has been devoted to them. Fungal β-D-glucans belong to a group of physiologically active compounds, collectively termed biological response modifiers (BRMs) (Bohn & BeMiller, 1995; Novak & Vetvicka, 2008). Due to their BRM activity these glucans serve as remedies or adjuvants in many pathological conditions, such as bacterial, viral or protozoal infections, and they are also potent antitumour drugs (Vetvicka & Novak, 2011). Contradictory data exist on the influence of molecular weight (M_w), degree of branching (*DB*), conformation and intermolecular associations of β-glucans on antitumour activity and on the mechanism(s) of their action as BRM (Chen & Seviour, 2007; Novak & Vetvicka, 2008; Wasser, 2002).

Until recently, biologically efficient β-D-glucans were supposed to have similar structure–the main chain of (1→3)-β-linked D-Glc*p* moieties to which some D-Glc*p* units are randomly connected by (1→6)-β-linkages (Chen & Seviour, 2007; Novak & Vetvicka, 2008). Range of *DB* about 0.2–0.3 is probably responsible for the highest antitumour activity, represented by lentinan, schizophyllan or yeast β-D-glucan; β-D-glucans with high or very low *DB* are substantially less active (Misaki, Kakuta, Sasaki, Tanaka, & Miyaji, 1981; Misaki et al., 1984). In native β-D-glucans, their fibrils are composed from organised parts in which the main chain is coiled to triple helix (Sletmoen & Stokke, 2008) and these regions are combined with single or double filaments (Ohno, Kurachi, & Yadomae, 1988; Okobira, Miyoshi, Uezu, Sakurai, & Shinkai, 2008; Saitô et al., 1987). However, the detailed structure of β-D-glucans from dissimilar sources differs and so does their biological activity. For example, antitumour activity of schizophyllan is supposedly conditioned by triple helix presence and M_w higher than 100 kDa (Kojima, Tabata, Itoh, & Yanaki, 1986). The triple helix, formed by three H-bonds in C-2 position and stabilised by side chains, is probably present only in high-molecular β-D-glucans: minimal M_w for stable triple helix of schizophyllan is 25–40 kDa, and below this value only single strands exist in aqueous solution. Moreover, the triple helix structure most likely should not be a solely effective form of β-D-glucan because alkali treatment, commonly used in isolation procedures, destroys this structure (Young & Jacobs, 1998). Certain recent opinions do not confirm established ideas of the necessity of high M_w and/or branching of biologically active β-D-glucans. In a series of papers (Descroix et al., 2010; Jamois et al., 2005; Saraswat-Ohri et al., 2011; Vetvicka et al., 2011) significant antitumour effects of non-branched and small-molecule oligosaccharides with (1→3)-β-frame are described.

These results imply a conclusion that effects of molecular size and structure of β-D-glucans on their biological, especially antitumour, activities will need further investigation. This conclusion can be undoubtedly applied to the other biologically active fungal glucans and their derivatives as well.

6. Chemically modified fungal glucans

Some medicinal applications, especially immunomodulation ones, need fungal glucans readily water-soluble, while many glucan preparations from fungal raw materials comprise mostly insoluble polysaccharides or their complexes. To improve the solubility of such preparations specific chemical modifications can be used. Carboxymethylation, sulphation, phosphorylation as well as some other modifications are common ways to prepare water-soluble derivatives of fungal glucans. Introduction of carboxymethyl and/or sulphate groups into β-D-glucan improved its water solubility significantly and enhanced the stiffness of the chains (Wang & Zhang, 2006). Moreover, such modifications of fungal glucans may induce or significantly enhance specific biological activities. The effectiveness of polysaccharide modification is usually monitored by NMR spectroscopy and by determination of the substitution degree (*DS*).

Carboxymethylation of glucans is usually made by the reaction of glucan suspension with chloroacetic acid at alkaline conditions. Ukai, Yoshida, Honda, Nagai, and Kiho (1992) described the preparation and analysis of the carboxymethyl derivatives of scleroglucan and (1→3)-α-D-glucan from *Agrocybe cylindracea*. Based on GC-MS analysis authors concluded that distribution of the carboxymethyl substituents depends on the type of glucan and its conformation. Wiater et al. (2011) prepared carboxymethylated derivatives of (1→3)-α-D-glucans from various fungal sources (*Lentinula edodes*, *Pleurotus ostreatus*, *Piptoporus betulinus and Laetiporus sulphureus*) and described their biological activities. Water-soluble carboxymethylated derivatives of insoluble (1→3)-β-D-glucan from the sclerotium of *P. cocos* (Wang & Zhang, 2006), branched (1→3),(1→6)-β-D-glucan from *S. cerevisiae* (Šolteš, Alföldi, & Šandula, 1993) and chitin–glucan from mycelium of *A.* niger (Dergunova et al., 2009) were also prepared and described. Carboxymethylation causes downfield shift of the substituted carbon signals by δ 8–9. The downshifted signals at δ 83.6 (C-2), 80.5 (C-4) and 71.5–72.3 (CH_2 of carboxymethyl) found in the ^{13}C NMR spectrum of modified *S. cerevisiae* β-D-glucan confirmed partial carboxymethylation at O-2, O-4 and O-6 positions (Šolteš et al., 1993).

Sulphation of fungal glucans is commonly achieved by the reaction with chlorosulphonic acid–pyridine or sulphur trioxide–pyridine complexes in dimethyl sulphoxide medium (Zhang, Zhang, & Cheng, 2002; Zhang et al., 2005). Reaction takes

Table 8
Chemical shifts of the ^{13}C resonance signals for native and sulphated fungal glucans.

Source (name)	Structure	C-1	C-2	C-3	C-4	C-5	C-6	Reference
Lentinus edodes	(1→3)-α-D-glucan	101.7, 100.6	71.1	83.2	70.6	72.8	61.1	Zhang and Cheung (2002)
(L-FV-II)		100.7, 103.8	71.9	84.0	70.4	70.3	61.3	
(SL-FV-II)[a]			**79.8**, **87.5**		**77.4**		**66.5**	
Pleurotus tuber-regium (TM8)	(1→3),(1→6)-β-D-glucan	103.0	72.5, 73.7	86.0–87.0 76.8	68.5	76.1	60.9	Zhang et al. (2003)
(S-TM8)[a]		103.1 101.5	72.4, 73.7 **79.4**, **80.3**	85.7–86.9 76.8	68.4 **77.4**	76.2	 **67.7**	

[a] Sulphated glucans.

place preferably at O-6, but is also possible at O-2, O-3 and O-4 (Zhang et al., 2002; Zhang, Zhang, Wang, & Cheung, 2003). Water-soluble sulphated derivatives were obtained from various fungal glucans: (i) (1→3)-α-D-glucans originating from mycelia of *P. cocos* (Huang, Zhang, Cheung, & Tan, 2006; Lin et al., 2004; Zhang et al., 2005) and from basidiocarps of *Lentinula edodes* (Zhang et al., 2002); (ii) (1→4),(1→6)-α-D-glucans from *Gastrodia elata* Bl. (Qiu et al., 2007); (iii) various branched (1→3),(1→6)-β-D-glucans, *i.e.*, those from *Pleurotus tuber-regium* (Tao, Zhang, & Cheung, 2006; Zhang et al., 2003; Zhang, Cheung, Ooi, & Zhang, 2004), *Russula virescens* (Sun, He, Liang, Zhou, & Niu, 2009), *Sacharomyces cerevisiae* (Williams et al., 1991), botryosphaeran (Mendes et al., 2009), grifolan (Nie, Shi, Ding, & Tao, 2006) and lentinan (Wang & Zhang, 2009). Depending on the polysaccharide structure and reaction conditions, the products showed *DS* in the range of 0.17–1.74. Sulphation causes strong downfield shift (δ 7–10) of the substituted carbon signals (Table 8, bold). Zhang et al. (2002) analysed ^{13}C NMR spectra of native (1→3)-α-D-glucan **L-FV-II** originated from *Lentinula edodes* and its sulphated derivative **SL-FV-II**. The downfield shifted resonance signals at δ 87.5 (C-2), 79.8 (C-2), 77.4 (C-4) and 66.5 (C-6) were assigned to sulphated carbons. Similarly, Zhang et al. (2003) compared ^{13}C NMR data of native (**TM8**) and sulphated (**S-TM8**) (1→3),(1→6)-β-D-glucans from *Pleurotus tuber-regium* and assigned the resonance signals at δ 80.3 (C-2), 79.4 (C-2), 77.4 (C-4) and 67.7 (C-6) to carbons sulphated at the corresponding positions.

Huang and Zhang (2011) described phosphorylation of water-insoluble (1→3)-α-D-glucan from mycelia of *Poria cocos* with H_3PO_4 in solution of LiCl and urea in dimethylsulfoxide. A ^{31}P NMR spectrum of the phosphated glucan exhibited several intense signals in the region of δ 0.4–1.3 ppm confirming that phosphate groups were bound to the polysaccharide at different positions. Water-solubility and chain stiffness of the phosphated derivative increased in comparison with the original α-D-glucan.

Several reports are devoted to comparing physical properties and biological activities of differently functionalised fungal glucans. Bao, Duan, Fang, and Fang (2001) reported aminopropylated, hydroxyethylated, sulphated, carboxymethylated, carboxymethylated-sulphated, and benzylamidated-carboxymethylated derivatives of linear (1→3)-α-D-glucan isolated from spores of *Ganoderma lucidum* (Fr.) Karst. Chen, Zhang, and Cheung (2010) prepared a carboxymethylated-sulphated derivative of (1→3)-β-D-glucan extracted from *Poria cocos*. The modified polysaccharide contained carboxymethyl and sulphate groups with *DS* of 1.05 and 0.36, respectively. Wang, Zhang, Li, Hou, and Zeng (2004) described preparation and analyses of five derivatives (sulphated, carboxymethylated, methylated, hydroxyethylated, and hydroxypropylated) of water-insoluble (1→3)-β-D-glucan isolated from fresh sclerotium of *Poria cocos*. Tao, Zhang, and Zhang (2009) reported sulphated and carboxymethylated derivatives of two water-soluble polysaccharide-protein complexes extracted from sclerotia of *Pleurotus tuber-regium*. Using ultrasonic treatment and subsequent chemical derivatisation, water-soluble carboxymethyl and sulphoethyl derivatives of insoluble baker's yeast (1→3),(1→6)-β-D-glucan were obtained with high yield by Šandula et al. (1999). By contrast, carboxymethylation of chitin-glucan complex isolated from mycelium of *Aspergillus niger* was less successful (*DS* = 0.3).

7. Conclusions

Many reports reviewed here clearly illustrate large structural variability of fungal glucans depending on raw materials as well as ways of isolation, purification and possible chemical modification. Among fungal glucans and their derivatives, large diversities in configuration, position and sequence of glycosidic bonds, molecular weight, branching and specific substitution were reported. Some of these polysaccharides are simple linear polymers, the other are more or less branched ones containing mono- and/or oligosaccharide side chains. Different anomeric structures, *i.e.*, α-, β- and combination of both these forms, were described for various fungal glucans. Glycoside bond positions, branching and molecular mass of these polysaccharides significantly varied as it was shown in many structural investigations. It is interesting to note that the common structural motifs, for example (1→3)- and (1→6)-linked β-D-Glc*p*, may have opposite location (in a backbone or side chains) for different fungal glucans. Evidently, structure parameters of fungal glucans are prerequisites of their physiological role in the fungal cell walls as well as of their physical, chemical and biological properties. Chemical modifications are often used to change these properties, first of all to obtain derivatives soluble in aqueous media. Structural diversity of fungal glucans permits to search novel macromolecular agents with potential qualities for various industrial and medicinal applications.

Acknowledgements

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