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HIGHLIGHT

Chemistry, physico-chemistry and applications linked to biological activities of β-glucans

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β-Glucans is the common name given to a group of chemically heterogeneous polysaccharides. They are long- or short-chain polymers of (1→3)-β-linked glucose moieties which may be branched, with the branching chains linked to the backbone by a (1→6)-β linkage. β-(1,3)-Glucans are widely distributed in bacteria, algae, fungi and plants, where they are involved in cell wall structure and other biological function. β-Glucans have been shown to provide a remarkable range of health benefits, and are especially important against the two most common conventional causes of death in industrialized countries, *i.e.* cardiovascular diseases (where they promote healthy cholesterol and blood glucose levels) and cancer (where they enhance immune system functions). This *Highlight* provides a comprehensive and up-to-date commentary on β-glucans, their chemistry, physico-chemistry, functional role in immunological responses, and possible applications as therapeutic tools. In addition, we discuss the mechanism behind their health benefits, which are not yet fully understood.

1 Chemistry and physico-chemistry of β-glucans

Glucans are high-molecular-weight polymers of glucose, broadly classified by their interchain linkage as being either α- or β-linked.[1] β-Glucans have a common structure comprising a main chain of β-(1,3)- and/or β-(1,4)-D-glucopyranosyl unit in non-repeating but non-random order, along with side chains of varying lengths. Fig. 1 summarizes the different structural types defined in glucans; they include linear β-(1,3)-D-glucans, linear β-(1,3;1,4)-D-glucans, side-chain-branched β-(1,3;1,2)-D-glucans, side-chain-branched β-(1,4;1,6)-D-glucans, side-chain-branched β-(1,3;1,6)-D-glucans, branch-on-branch β-(1,3;1,6)-D-glucans, cyclic β-(1,2)-glucans and cyclic β-(1,3;1,6)-glucans. These polysaccharides are widely distributed in bacteria, algae, fungi and plants, where they are involved in cell wall structure and other biological functions, all discussed below.[2] Table 1 lists the sources of these polysaccharides.

In the following we will describe some examples of the different structural types with the relative sources.

The simplest glucan is linear and unbranched β-(1,3)-D-glucan (Fig. 1a), found both among the prokaryotes and eukaryotes. Well known examples are curdlan and paramylon. Curdlan was the first bacterial β-(1,3)-glucan to be identified.[3,4] It is a neutral gel-forming polymer first detected in *Agrobacterium* biovar 1. Curdlan production was also detected in few *Rhizobium* strains, and in species of the Gram-positive genus *Cellulomonas*. It is a secondary metabolite biosynthesized in the post-stationary growth phase during conditions of N-starvation. This polymer is insoluble in water, alcohols, and most organic solvents, but dissolves in dilute bases (0.25 M NaOH), DMSO and formic acid. Curdlan in 0.3 N NaOH has an average molecular weight in the range 53–2000 kDa.[3] Curdlan can be readily detected by the formation of dark blue colonies on media containing aniline blue, a β-(1,3)-glucan-specific dye. Curdlan has the following principal roles: a sink for fixed carbon when the C/N ratio is severely unbalanced; use as a C reserve; enhancement of cell-to-cell interactions, leading to the formation of flocks, biofilms and surface pellicles (which can provide protection from hydrodynamic shear and heat stress); allowing access to nutrients; and allowing escape from predatory amoebae.

Paramylon, similarly to curdlan, is a high-weight polymer which consists of a fibrillar β-(1,3)-D-glucan deposited as granules in the cytoplasm of euglenoids such as *Euglena* and *Astasia*, where it functions as a storage polysaccharide.[5] X-ray diffraction and density measurements indicate that paramylon has a very high level of crystallinity in the native state (approaching 90%), whereas curdlan powder is only 30% crystalline.[6] Because of this high crystallinity, paramylon granules are unique among the carbohydrate storage products in plants and algae groups. It has no solubility in water at ambient temperatures, and its molecular weight is estimated to be larger than 500 kDa.[7]

Another simple structural type is that of the linear β-(1,3;1,4)-D-glucans (Fig. 1b). These compounds occur mostly in the non-lignified cell walls of cereal grains,[8] vegetative organs of grasses

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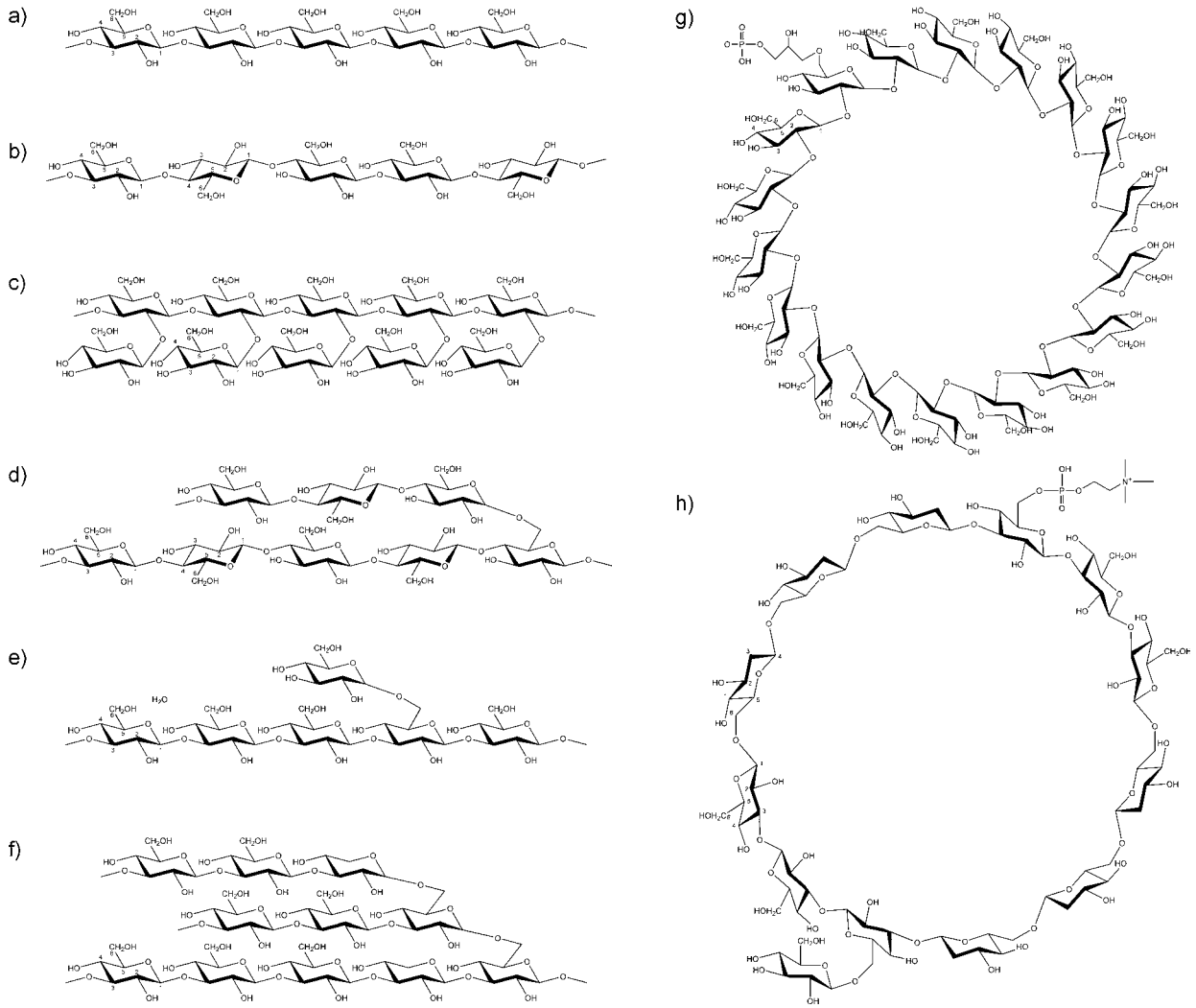

Fig. 1 Structures of β-glucans. See text for details.

(family Poaceae),[9] but also in the walls of some green[10] and red algae,[11] some bryophytes, and lichens.[12] Glucans from barley, oats, or wheat are concentrated in cell walls of the endosperm and the aleurone layer of barley, oats, wheat, sorghum and other cereals.

Levels of β-glucans can vary dramatically between species, varieties and environmental conditions, but usually range from 2 to 6% dry weight. Among all cereal grains, oats and barley contain the highest levels of β-glucans, with the latter at 2–14% dry weight. Over 90% of the β-D-glucosyl residues are arranged as blocks of two or three adjacent (1,4)-linked units separated by a single (1,3)-linked unit, which forms the two types of building blocks of cereal β-glucans: a cellotryosyl unit and a cellotetraosyl unit. The distribution of the building blocks is believed to be random. The remaining 10% of the polymer chain is mainly composed of longer cellulosic sequences ranging from 5 up to 14 β-D-glucosyl residues. The ratio between the trisaccharide and tetrasaccharide units constitutes a 'fingerprint' of a particular grain, and is 4.5 in wheat, 3.0 in barley and 2.3 in oats.[13]

Molecular weight of β-glucan in the cell wall matrix of cereals has so far not been measured. The apparent molecular weight obtained for isolated β-glucans is scattered in the range of 10–1000 kDa, depending on sources, methods of isolation, degree of aggregation and determination techniques.[13] Although they are highly soluble, when ingested they are not hydrolyzed in the small intestine of human digestive system.[14]

Among the branched structures, the simplest is the branched β-(1,3;1,2)-D-glucan (Fig. 1c) present only in the type 37 capsule of *Streptococcus pneumoniae*. This polymer has a β-(1,3)-glucan backbone with (1,2)-linked β-glucopyranosyl side-branches at each glucopyranosyl residue, giving a crowded, comb-like structure. It is soluble in water and DMSO.[4]

Krestin, known as polysaccharide K (PSK), prepared from the basidiomycete *Coriolus versicolor* (strain CM-101) is a β-glucan–protein complex containing 25–38% protein residues. It is a β-(1,4)-D-glucan with (1,6)-β-glucopyranosidic side chains for every fourth glucose unit (Fig. 1d). It has a structure with branches at 3- and 6-positions, in a proportion of one for every few residual groups of 1,4-bonds, and an average molecular weight of 94 kDa.[15,16] Extracts from *C. versicolor* strain Cov-1 contain another proteoglycan named polysaccharide peptide (PSP). Essentially PSK and PSP are similar, except for the presence of other monosaccharides, namely fucose in PSK, and rhamnose and arabinose in PSP.[17]

Table 1 Sources of β-glucans

Structure	Source		Common name
Linear (1,3) β-glucan [Fig. 1a]	Bacteria	*Agrobacterium* spp. *Rhizobium* spp. *Cellulomonas* spp. *Bacillus* spp. *Alcaligenes faecalis*	Curdlan
	Fungi	*Poria cocos*	Pachyman
	Lichens	*Stereocaulon ramulosum* *Ramalina* spp. *Cladonia* spp.	
	Algae	*Euglena* spp. *Astasia longa* *Pavlova mesolychnon* *Peranema trichophorum*	Paramylon
	Plants	*Vitis vinifera* *Gossypium arboretum* *Nicotiana alata*	Callose
Linear (1,3;1,4) β-glucan [Fig. 1b]	Lichen	*Cetraria islandica*	Lichenin
	Algae	*Ulva lactuca* *Monodus subterraneus* *Micrasterias* spp.	
	Plants	*Hordeum vulgare* *Avena sativa* *Triticum vulgare* *Equisetum arvense*	
Side-chain-branched (1,3;1,2) β-glucan [Fig. 1c]	Bacteria	*Streptococcus pneumonie* Type 37	
Side-chain-branched (1,4;1,6) β-glucan [Fig. 1d]	Fungi	*Auricularia auricula-judae* *Coriolus versicolor*	Krestin
Side-chain-branched (1,3;1,6) β-glucan [Fig. 1e]	Fungi	*Pleurotus ostreatus*	Pleuran
		Lentinus edodes	Lentinan
		Sclerotium rolfsii	Scleroglucan
		Schizophyllum commune	Schizophyllan
		Grifola frondosa	Grifolan
		Phytium apinadermatum *Achyla bisexualis*	Mycolaminarin
	Algae	*Chaetoceros mülleri* *Ochromonas* spp. *Haramonas dimorpha* *Phaeodactylum tricornutum* *Coscinodiscus nobilis* *Thalassiosira pseudonana*	Chrysolaminarin
		Laminaria spp. *Eicenia* spp.	Laminarin
Branch-on-branch (1,3;1,6) β-glucan [Fig. 1f]	Fungi	*Saccharomyces cerevisiae* *Schizosaccharomyces pombe* *Aspergillus fumigatus* *Candida albicans*	
Cyclic (1,2) β-glucan [Fig. 1g]	Bacteria	*Agrobacterium tumefaciens* *Sinorhizobium meliloti* *Rhizobium meliloti* *Mesorhizobium loti* *Brucella* spp.	
Cyclic (1,3;1,6) β-glucan [Fig. 1h]	Bacteria	*Bradyrhizobium japonicum* *Rhizobium loti* *Azorhizobium caulinodans* *Azospirillum brasiliense*	

Another branched structural type is branched β-(1,3;1,6)-D-glucan (Fig. 1e) found in different groups of algae and in ascomycetes and basidiomycetes. β-Glucans in algae are present as storage polysaccharides or wall components.[18] They have been identified as water-soluble polysaccharides in brown algae (laminaran, also called laminarin), diatoms (chrysolaminarin),

chrysophytes (chrysolaminarin, also called leucosin), and other heterokonthophyta.[19–21]

Laminarans may be among the most investigated glucans; these polymers are extracted from kelp (*Laminaria* sp., *Eicenia* sp.) as a by-product from production of alginic acid. They consist of glucopyranosyl residues linked by β-1,3-D-glycosidic bonds or β-1,6-D-branched β-1,3-D-glycosidic bonds; laminaran from *Eicenia bicyclis* has a molecular weight of 6.17 kDa,[22] while laminaran from *Laminaria digitata* has a molecular weight of 5.85 kDa.[22] Laminarans are easily soluble in neutral water, and can be fermented by human fecal bacteria easier than other brown algal polysaccharides, such as alginate, fucoidan and cellulose.[23,24]

Several fungal species produce β-(1,3;1,6)-D-glucans.[25,17] All these polymers possess a main chain of β-(1,3) linked glucose residues branched with β-(1,6)-D-glucosyl units. Wide variations can be found in the degree of branching, as residues may be attached regularly or randomly. Examples of these high molecular weight polymers are: schyzophyllan (∼450 kDa), derived from *Schizophyllum commune*,[26] and scleroglucan (∼2200 kDa), from *Sclerotium rolfsii*,[27] both having the same structure consisting of a main linear chain a β-(1,3)-D-glucopyranosyl units with a β-glucopyranosyl group linked (1,6) to every third residue of the main chain; lentinan, from *Lentinula edodes*,[28] composed of five β-(1,3) glucose linear residues and two β-(1,6)-D-glucopyranoside side branches (∼400–800 kDa); grifolan (∼9000–25 000 kDa) from *Grifola frondosa*,[29] a β-(1,3)-D-glucan backbone with every third residue bearing a single (1,6)-β-glucopyranosyl side branching unit; and sparan (∼510 kDa) from *Sparassis crispa*, a β-(1,3)-D-glucan backbone with two β-(1,6)-D-glucosyl and one (1,2)-β-D-glucosyl side branch every ten residues.[30] The glucans isolated from these basidiomycetes are water-soluble.

β-(1,3;1,6)-D-Glucans are present also in the hemyascomycete *Saccharomyces cerevisiae*, with a branch-on-branch (Fig. 1f) structure. Its cell wall, which in terms of dry weight may account for about 10–25% of the total cell mass, may contain 30–60% of its mass in glucans.[31] The β-(1,3)-D-glucan is found as a branched polymer with β-(1,6) interchain link, and a molecular weight of about 190–200 kDa. It forms the core of the wall, and its non-reducing ends are cross-linked to the reducing end of chitin chains through a β-(1,4) linkage, and this complex in turn is bound to β-(1,6)-glucan or mannoproteins. This structure makes the polysaccharide insoluble in both water and hot alkali.[32,33]

Fig. 1g shows the cyclic structure of the β-(1,2)-glucans present in a variety of *Agrobacterium* and *Rhizobium* species. These periplasmic cyclic glucans vary in ring size, from 17 to 25 residues in *R. leguminosarum* and *A. tumefaciens*, and up to 40 residues in *R. meliloti*.[34] These glucans have been shown to be important for a number of symbiotic and pathogenic plant–microbe interactions, and act as a general signaling molecule during plant infection.[34] They are required for effective nodule invasion in symbiotic nitrogen-fixing *R. meliloti* and for crown gall tumour induction in *A. tumefaciens*. Moreover, they induce systemic suppression, a counter-defensive strategy that may facilitate pathogen spread in plants.[35]

Fig. 1h shows the cyclic structure of the β-(1,3;1,6)-D-glucans present in legume symbionts. These water-soluble glucans are found in the periplasm of *Bradyrhizobium japonicum*, *Rhizobium loti*, *Azorhizobium caulinodans*, *Bradyrhizobium japonicum*, and *Azospirillum brasilense* in both the free-living and bacteroide forms. They consist of a ring of 10–13 units, with different proportions of (1,3)- and (1,6)- linkages.[36,37] Like the (1,2) cyclic glucans, they have been implicated in osmotic regulation and suppression of plant defense response.

β-Glucans can vary in terms of physico-chemical parameters, such as backbone structure, frequency and length of branching, molecular weight (from 10^2 to 10^6 Da), the charge of their polymers and structure in aqueous media, degree of polymerization (*i.e.* the number of repeat units building the polymer), higher-order conformations (which include random coil, single helix, and triple helix), and solubility.[38,39]

Since higher-order structure and solubility are assumed to be of importance in the biological recognition and effects of glucans, these two parameters will be discussed below.[40,41]

The β-(1,3;1,4)-glucans from all cereals are relatively long molecules that adopt an extended random coil conformation in aqueous media[13]; dynamic light scattering measurements demonstrate that intermolecular association among molecules through hydrogen bonding is possible, which leads to the formation of aggregates imparting stiffness to the polymer chain, and high solution viscosity.[42]

In the case of β-(1,3) glucan chains, the spatial organization is governed by the glycosidic linkage pattern and the conformation, because the six-membered chair-form ring of the glucopyranosyl molecule is rather rigid, and most of the flexibility of the glucan chain arises from rotations about the bonds of the glycosidic linkages.[43] According to Burton and Brant, β-(1,3) glucans can be described as an irregular pseudohelical trajectory with a periodicity of about six glucose residues.[44]

Following rigorous purification, the structure of glucans such as curdlan,[45] paramylon,[46] laminaran,[47] lentinan,[28] scleroglucan[48] and schizophyllan[49] have been identified by means of X-ray crystallography, terahertz or FRET spectroscopy, which indicate that these glucans have a triple-helix backbone conformation in the solid state. The difference in the β-(1,3)-glucan structures occurs primarily in the side chain. Curdlan, together with paramylon, is structurally the simplest member of the β-(1,3)-glucans with no glycosyl side chain, while laminaran, lentinan, scleroglucan and schizophyllan have β-(1→4) or β-(1→6)-D-glycosyl side-chains exposed toward the exterior of the helical structure.

The triple-helix structure is stabilized by three types of hydrogen bonding: intermolecular hydrogen bonding formed between the different chains in the same *x*–*y* plane (hexagonal hydrogen bonding), intramolecular hydrogen bonding formed between adjacent O2 atoms in the same chain (right-handed helical hydrogen bonding), and intermolecular hydrogen bonding formed between different chains in a different *x*–*y* plane (left-handed helical hydrogen bonding). X-ray fiber diffraction data reveal that the triple-helix structure of unbranched and branched (1,3)-glucans is characterized by a 'pitch' (defined as the length between the six main chain glucose units along the helix *c* axis) of 1.74 nm per 6 glucose residues.[50,43]

Although the triplex structure is stable over a broad range of temperatures in aqueous solution at physiological pH, the hydrogen bonds can be destabilized – *i.e.* the triplex structure can be forced to undergo a strand-separation transition

(denaturation) – by highly alkaline solutions (pH 12), solvents such as DMSO, or by increasing the temperature above the triple-helix melting temperature (135 °C).[51] During this transition, a dynamic equilibrium between the breaking and formation of hydrogen bonds occurs. The number of interacting residues required to suppress complete strand separation is independent of the degree of polymerization of the strands. On the other hand, the probability that a region of hydrogen bonds within the original triplex is present sufficiently long enough to stabilize it increases with increasing molecular weight of the sample. Such a molecular weight effect is observed both for helix dissociation induced by high pH or high temperature. The higher the molecular weight, the higher pH or temperature is needed in order to induce dissociation of the triple helix. Upon restoration of the thermodynamic conditions favoring helix conformation, triple helices are able to reform spontaneously, although a renatured triplex structure may bear imperfectly assembled regions.[43,52]

Different immunological effects and complexation abilities may be related to the different structures of untreated, denatured, and renatured molecules of β-(1,3)-D-glucans, and incorrect conclusions can be easily drawn when comparing results obtained by using such diverse β-glucans. Hence, whether single-helix or triple-helix β-(1,3)-D-glucan are the most biologically active is still an unresolved issue, and the literature appears inconsistent and often contradictory.[53,54,40] Despite intensive research efforts, there is therefore still no consensus on the basic structural requirements for biological effects, and the higher-order structure of the biologically active β-(1,3)-D-glucan component is not well established.

Structure has an impact also on the water solubility of β-glucans; the side-chains prevent the formation of large insoluble aggregates of triple helices through hydrogen bonding, and the projection of these chains to the outside of the helix hinder the close lateral packing of the strands present for example in curdlan or paramylon, while the charge on them acts to 'untie' the triple-stranded structure. Glucan solubility in aqueous solution can be enhanced by depolymerization by acidic or alkali hydrolysis, enzymatic degradation, ultrasonic treatment, as well as by sulfation and phosphation. Of these methods, sulfation is preferred due to the positive impact on the biological effects (*e.g.* immunological potency) on human and animal health.[55]

Common sources of experimentally used β-glucans that have achieved particular clinical relevance include yeast (typically *Saccharomyces cerevisiae*) and fungi (mainly *Lentinus edodes*, *Coriolus versicolor* and *Schizophyllum commune*). In Japan, lentinan from *L. edodes* and krestin from *C. versicolor* are prescriptive medications for use as oral-adjunct immunotherapeutic agent together with chemotherapy and/or radiotherapy and surgical treatment of colorectal and gastric cancers.[56–59]

A less investigated β-glucan is the paramylon from *Euglena gracilis*, which can be considered to be a quite peculiar case. *Euglena* can accumulate large amounts of paramylon, up to 95% of the cell mass, when grown in the presence of adequate carbon sources under heterotrophic growth conditions (Fig. 2a).[60] As mentioned earlier, paramylon has an unusually high crystallinity for a natural macromolecule, and is an extremely pure β-glucan, as shown in the NMR spectrum, which indicates that it consists of 100% glucose (Fig. 2b). The crystallinity of paramylon is due

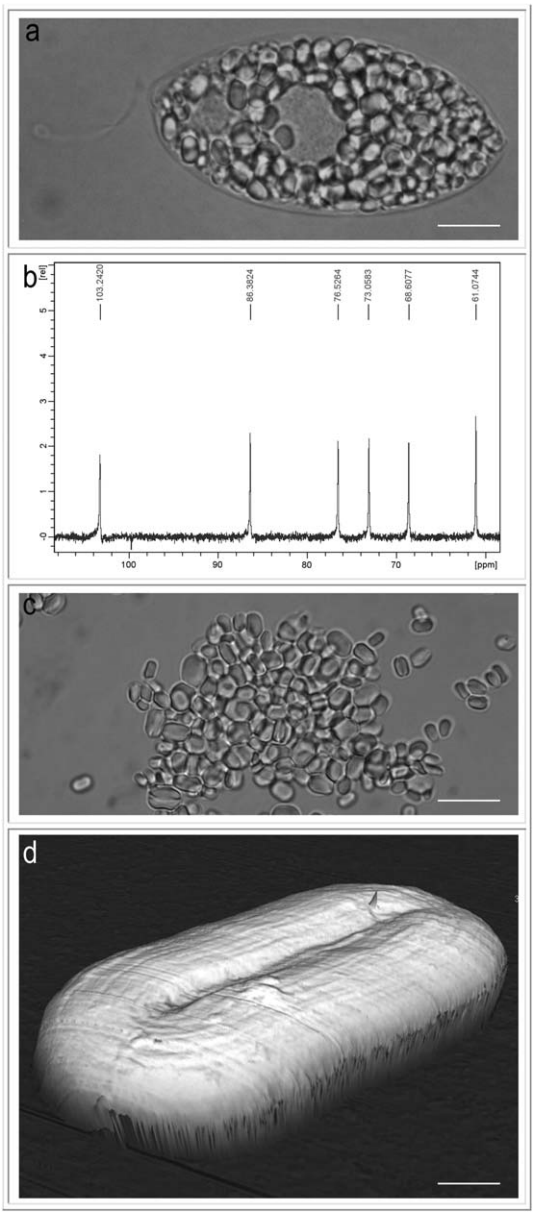

Fig. 2 a) *Euglena gracilis* cell completely filled with paramylon granules (scale bar 10 μm); b) NMR spectrum of paramylon; c) isolated paramylon granules (scale bar 10 μm); d) scanning electron micrograph of a paramylon granule showing the highly ordered structure of concentric layers (scale bar 0.5 μm).

to higher-order aggregates of microfibrils, measuring 4–10 nm, composed of unbranched triple helices of β-(1,3)-D-glucan chains.[46] Its high crystallinity is an advantage, in that paramylon granules can be isolated at very low cost and in an efficient manner by simply disrupting the cells and purifying the granules by successive washing with a low concentration of detergent (Fig. 2c and 2d).[63] Paramylon has been proven to potentiate the resistance of a brine shrimp of the genus *Artemia* to stress conditions resulting from poor growth medium quality and daily handling, and to enhance reproductive success of the shrimps.[61] Moreover, glucose-tolerance tests performed in diabetic mice five weeks after diabetes induction showed a blood glucose concentration lower in animal feds with paramylon-containing food

with respect to control animals (unpublished results). Recently published results by Sugiyama *et al.*[62] have demonstrated that orally administered paramylon exhibits protective action on acute hepatic injury induced by CCl_4 *via* an antioxidative mechanism. These same authors[63] demonstrated also that the oral administration of paramylon inhibits the development of atopic dermatitis-like symptoms in mice, providing an effective alternative therapy to prednisolone. All these results support the potential use of paramylon in different fields of application.

Euglena can be easily cultivated under autotrophic, heterotrophic or mixotrophic conditions, and under extremely low pH conditions (pH 2.5–3.5)[64,65] These characteristics make *Euglena* extremely attractive as low-cost glucan source, since hydrates of agricultural wastes or wastewater from sugar industries could be employed as carbon source for paramylon accumulation. Both bioreactors and open systems could be used for biomass production of this alga.

2 Applications linked to biological activities: medical and pharmaceutical

Much of the interest in the use of cereal β-glucans has stemmed from their use as a functional dietary fiber. This term is used to collectively describe a group of substances in plant material which resist human digestive enzymes. Until recently dietary fiber was divided in two types: hydrosoluble and insoluble. However, this categorization does not accurately represent the properties of all fibers, and has been abandoned for a more accurate categorization based on the physiological effects of each dietary fiber, which depend mainly on the viscosity and fermentability. Hence fibers are divided into (i) dietary fibers, which are non-digestible carbohydrates and lignins intrinsic to and intact in plants, and (ii) functional fibers, which are physiologically beneficial non-digestible carbohydrates. The sum of dietary fibers and functional fiber is termed total fiber.[65,66] β-Glucans can be defined as non-digestible carbohydrate, and have been used in several clinical trials to test their general effects on health and clarify the mechanisms responsible.[67]

Recently, these compounds have been recognized as having a complex role as immunostimulating agents.[68,69] Trials showed that β-glucans are especially important against the two most common conventional causes of death in industrialized countries, *i.e.* cardiovascular diseases (as fibre, promoting healthy cholesterol and blood glucose levels)[70] and cancer (as immunomodulatory substances stimulating specific receptors on immunocompetent cells).[71] On the basis of numerous clinical studies, in 1997 the US Food and Drug Administration (FDA) approved a health claim that oat β-glucans at a level of 3 g per day may reduce cholesterol and lower risk of coronary heart diseases. The claim was amended in 2006 by adding barley as an additional source of soluble β-glucan fiber eligible for the same purposes.

Cardiovascular diseases are closely connected to lipid metabolism pathologies. β-Glucans have been shown to decrease low-density lipoprotein (LDL) cholesterol and increase high-density lipoprotein (HDL), possibly alleviating dyslipidemia.[72,73] The mechanism for β-glucans to lower levels of LDL is considered to be mediated by the ability of β-glucans to bind to bile acids. Bile acids, acidic steroids synthesized in the liver from cholesterol, are actively reabsorbed by the terminal ileum and undergo enterohepatic circulation. By trapping bile acids within micelles in the intestine, their delivery in the feces is increased. The increased exclusion of bile acids in turn activates cholesterol 7α-hydroxylase (7α-HC) and upregulates low-density lipoprotein receptor (LDLR), thus increasing transport of LDL into hepatocytes, and the conversion of cholesterol into bile acids.[74] By this means, levels of cholesterol can be reduced.

Cholesterol-lowering occurs also as a secondary reaction of microbial fermentation of β-glucans in the large intestine; β-glucans are not digested in the small intestine, but are fermented by the colonic microflora in the large instestine.[75] This fermentation results in the formation of short-chain fatty acids (SCFAs), primarily acetate, propionate and butyrate. Propionate in turn reduces cholestereol by suppressing cholesterol synthesis in the liver. *In vitro* evaluation of bile-acid binding capacity and fermentation was performed on oat β-glucan hydrolysates with different molecular weights, namely 156, 371 and 687 kDa.[74] The results showed that the lower the MW of the β-glucan fraction, the lower the viscosity of the solution, and the greater the *in vitro* bile-acid binding. *In vitro* fermentation of the hydrolysates with high, medium, and low MW lowered pH as a result of the SCFA production. The low-MW β-glucan produced more SCFAs than the high-MW β-glucan after 24 h of fermentation. All MW fractions produced more propionate than lactulose. The authors suggested that the greater SCFA production was due to the greater water solubility of the low-MW β-glucan fraction with respect to the medium- and high-MW fractions.[74] A study conducted on ileostomized volunteers showed that oat bran with native β-glucan given as breakfast cereals (11.6 g β-glucan per day) increased median excretion of bile acid by 144% within 24 h of consumption, and this increase could be detected by an increase in the serum concentration of 7α-hydroxycholesterol by 57% within 24 h of consumption.[76]

The decreased cholesterol absorption may also be influenced by a low-energy intake due to a high-satiety effect produced by β-glucans[53,54] thanks to their ability to increase the viscosity of the content of the small intestine, which in turn is related to the MW and amount of β-glucan solubilized in the small intestine.[77,78] Glucans exhibit high viscosity at very low concentration (1%), which is stable over a wide range of pH values. To create significant viscosity, β-glucans have to be soluble, with molecular weights ranging from 26.8 to 3000 kDa. A recent double-blind randomized clinical trial showed that an extruded breakfast cereal providing 3 g oat β-glucan per day with a high MW (2210 kDa) or medium MW (530 kDa) both lowered LDL cholesterol by 5% (0.2 mmol L^{-1}), but the efficacy was reduced by 50% when the MW was reduced to 210 kDa.[79]

The increased viscosity of the chyme (the partly digested food expelled by the stomach) in the gut slows gastric emptying time, lengthens the passage time in the small intestine, interferes with the mixing of food stuffs and digestive enzymes, and alters the diffusion and interaction of nutrients with the mucosal surface, and hence their absorption rate. Vitaglione *et al.*[80] demonstrated the ability of β-glucans to modulate appetite moods and to reduce energy intake by influencing satiety-related hormones, such as ghrelin, which increases food intake, and peptide YY (PYY), which acts as a suppressor of food intake. The authors showed that bread formulated with 3% w/w barley β-glucans is able to modulate appetite by

decreasing hunger and increasing fullness and satiety, and reducing by 19% the energy intake in the subsequent meal. These features were associated with a reduction of blood glucose and ghrelin concentration and with an increase of PYY over the 3 h between breakfast and lunch.[80]

β-Glucans have been found to be effective in controlling glycometabolism. Both oat and fungal β-glucans reduce blood glucose concentrations after oral administration in animal experiments and clinical trials.[81] Though the mechanisms by which these fibers improve markers of glucose and insulin metabolism is poorly understood, the leading theory is that these fibers increase the viscosity of the intestinal content, causing reduced postprandial insulin and glucose levels.[82,83] Over time, lower ambient insulin levels improve cellular insulin sensitivity, resulting in improved glucose metabolism.[84] The effect of β-glucans to reduce blood glucose could be also mediated by delaying stomach emptying so that dietary glucose is absorbed more gradually.[85] After ingestion of the glucans, the peak of the blood glucose level is smoothed, and the shape of the plasma glucose response curve is much flatter. These changes reduce the feeling of hunger caused by rapid decrease in blood glucose. Thus, β-glucans may decrease appetite and reduce food intake.[86]

Glucans do not attack tumour cells directly, but exert their antitumour effects by stimulating specific and non-specific immune responses in the host.[71,87] The ability of our immune system to immediately recognize and respond to infection is reliant upon germ-line-encoded pattern recognition receptors (PRRs).[38,88] PPRs recognize pathogen-associated molecular patterns (PAMPs) on microorganisms, which, while not unique to pathogens, are critical for microbe survival and are not found in the metazoa. PAMPs include lipotechoic acid (Gram-positive bacteria), LPSs (Gram-negative bacteria) and β-glucans (bacteria and fungi).[68] These mechanisms allow a small set of receptors to recognize a wide variety of microbes and microbial products. PAMPs can trigger immune responses in isolated form, and there has been much interest in their development as adjuvants and as immunotherapeutic agents. PRRs that recognize invading microbes or their toxic products do not undergo somatic mutation, as do receptors of the adaptive immune system. PRRs are expressed on, but not limited to, cells of the innate immune system. β-Glucans are themselves PAMPs that interact with PRRs to mediate their immunostimulatory activity.[69] Previous reports indicate that the physicochemical properties of glucans (*e.g.* primary structure, polymer size, surface charge, solution conformation and side-chain branching) may be important determinants for recognition and interaction with PRRs in the innate immune system.[53] The consequences of β-glucan recognition likely depend on the cell types involved and the receptor(s) engaged. In humans, a number of such receptors have been identified. These are dectin-1, complement receptor 3 (CR3), scavenger receptors, lactosylceramide, and the toll-like receptor (TLR).[89] These receptors are found on monocytes, macrophages, neutrophils, eosinophils, dendritic cells, NK cells and Gr-1-positive populations of splenic T lymphocytes. They are also found on non-immune cells such as endothelial cells, alveolar cells, and fibroblasts.[89] We will describe below only dectin-1 and CR3 receptors, because the interaction between β-glucans and these receptors is more well-established than that of the other receptors.

Dectin-1 is considered the primary PRR for glucans;[90] it is a type II transmembrane receptor containing a single extracellular C-type lectin-like carbohydrate recognition domain (CRD) and a tyrosine-based activation motif (ITAM) in the cytoplasmic tail. Dectin-1 is expressed at high levels on blood and splenic monocytes, neutrophils and alveolar and inflammatory macrophages, and at lower levels by dendritic cells and a subset of T lymphocytes.[89] Using murine recombinant dectin-1, and natural and synthetic water-soluble β-glucan, it was demonstrated that this receptor is highly specific for glucans that have a β-(1,3)-D-glucopyranosyl backbone, that it does not recognize non-β-linked carbohydrate polymers (*e.g.* mannan or pullulan), and that it does not interact with plant-derived glucans (*e.g.* barley glucan) with a mixed linkage polymer backbone characterized by alternating regions of (1,3)-β and (1,4)-β linkages.[91] The specificity of the interaction was further demonstrated by the observation that a (1,6)-β-linked glucan is not recognized by dectin-1. While dectin-1 is highly specific for β-(1,3)-D-glucans, it does not recognize all β-(1,3)-D-glucans equally; structural analysis demonstrated that glucan backbone chain length and β-(1,6) side-chain branching strongly influenced dectin-1 binding affinity. Recognition of glucan ligands requires a backbone chain length of at least seven glucose subunits and at least one side-chain branch. On the basis of the crystal model of dectin-1, it was suggested that the binding pocket of this receptor may be a groove that accommodates the helical structure of glucans, with a unique structure that preferentially interacts with the branched structure.[91]

CR3, identified over 20 years ago, is a type of membrane glycoprotein consisting of two non-covalently linked α and β subunits.[92] It is a promiscuous PRR with two separate binding sites, one carbohydrate-binding lectin site for β-1,3-glucan and a second site for the complement cleavage fragment iC3b. CR3 is highly expressed on neutrophils, monocytes, and natural killer (NK) cells, whereas less is present on macrophages.[92] It mediates the diapedesis of phagocytes and NK cells into sites of inflammation, and triggers phagocytosis and degranulation in response to microorganisms or immune complexes opsonized with the iC3b.[93] CR3 is not triggered to mediate phagocytosis or cytotoxicity by ligation to cells bearing only iC3b, since its activation requires its dual ligation to both β-(1,3)-glucan and the iC3b-deposited adjacent to the β-(1,3)-glucan on fungal cell walls by the complement system. When phagocyte CR3 binds to iC3b on bacteria or yeast, phagocytosis and degranulation are triggered because of simultaneous recognition of iC3b and specific microbial polysaccharides.[94] By contrast, when phagocyte or natural killer (NK) cell CR3 adheres to iC3b on erythrocytes or tumor cells that lack CR3-binding membrane polysaccharides, neither lysis nor cytotoxicity are stimulated.[95]

Many human tumors generate an immune response that results in the deposition of antibody and iC3b on membrane surfaces, which in turn serves as a specific target for cells bearing CR3 that have been primed with soluble β-glucan. Various tumor models were described in which specific mAb given alone had little or no effect on tumor regression and yet mediated complete remission when given together with either oral or intravenous β-(1,3)-D-glucan.[96,97]

The mechanism of the function of oral and intravenous β-(1,3)-D-glucan was shown to be similar. Although intravenous soluble yeast β-(1,3)-D-glucan is delivered directly to the CR3 on

circulating granulocytes, orally administered β-(1,3)-D-glucan goes through an intermediate step in which macrophages process and deliver soluble β-(1,3)-D-glucan to the CR3 of granulocytes in the bone marrow.[71] In addition to priming granulocyte CR3, oral β-(1,3)-D-glucan primes the CR3 of tissue macrophages and probably also the CR3 of marrow monocytes and NK cells.[94] Oral uptake and distribution of barley or yeast β-(1,3)-glucan was demonstrated to occur *via* gastrointestinal macrophages tracking the oral uptake and *in vivo* processing of fluorescein-labeled glucan by intestinal epithelial cells and gut-associated lymphoid tissue (GALT).[94,98] The GALT has a surveillance function that results in the phagocytosis, presentation and destruction of perceived pathogens; it contains diverse leukocyte populations, including macrophages, dendritic cells, T and B lymphocytes. The GALT is a privileged site in the larger immune system that operates *via* two intertwined dynamic networks.[69] The innate or non-specific immunity represents the basic resistance to disease that an individual is born with; it is a relatively rapid non-specific system with no capacity for memory. Innate defense mechanisms provide the first line of host defense against invading microbial pathogens and also provide protection against some tumor cells until an acquired or specific response develops. This acquired or specific immunity requires activity of a functional immune system, involving cells called lymphocytes and their products. Acquired immunity does not operate independently of innate immunity, rather the specific immune response supplements and augments the non-specific defense mechanisms, producing a more effective total response.[99]

The movement of soluble glucan from the gastrointestinal tract into the systemic circulation mainly depend on the presence of dectin-1 positive cells in the GALT, though also intestinal epithelial cells not expressing the receptor were observed to uptake and internalize labeled glucan. Particulate β-glucan is uptaken by GALT-associated macrophages, which transport glucan to various sites throughout the body (lymph node, spleen, bone marrow) and slowly degrade the particulate, most likely *via* oxidative pathway (macrophages do not have glucanase), and release a bioactive, smaller soluble glucan of about 25 kDa.[71] Bone marrow granulocytes use CR3 to take up the soluble β-glucan released by macrophages; the finding of CR3 surface-bound β-(1,3)-glucan on isolated peritoneal granulocytes demonstrated that membrane CR3 serves as a receptor for β-1,3-glucan *in vivo*. Although macrophages and granulocytes may also use dectin-1 to capture soluble β-(1,3)-glucan, only CR3 with bound β-glucan triggers cytotoxic degranulation in response to iC3b-coated tumors.[93]

Glucan also enhances maturation of dendritic cells (DC), derived from monocytes, with significant interleukin production, and a strong antigen-presenting capacity to T cells for activation of immune responses.[71,100] As immunostimulating agent that acts through the activation of macrophages and NK cell cytotoxicity, β-glucan can inhibit tumor growth in the promotion stage too.[101] NK cells are directly cytotoxic to tumor cells, and play a primary role in regulating immune responses. Carcinoma-bearing mice treated with extracts of *Grifola frondosa* showed a marked suppression of tumor growth, associated with increases in cytokine release by spleen cells and cytokine expression in NK cells. Furthermore, there was an increase in macrophage-derived interleukin, which serves to activate NK cells.[102]

The anticarcinogenic activity of β-glucan could also be mediated through a anti-angiogenesis pathway.[103] Angiogenesis is crucial to tumor growth and metastasis, and interruption of this process is a prime avenue for therapeutic intervention of tumour proliferation. Sarcoma S180 tumour-bearing mice treated with PSP from *Coriolus versicolor* mycelia showed a reduction of vascular density and tumour weight.[104] Mice were fed with PSP solution in their drinking water (35 mg per day per mouse). The study demonstrated that the anti-tumour activity of PSP resides in its angiogenic properties *via* suppression of vascular endothelial cell growth factor (VEGF) gene expression, resulting in a deprivation of angiogenic stimulation to the tumour growth.[104] PSP was tested on patients who had completed conventional treatment for advanced non-small-cell lung cancer (NSCLC).[105] Patients were given three capsules (340 mg each) of purified PSP three times daily for 28 days. After the treatment there was a significant improvement in blood leukocyte and neutrophil counts, serum IgG and IgM, and percent of body fat among the treated patients, but not in the control. Although the treated patients did not improve with regard to cancer-related symptoms, the treatment appeared to be associated with slower deterioration in patients with advanced NSCLC.[59,105] Extracts of *Grifola frondosa* were tested also on cancer patients in stages II–IV. Liquid extract was orally administered twice daily for 3 weeks. Cancer regression or significant symptom improvements were observed in liver cancer, breast cancer, and lung cancer patients, while a minor improvement was present in leukaemia, stomach and brain cancer patients.[106]

Hence, β-glucans could influence the initiation phase of carcinogenesis, and inhibit promotion and progression of the tumours, by activating NK cell function, and interfering with tumour angiogenesis.[107]

β-Glucans can be used also as adjuvants to cancer chemotherapy and radiotherapy.[108] Anticancer drugs impair blood-forming functions important for maintaining the defense system of patients. Chemotherapy has neutropenia as major side effect, which in turn may accelerate risk of infections. β-Glucans enhance haematopoietic responses in animal models under chemotherapy, by increasing interleukin-6 concentration.[109] In the case of radiotherapy, β-glucans could increase serum level of radioprotective cytokines, while decreasing the level of radio-induced tumour necrosis factor, increased as a consequence of tissue injury and anemia due to radiation.[110]

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