Beta-glucans in higher fungi and their health effects

Otakar Rop, Jiri Mlcek, and Tunde Jurikova

*Together with chitin, the β-glucans are components of mycetes' cell walls. A high level of biological efficiency has been found in β-glucans, especially β-1,3-D-glucans and β-1,6-D-glucans isolated from some basidiomycetes. (Biological efficiency refers to the relative ability of β-glucans to promote a desired response, for example to induce leukocyte activation and to produce inflammatory mediators.) These polysaccharides increase the number of Th1 lymphocytes, which help protect organisms against allergic reactions. A number of β-glucans, for example pleuran from Oyster (*Pleurotus *spp.) mushrooms or lentinan from Shiitake (*Lentinus edodes*) mushrooms, have shown marked anticarcinogenic activity. In addition to having an immunity-stimulating effect, β-glucans may participate in physiological processes related to the metabolism of fats in the human body. Their application results in a decrease in the total cholesterol content in blood and may also contribute to reductions in body weight.*

INTRODUCTION

Glucans are polysaccharides that contain glucose as a sole monomer unit.[1] This group of polysaccharides involves glycogen, cellulose, and dextran. Their general formula is $(C_6H_{12}O_5)_n$.[2]

Among an immense number of theoretically possible combinations, the number of natural saccharides is relatively limited (approx. 300). Homopolymers of D-glucose (polyglucose, glucans) are the most frequent. Their diversity results from the different bonds among glucopyranose units. Condensation can take place with any hydroxylic group of any carbon atom and can result in the conformation of either α- or β-anomers, because there are at least eight different ways in which two monomer units can link. The diversity of glucans is further increased by substitutions of sugar rings and by branching of chains. However, among many theoretically possible polymers of glucose, only a few are found in nature.[3]

Polysaccharides called β-glucans as well as β-1,3-D-glucans or β-1,4-D-glucans (earlier, also lichenins) are present in the cell walls of higher plants and also in the seeds of some cereals (e.g., barley and oats). Related polymers, which are also called β-glucans and/or β-1,3-D-glucans and β-1,6-D-glucans, are synthesized by fungi, molds, and yeasts.

The range of relative molecular weights of β-glucans is very wide and fluctuates (depending on origin) from tens to thousands of kilodaltons. The solubility of β-glucans in water is dependent, above all, on their structure, and this is associated with their origin. Their solubility increases with temperature. Protein-bound glucans are insoluble. After partial hydrolysis, their molecules can produce gels. However, native molecules lack this capability, so it can be said that β-glucans represent partly soluble and partly insoluble food ingredients. The basic structure of β-glucans with combination bonds (1→3), (1→4) is presented in Figure 1.[4]

Edible fungi are known to have been used thousands of years ago in ancient civilizations for their healing powers as well as to increase human longevity.[5] Some populations also knew of their hallucinogenic effects. Today, fungi are appreciated and consumed primarily for

Affiliations: *O Rop* and *J Mlcek* are with the Department of Food Engineering, Faculty of Technology, Tomas Bata University in Zlin, Czech Republic. *T Jurikova* is with the Institut of Natural and Informatics Science, Faculty of Central European Studies, Constantine the Philosopher University in Nitra, Slovak Republic.

Correspondence: *O Rop*, Department of Food Engineering, Faculty of Technology, Tomas Bata University in Zlin, namestí T.G. Masaryka 275, 762 72 Zlin, Czech Republic. E-mail: rop@ft.utb.cz, Phone: +420-576-031-129, Fax: +420-576-031-111.

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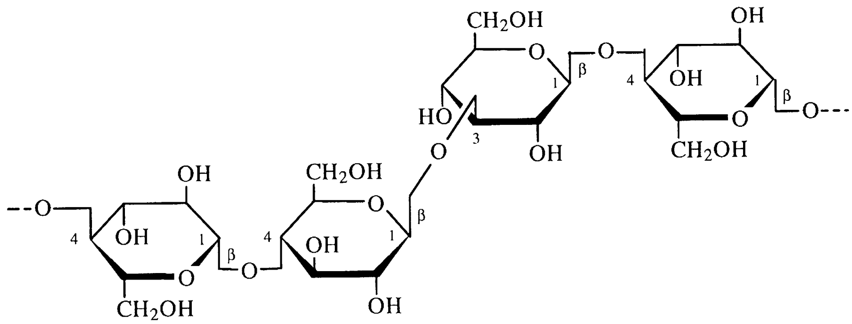

Figure 1 **The basic structure of β-glucans with combination bonds (1→3), (1→4).**
Data from Velisek (1999)[4].

their sensory and nutritional properties; they are also used increasingly in medicine and in the pharmaceutical industry. Fungi show favorable dietetic properties thanks to their low caloric value, low fat content, and high levels of proteins, minerals, and some polysaccharides.[6]

Many species belonging to the basidiomycetes group are used as food additives, which should strengthen the immune system of humans. This theory is supported by results of many research studies that tested the biological activities of higher fungi. These studies resulted in findings indicating that some substances contained in edible fungi show positive effects on cellular activities and on secondary production of chemical compounds that can strengthen the human immune system and support the treatment of a number of diseases.[7] Regarding the popularity and frequency of fungi consumption, many authors mention the positive effects of the compounds mentioned above, but they also emphasize the risk associated with their consumption. This risk results from the noxious substances that fungi sometimes contain; heavy metals are of particular concern, because fungi show a generally high ability to accumulate them and to mediate their penetration into the food chain.[8]

For centuries, the most extensive knowledge and use of fungi for healing was in traditional Chinese medicine.[9] Presently, increasing attention is being paid to polysaccharides, which are an integral component of fungi.[10] The cell walls of fungi contain two polymers, chitin and β-glucan. In molecules of both chitin and β-glucan, individual chains are linked with hydrogen bridges so that covalent bonds form between both polymers.[11] This process results in the formation of a strong cell wall, in which chitin fibers are interlinked, creating a network within a glucan matrix.[12] In addition to these fundamental building blocks, it is possible to find smaller amounts of some other saccharides in fungi.[13] So, for example, we can find increased levels of disaccharide trehalose in Trunk Rot (*Pholiota adiposa*) mushrooms, and mannan oligosaccharides are an important component of Shiitake (*Lentinus edodes*) mushrooms.[14]

Glucans β (1→ 3), β (1→ 4), and β (1→ 6) are a key reason fungi are used as food additives and in pharmacology; they have also shown beneficial effects when used in the treatment of various diseases.[15] The objectives of this review are to present a survey of the β-glucans found in basidiomycetes as well as to provide general information about their properties and their effects on animal and human organisms.

OCCURRENCE OF β-GLUCANS IN FUNGI

The contents and ratios of individual saccharide components in fungi are determined, above all, by genetics (i.e., they are species[16] or even cultivar dependent[14]). Nowadays, Shiitake (*Lentinus edodes*) and some members of the genus Oyster (*Pleurotus* spp.) are acknowledged to be some of the most important sources of β-glucans; in Shiitake, glucan lentinan is the effective substance,[17] while in Oyster mushrooms, the effective substance is called pleuran.[18] Glucans differ in the structure of their side chains, which are specific to individual fungal species. In fungi, β-glucans are present either in their water-soluble or -insoluble form. The biological activity of the water-soluble form, however, has been shown to be much greater in humans and animals and to have a more pronounced effect on their immune systems.[19] The average levels of β-glucans in some species of Oyster mushrooms and in Shiitake are presented in Table 1.

Glucans are also present in many other fungal species. For instance, in some members of the genus *Boletus* β-glucans comprise 2–13% of digestible dry matter.[16] A survey of the most important biologically active glucans and of the fungi in which they occur is presented in Table 2.

Regarding other members of the basidiomycetes group that possess β-glucans in sufficient amounts to enable their use for pharmaceutical purposes, the following are worth mentioning: Judas's ear (*Hirneola auricula judae*), *Stropharia aeruginosa* mushroom, Black Poplar mushroom (*Agrocybe aegerita*), or Honey fungus

Table 1 **Average β-glucan content and percentages of water-soluble and water-insoluble fractions in Shiitake (*Lentinus edodes*) and some members of the Oyster genus (*Pleurotus* spp.).**

Latin name of fungus	Content of β-glucans (mg.100/g DM)	Water-soluble percentage β-glucans	Water-insoluble percentage β-glucans
Pleurotus ostreatus	38	37.8%	62.2%
Pleurotus eryngii	38	16.8%	83.2%
Pleurotus pulmonarius	53	18.7%	81.3%
Lentinus edodes	22	46.1%	53.9%

Data from Manzi and Pizzoferrato (2000)[17].

(*Armillaria mellea*).[21] Recent studies on β-glucans also investigated the Himematsutake mushroom (*Agaricus brasiliensis*), which contains, on average, 42 mg of β-glucans per 100 g of dry matter.[22]

In addition to varying according to species, the content of β-glucans can be influenced by other factors, particularly the mushrooms' growing conditions. The highest levels of water-soluble polysaccharides can be found in basidiomycetes cultivated on a substrate with the C/N ratio 40:1. For Oyster mushrooms (*Pleurotus* spp.), the most favorable pH of the substrate is 5.5; however, the incubation temperature is also important, because it is species-specific.[23] When growing Shiitake mushrooms (*Lentinus edodes*), good results were obtained on substrates with a high content of polyphenolic compounds, because they induce an increased synthesis of β-1,3-glucan synthetase in their fruiting bodies. For that reason, it is recommended that small amounts of olive pomace be added to the growing substrate.[24]

The β-glucan content also depends on the degree of fruiting body maturity. So, for instance, champignons show the highest levels of these compounds immediately prior to the period in which the spores begin to ripen.[25]

Under certain circumstances, the biological efficiency of β-glucans may be promoted by the presence of their complexes with proteins. So, for example, the Shiitake (*Lentinus edodes*) mycelium contains a glycoprotein called LEM (Lentinan edodes mycelia), while in Lingzhi mushrooms (*Ganodema lucidum*) we can find highly active immunity-stimulating glycoproteins called fungal immunomodulatory proteins (FIMs) and Ganoderma polysaccharides peptide (GPP). Proteoglucans polysaccharide peptide (PSP) and polysaccharide-krestin (PSK), which are present in Turkey Tail (*Trametes versicolor*) or in Split Gill mushrooms (*Schizophyllum commune*), are used as cancerostatics.[24] Results obtained in experiments with proteoglucan ATOM (antitumor organic substance Mie), which is present in Himematsutake mushrooms (*Agaricus brasiliensis*), are also very promising.[20]

Table 2 **Select *Basidiomycetes* species containing biologically active β-glucans.**

Latin name of fungus	Name of β-glucan
Pleurotus spp.	Pleuran
Lentinus edodes	Lentinan
Schizophyllum commune	Schizophylan
Ganodema lucidum	Gl – 1
Trametes versicolor	Krestin
Grifola fondosa	Grifolan
Flammulina velutipes	Flammulin

Data from Jablonsky (2005)[20].

EFFECTS OF β-GLUCANS ON ANIMALS AND HUMANS

Although the chemical structure of β-glucans in the cell walls of fungi has not been examined completely, it is probable that they appear primarily in the form of glucose chains, which are twisted and create a single or a triple helix. The biological efficiency of these two forms is different. The most important and cardinal feature seems to be the capability of single-helix glucans to link themselves to immunoglobulins present in blood serum.[25]

When studying the processes that occur during the course of immune system stimulation, the most positive effect was obtained with β-1, 3-glucan, which is the glucan found most frequently in yeast cell walls.[26] Although the principle underlying β-glucan's effects is not fully known, it seems that its biological activity is based on its interaction with specific β-glucopyranose receptors present on leukocytes.[27] This interaction is stimulated not only by the conformation of the glucan molecule but also by the degree of its water solubility. Water-soluble glucans show much better efficiency.[28] Increased activity is further augmented by a higher degree of substitution. In studies on the use of β-glucans to support the immune system, very good results were obtained with β-1, 3-glucan, which has a structure that corresponds with a single helix and which also has hydrophilic groups present on the chain's surface. The highest degree of biological activity of glucan polymers was observed at branching degrees ranging from 0.20 to 0.33.[29]

The molecular weight of β-glucans is another factor affecting their ability to interact with the surface of leukocytes,[28] with a higher molecular weight being more advantageous.[30] β-glucans consumed by humans in their food show varying degrees of resistance to digestive enzymes.[31] In the human body, intensive oxidation of β-glucans is permanently underway, which results in the formation of temporary active metabolites (these are less effective than β-glucans themselves), which slowly change into non-active forms.[32] Certain levels of non-degraded β-glucans have been detected in liver and spleen; in these organs, they can persist for a longer period and preserve their biological activity.[28]

In the human body, the effects of β-glucans are dependent on the pH at which they interact with leukocytes. If the pH reaction is alcalic, the structure of a triple-glucan helix is split and only simple helices are formed. A neutralization of the glucan solution increases the share of molecules with a single helix; such molecules then show a high ability to attach themselves to some proteins and to form complexes, which then stimulate production of antibodies by macrophages.[33] In an acidic environment, hydroxylic groups occurring on the surface of chains are disrupted and the biological efficiency of β-glucans is reduced. The intensity of their action may also be reduced in the presence of epoxy groups.[34]

Linkage between β-glucans and proteins can be found not only in fungi and higher plants but also in some invertebrates. For instance, scientists isolated from horseshoe crab *Tachyleus tridentatus* a protein that was able to bind itself very easily with β-glucans originating from basidiomycetes (e.g., with lentinane or schizophylane), thereby increasing their biological activity.[35]

MECHANISM OF ACTION OF β-GLUCANS

Molecules of β-1,3-glucan are relatively resistant to the acid in the stomach. Following oral administration, β-glucans slowly pass into the duodenum and are entrapped by macrophage receptors present in the intestinal wall. These receptors are of protein-like nature and show an ability to distinguish among at least seven saccharide units. These receptors are produced by bone marrow and occur on the macrophage membranes, probably from the beginning of their ripening and into the course of their differentiation.[27]

As soon as a glucan molecule meets a group of glucan receptors, it is activated and produces such bactericidal compounds as lysozyme, reactive oxygen radicals, and N-oxides. Thereafter, the cells begin to produce several cytokines, which activate surrounding phagocytes and leukocytes that are responsible for inducing specific immunity.[36] Thus, glucans induce not only a local activation of cells but also an overall (i.e., systemic) response of the organism because cytokines are produced by cells migrating from the site at which they reacted with glucans.[37]

Glucans also play a very important role in promoting the activity of helper lymphocytes known as Th1 and Th2 effectors. Th1 lymphocytes control immunity against intracellular parasites while Th2 effectors control immunity against extracellular pathogens. A disturbance of their balance and a predominance of one of these two populations may trigger an autoimmune response. For example, predomination of a Th2 population is associated with the development of allergy. Glucans, however, create conditions supporting Th1 lymphocytes. So, for example, it was demonstrated that grifolan showed an ability to promote the formation of Th1 lymphocytes to the detriment of Th2 cells.[38] Each subpopulation of lymphocytes produces a characteristic spectrum of cytokines. Th1 lymphocytes synthesize the cytokines interferon gamma (INF-γ gamma) and interleukin 2 (IL-2). On the other hand, Th2 lymphocytes produce cytokines IL-4, IL-5, and IL-6.[11]

However, in spite of the data presented above, present knowledge of glucans' complete mechanism of action within the body is still considered insufficient and further research is required.[30]

IMPORTANCE OF β-GLUCANS ORIGINATING FROM BASIDIOMYCETES FOR ANIMALS AND HUMANS

Grifolan is one of the most effective β-glucans. This compound shows a marked effect promoting the activities of macrophages.[39] Thus, it increases production of IL-1, since IL-1 is produced just by macrophages. Aqueous extracts of grifolan have been shown to increase insulin production by as much as 25%; for that reason, this glucan seems to be a substance that could contribute to the treatment of diabetes.[40,41] In addition to promoting IL-1 production, macrophages are able to produce IL-6 and IL-8, which represent important activators of other leukocytes.[42] Both these substances promote cell division and thus increase the numbers of leukocytes in blood.[11]

Also the Cauliflower mushroom (*Sparassis crispa*) seems to be a prospective fungus species because the application of its aqueous extracts showed a positive effect on production of both IL-6 and IL-8. In humans, the result of this action is an increase in the numbers of monocytes and granulocytes in blood circulation.[43] A positive effect of the aqueous extracts of Cauliflower mushroom on the speed of bone marrow renewal was also observed in mice.[42] In experimental animals, higher numbers of monocytes and granulocytes were observed not only in blood but also in spleen. The weight of this body organ increased in accordance with the duration of treatment with β-glucans.[44]

Grifolan-induced activation of macrophages is associated with increased synthesis of N-oxides in these cells,[26] and precisely these oxides represent one of the basic antibodies produced by macrophages.[45] Grifolan shows the highest efficiency in an alkaline environment and in oxidative conditions, when it promotes the formation of free oxygen radicals; these then cause the oxidation of lipoprotein membranes in pathogenic cells.[46]

Grifolan has been used to successfully suppress the pathogenic fungus *Candida albicans*, which can cause infections of oral cavity membranes and even systemic infections (of lungs, lymphatic nodes, liver, and spleen).[47] In mice, a very strong effect of grifolan was observed when suppressing inflammation of mucose membranes of the respiratory tract.[48] The effect of grifolan is supported by simultaneous use of vitamin C.[46]

Other β-glucans produced by basidiomycetes can also show a positive effect on the production of antibodies by leukocytes. In vertebrates, for instance, lysozyme bactericide components of a protein nature are promoted by schizophylan originating from Split Gill fungus (*Schizophyllum commune*).[49] A less marked promoting effect of krestin on the activity of leukocytes was observed in Turkey Tail mushroom (*Trametes versicolor*).[50]

As far as β-glucans (e.g., pleuran) are concerned, they have been shown to have a significant effect on decreasing cholesterol levels in the blood of rats[51] and hamsters.[52] In humans, fungal β-glucans reduced both the overall level of cholesterol[53] and the level of LDL cholesterol in blood.[54] While they reduced the level of free fatty acids, however, they also slightly increased the level of HDL cholesterol.[55] Lower levels of cholesterol were probably correlated with increased amounts of leptin.[56] The synthesis of leptin was supported solely by supplements of β-glucans, and it should be noted that very good results were obtained thanks to polysaccharides originating from Straw mushroom (*Volvariella volvacea*).[57] This mushroom contains approximately the same amounts of biologically highly efficient β-glucan in all its parts.[58] Leptin is a protein-like substance, which is produced by fat cells of the subcutaneous connective tissue and is commonly found in blood. Leptin receptors can be found in some parts of the hypothalamus, where they control the feelings of hunger and satiety.[59] Thus, it is probable that leptin mediates feedback in the brain related to the fat content within the body.[11] This indicates that leptin and its effects on the nervous system may offer a promising way to treat obesity.[60]

From a commercial standpoint, pleuran from Oyster (*Pleurotus ostreatus*) mushrooms and lentinan from Shiitake (*Lentinus edodes*) mushrooms are currently the most frequently used β-glucans. Both of them show positive effects on the intestines. They increase the resistance of intestinal mucosa to inflammation[61] and inhibit the development of intestinal ulcers.[62] Lentinan also shows a positive effect on peristalsis.[63]

The mechanism of action by which pleuran and lentinan protect the intestinal mucosa is not yet known.[51] Pleuran reduces the amount of conjugated dienes in the intestines, liver, and erythrocytes,[64] but, in general, it has a minimizing effect on the activity of antioxidative enzymes[51] and practically no influence on peroxidation of fats within the body. Also, the effect of pleuran on reducing blood cholesterol levels is negligible.[65] However, it was demonstrated that lentinan markedly promoted antioxidative enzyme function. In addition to their well-known effects on heterogenous organisms, they also cause a decrease in the transcription intensity of genes coding the synthesis of some mycotoxins. In this regard, the best known is their inhibitory effect on the production of aphlatoxins by the mold *Aspergillus niger*.[66]

In vitro and clinical experiments have demonstrated that the consumption of fruiting bodies of basidiomycetes can help prevent the onset of oncogenesis[20]; moreover, it has been demonstrated that fungal glucans possess antineoplastic activity.[67] Histological analyses of tissues sampled from tumors demonstrated not only tumor disappearance but also increased levels of activated macrophages.[24] Results of recent studies are very promising, particularly regarding the treatment of both melanoma and basal oncogenic cells.[68] A marked antineoplastic activity was recorded, above all, in the case of lentinan[69] but also of grifolan[38] and pleuran.[70] When curing tumorous diseases, it is important to quickly restore the cell immunity that has been destroyed by radiation and chemotherapy and, to this end, fungal β-glucans seem to be a very promising means.[24]

In the near future, research will likely be focused on some other fungal species beyond the basidiomycetes in which glucans are currently known to exist. In this context *Phellinius linteus*, a fungus traditionally used in Asian medicine, which shows anti-cancer effects similar to those of Shiitake, seems to be a very promising candidate.[71] Also, some champignon species, e.g., Himematsutake (*Agaricus blazei* syn. *Agaricus brasiliensis*) mushrooms, show anti-cancer effects.[72] For example, the β-glucan isolated from this fungal species has been shown to suppress carcinogenic growth in human ovaries and in the respiratory tract of rats.[73] The aqueous extract of this champignon is also interesting because it shows preventive effects against the formation of metastases.[20] In mice, a marked increase in the champignon's anticarcinogenic activity was supported by zinc supplementation of their food.[74] Himematsutake (*Agaricus brasiliensis*) mushrooms are currently very popular in Brazil, Japan, and China, while in Europe their use as an edible foodstuff or as a source of pharmaceutically effective substances is rare.[75]

CONCLUSION

β-glucans, which show marked immunity-stimulating effects in both humans and animals, have been found in numerous fungal species. Based on recent findings, they appear to be promising for aiding in the cure of tumorous diseases. They help reduce cholesterol levels in blood and positively influence the metabolism of fats and sugars within the human body. They contribute to improved resistance against allergies by increasing the numbers of Th1 lymphocytes in blood. In terms of biological activity, β-1,3-D-glucans and β-1,6-D-glucans, which are contained in Oyster, Shiitake, and Split Gill mushrooms, as well as other basidiomycetes, are considered to be the most effective. Among them, the greatest attention is being paid to Himematsutake mushrooms.

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Declaration of interest. The authors have no relevant interests to declare.

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