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Review Article

Chitosan and Chitosan Oligosaccharides: Applications in Medicine, Agriculture and Biotechnology

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Abstract: Chitosan and its oligosaccharides (COS), which are known to possess multiple functional properties, have attracted considerable interest due to their biological activities and potential applications in medicine, agriculture, environment, biotechnology and pharmaceutical industries. In this review, we have summarized the findings of previous investigations that have focused on biological and industrial properties of chitosan and COS.

Keywords: Chitosan, Depolymerisation, COS, Anti-Obesity, Antitumor Activity

1. Introduction

Natural biopolymers have several advantages, such as availability from replenishable agricultural or marine food resources, biocompatibility, and biodegradability, thereby leading to ecological safety and the possibility of preparing a variety of chemically or enzymatically modified derivatives for specific end uses. Furthermore, nontoxic, and nonallergenic nature of chitosan especially encourages its potential use as a bioactive material [1].

Chitosan has been employed in photography, biotechnology, cosmetics, food processing, biomedical products (artificial skin, wound dressing, contact lens, etc.), system of controlled liberation of medicines (capsules and microcapsules), treatment of industrial effluents for removal of metallic and coloring ions [2], waste water treatment, membrane technology, pulp and paper, food industry and agriculture.

In agriculture, there is a worldwide trend to use chitosan as an alternative compound because of its fungicidal effects and elicitation of defense mechanisms in many plant tissues. It is also useful in other ways including used as a coating material for prolonging postharvest life and limit fungal decay on strawberry and bell pepper. Chitosan is registered as a pesticide [3].

Recent studies on chitosan depolymerisation have drawn considerable attention, as the products obtained are more water-soluble [4]. Chitosaccharides have been proven to have a wide variety of applications in the biomedical, pharmacological, agricultural and biotechnological industries. Therefore, recent studies on chitosan have attracted interest in converting it to more soluble chitooligosaccharides, called COS, which possess a number of interesting biological activities, such as antibacterial, antifungal, neuroprotective and anti-inflammatory activity, antitumor properties as well as immune enhancing effects on animal health [4-5].

2. Structure and Properties of Chitosan

Chitosan, a natural cationic polysaccharide, consisting of a copolymer from β-(1→4)-2-acetamido-D-glucose and β-(1→4)-2-amino-D-glucose units (figure 1). Chitosan is the most abundant basic biopolymer and is structurally similar to cellulose, which is composed of only one monomer of glucose and differs from chitin by the degree of deacetylation (DD) [2, 6].

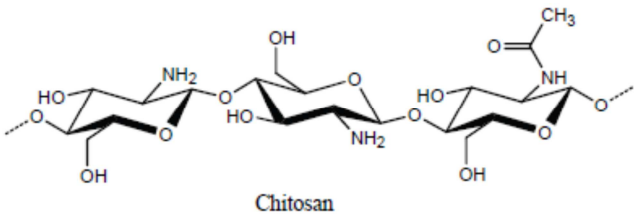

Figure 1. *Structures of chitosan [2].*

Chitosan has three types of reactive functional groups, an amino/acetamido group as well as both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively. It is divided into three crystal types: α, β and γ type [6]. Chemical modifications of these groups have provided numerous useful materials in different fields of application [1]. Chitosan is insoluble in water, organic solvents and aqueous bases and it is soluble after stirring in acids (acetic, nitric, hydrochloric, perchloric and phosphoric,…) because the amino groups (pKa from 6,2 to 7,0) are completely protonated in acids with pKa smaller than 6,2 [1-2]. Chitosan has the ability to form films, to react with polyanions as well as to chelate and remove metal ions. The positive charge of chitosan confers to this polymer numerous and unique physiological and biological properties with great potential in a wide range of industries such as cosmetology, food, agriculture, biotechnology, pharmacology and medicine [7].

Chitosan is a major component of the shells of crustaceans and it is found commercially in the waste products of the marine food processing industry. It is extracted from fungal cell walls, from the exoskeleton of crustaceans and insect and it is commercially produced by alkaline deacetylation of chitin [4, 7-8].

3. Chitosan Oligosaccharides (COS)

Chitosan oligosaccharides have recently been produced by several methods, such as enzymatic and acidic hydrolysis. The molecular weights of COS are 10 kDa or less and are readily soluble in aqueous solutions and have lower viscosity under physiological conditions because of shorter chain lengths and free amino groups in D-glucosamine units, making COS perform valuable biological activities at the cellular or molecular level [9-10].

Many nonspecific enzymes, such as cellulases, lipases and proteases as well as chitosanases, have been used to prepare COS [10]. Hydrolysis of chitosan can progress by use of acid as hydrochloric acid, acid with electrolytes, nitrous acid, phosphoric acid, hydrofluoric acid, or oxidative reductive methods with hydrogen peroxide or persulfate. Other acids as lactic acid, trichloroacetic acid, formic acid, and acetic acid have also been studied for their degradative effect on chitosan. These treatments also result in the formation of secondary compounds that are difficult to remove [1]. Also, chemical hydrolysis has several disadvantages in terms of low production yields and a high risk of environmental pollution [9].

Chitosan oligosaccharides contain three types of reactive functional groups: an amino/acetamido group, a primary hydroxyl group, and a secondary hydroxyl group at the C-2, C-3, and C-6 positions, respectively. The amino contents, DD, and molecular weight are the main reasons for the differences between their structures and physicochemical properties, which are also correlated with their biological functions [9].

4. Medical Application of Chitosan and Chitosan Oligosaccharides (COS)

Chitosan and COS are considered one of the most valuable polymer for biomedical and pharmaceutical applications due to its biodegradability, biocompatibility, antimicrobial, non-toxicity, anti-tumor properties and others biological activities. Chitosan have hypo-cholesterolemic activity [11-12]. There are several hypotheses for cholesterol reduction by chitosan, the entrapment of the viscous polymer solution is suggested to reduce the absorption of fat and cholesterol in food. The absorption of chitosan on the surface of the emulsified lipid, forms a protective coating which may prevent the lipase or co-lipase from adsorbing the droplet surfaces and gaining access to the lipids [9]. The hypo-cholesterolemic activity of chitosan was higher when its DD was higher (90% deacetylated) at the same molecular weight, which might be due to the electrostatic attraction between chitosan and anionic substances such as fatty acids and bile acids. Additionally, chitosan significantly lowered serum total triglyceride (TG), total cholesterol (TC) and low-density-lipoprotein cholesterol (LDL-C) concentrations and elevated the high-density-lipoprotein cholesterol (HDL-C) level. The liver hepatic and lipoprotein lipase activities were also reduced by chitosan, which indicated that chitosan could regulate lipase activity, but also reduce serum lipid levels and liver-fat accumulation [10].

Several studies have shown that chitosan oligosaccharides (COS) have anti-obesity effects [13-14]. The COS significantly decreased lipid accumulation, a marker of adipogenesis, in a dose dependent manner. The low molecular mass COS (1- 3 kDa) were the most effective at inhibiting adipocyte differentiation. Moreover, mRNA expression levels of both CCAAT/enhancer-binding protein (C/EBP) α and peroxisome proliferator-activated receptor (PPAR) γ, the key adipogenic transcription factors, were markedly decreased by COS treatments. COS also significantly downregulated adipogenic marker proteins such as leptin, adiponectin, and resistin. COS plays a role as anti-obesity agents by inhibiting adipocyte differentiation mediated through the downregulated expression of adipogenic transcription factors and other specific genes [15].

Chitosan oligosaccharides participate in scavenging intracellular radicals and also suppressed NF-κB activation. COS protected Chang liver cells against oxidative damage induced by t-BHP via inhibiting production of ROS and lipid peroxidation and the elevation of the levels of antioxidant enzymes. COS contribute to attenuation of allergic reactions and COS with MW from 1 to 3 kDa possess the highest inhibitory effects on degranulation and cytokine generation of mast cells. The antitumor activity of COS was due to its cationic property exerted by amino groups, and the molecular weight also plays a major role for the antitumor activity [1]. The electrostatic interaction between the charges of anticancer products used, and charged functional residues existing on the internal components, and the tumor cell surface may be responsible for the antitumor effect of COS. Highly charged

COS derivatives reduce the viability of cancer cells, regardless of their positive or negative charge. Further studies by fluorescence microscopic observations and DNA fragmentation have revealed that necrosis was the main cause of the anticancer effect of highly charged COS [9].

In human hepatocellular carcinoma cell line, HepG2, COS inhibit cell proliferation, reduce the percentage of cells in S-phase, and decrease the rate of DNA synthesis in the cells. Analysis of expression of cell cycle-related genes revealed that p21 was upregulated, while proliferating cell nuclear antigen (PCNA), cyclin A, and cyclin dependent kinase (CDK)-2 were downregulated [16-17]. For colon cancer, COS exerts its chemopreventive effect against colon cancer by increasing quinine reductase (QR) and glutathione-S-transferase (GST) activities and glutathione (GSH) levels and by inhibiting ornithine decarboxylase (ODC) activity and cyclooxygenase-2 (COX-2) expression. Also COS pretreatment inhibited pro-inflammatory cytokine-mediated nitric oxide (NO) production, inducible NO synthase (iNOS) expression, and invasiveness of HT-29 cells[16].

The immunostimulant property of COS is also thought to be responsible for the antitumor activity. Chitosan oligomers were effective in enhancing the migratory activity of macrophages. COS enhance acquired immunity by accelerating T-cell differentiation to increase cytotoxicity and maintain T-cell activity. COS can stimulate the production of cytokines such as interleukin-1 (IL-1b) and tumour necrosis factor-alpha (TNF-α), while chitosan may bind with some substances involved in the migration of macrophages, leading to migration reduction of macrophages [9].

Chitosan has also been reported to prevent increases in blood pressure. A high-salt diet can raise blood pressure because Cl^- activates angiotensin-converting enzyme (ACE), while chitosan can bind Cl^- and remove it, preventing the blood pressure from rising [10]. Also, chitosan has wide spectrum of activity and high killing rate against Gram-positive and Gram-negative bacteria but lower toxicity toward mammalian cells. When chitosan was administered orally in mice, the LD50 was found to be in excess of 16 g/kg [1].

Bacterial lipopolysaccharide (LPS), one of the principal components of the outer membrane of Gram-negative bacteria, has been recognized as a key molecule in the pathogenesis of sepsis and septic shock. It is composed of O-antigen, core oligosaccharide and lipid A. Lipopolysaccharide, a potent activator of the immune system, elicits the production of pro-inflammatory mediators in immunocytes. Toll-like receptor 4 (TLR4) and myeloid differentiation factor (MD) 2 receptor complex is required for recognition and signaling of LPS. Chitosan oligosaccharides are potential inhibitive effector of LPS, inhibits binding of LPS to TLR4/MD-2 receptor complex, thus attenuate activation of mitogen-activated protein kinases (MAPKs) and decrease nuclear translocation of nuclear factor-κB (NF-κB). Chitosan oligosaccharides reduce the production of pro-inflammatory mediator, such as IL-1 and nitric oxide (NO) [18].

Chitosan antibacterial action was related to the presence of a polycationic structure below pH 6 [5]. Below its pKa (6,3-6,5), the amino group (C2 of chitosan glucosamine) is positively charged. This charge is able to interact with the negatively charged components at the surface of the bacterial cell walls. This binding or interaction leads to a rupture or leakage of proteins and intracellular constituents of the microorganism in the medium [19]. The physical state of chitosan and its MW are also of great importance in its action mode. It has been reported that the use of a low molecular weight (LMW) water soluble chitosan or nanoparticles that can penetrate the microbial cell wall, exhibit another form of antimicrobial activity, as they combine to DNA and inhibit mRNA synthesis and DNA transcription [5].

5. Agricultural Application

Chitosan has a wide variety of applications in agricultural [7]. Chitosan is naturally-occurring compounds that have potential in agriculture with regard to controlling plant diseases [20]. Chitosan effects on plant response were first characterized as an elicitor. It was shown to be able to activate plant defensive genes through the octadecanoid pathway [21]. This molecule was shown to display toxicity and inhibit fungal growth and development. They were also reported to be active against viruses, bacteria and other pests. Fragments from chitosan are known to have eliciting activities leading to a variety of defense responses in host plants in response to microbial infections, including the accumulation of phytoalexins, pathogen-related (PR) proteins and proteinase inhibitors, lignin synthesis, and callose formation [20]. Chitosan oligomers (DP3-6) had no eliciting activity, while higher chitosan oligomers, e. g., octamers, were efficient elicitors for inducing pisatin accumulation and inhibiting fungal growth. These results suggest that elicitor activities of chitosan oligomers are highly dependent on their polymerization and the presence of N-acetylglucosamine [10].

Chitosan can be used as a seed-coating agent to control *Agrotis ypsilon*, soybean pod borer, and soybean aphid. Coating based on chitosan was used as a feeding deterrent and for enhancing the germination and quality of soybean seeds. Chitosan coating had a significant effect on antifeeding against pests, also chitosan coating increased seed germination, plant growth, and soybean yield efficiently [22]. The chitosan seed treatment induced a decrease in disease severity and enhanced quantitative yield parameters, suggesting the possibility of the use of chitosan as a seed treatment in crop protection in order to improve the plant defense response [23].

Supplementation of hairy root cultures of *Brugmansia candida* with chitosan increase the content of root scopolamine and hyoscyamine, elicited a rapid deposition of the β-1,3-glucan, callose in the cell walls of *Petroselinum crispum*, enhance the production of hernandulcin, a minor constituent of the essential oil obtained from the aerial parts of *Lippia dulcis Trev*, increases the growth rates of roots and shoots of daikon radish (*Raphanus sativus L.*), enhances

growth factors in terms of the average values of flower-stem length, the number of growing leaves, including leaf width and length as well as the number of flowers per bush and increases the yield and marketability of soybean sprouts. Chitosan can reduce disease severity in orchids, by increasing the activity of PAL (phenylalanine ammonia-lyase) and PPO (polyphenol oxidase), increased biosynthesis of phenolic compounds or induced secondary metabolites and systemic acquired resistance. Also, increased disease resistance may be mediated in part via an increase in the concentrations of jasmonic acid, resistance to disease infections may also involve closure of stomata by ABA (abscissic acid), while chitosan-oligosaccharides advance flowering time and increased flower numbers of passion fruit (*Passiflora edulis Sims*) [3]. In addition, chitosan has been described as a plant antivirus [1].

6. Biotechnological Industries Application

Chitosan has a wide variety of applications in biotechnological industries [7]. Several derivatives of chitosan were shown to inhibit *E. coli*, *Staphylococcus aureus*, some *Bacillus* species, and several bacteria infecting fish. Fungicidal activity of chitosan has been documented against various species of fungi and oomycetes. Some of the derivatives also repressed spore formation. Chitosan is able to permeabilize the plasma membrane of *Neurospora crassa* and kills the cells in an energy-dependent manner. Twenty four derivatives of chitosan were shown to have significant insecticidal activity when administered at a rate of 5 $g \cdot kg^{-1}$ in an artificial diet. The most active derivative; N-(2-chloro-6-fluorobenzyl)chitosan; caused 100% mortality of larvae and its LC50 was estimated at 0.32 $g.kg^{-1}$. All synthesized derivatives highly inhibited larvae growth as compared to chitosan by 7% and the most active derivative was the O-(decanoyl)chitosan, with 64% growth inhibition after 5 days of feeding on the treated artificial diet [20].

Chitosan has also been investigated as a metal-recovering agent in industry and has been noted for its application as a film-forming agent in cosmetics, a dye binder for textiles, a strengthening additive in paper, and a hypolipidic material in diets [1]. Chitosan nanoparticles can be used as possible molecular transporters into plastids like the chloroplast. Interesting results were obtained by Wang et al.[24], who prepared QD-labeled Chitosan-DNA complexes to monitor nanoparticle-mediated genetic transformation of cultured cells of *Jatropha curcas*. The potential of use of chitosan for genetic transformation is suggested by its capability to form, through electrostatic interactions, a complex where DNA is protected from nuclease degradation [25].

7. Conclusion

Chitosan and chitosan oligosaccharides (COS) possess various biological activities. These activities depend largely on the physicochemical properties, including the degree of deacetylation (DD) and the degree of polymerisation (DP).

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