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APPLICATIONS AND ENVIRONMENTAL ASPECTS OF CHITIN AND CHITOSAN

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ABSTRACT

A survey on the properties of the natural nitrogen-containing polysaccharides chitin and chitosan is given. Outstanding features are the materials' mechanical and chemical properties which offer numerous, largely unexplored applications in technology, chemistry, medicine, and agriculture. Derivatives of chitin and chitosan are accessible by reactions of the hydroxy and amino groups with appropriate reagents. Various types of gels, membranes, and fibers, including polycationic and water-soluble materials, can be formed. Production of chitin and chitosan from waste crab shells involves environmentally safe processes. The polysaccharides are recycled in nature by enzymatic degradation and reuse of *N*-acetylglucosamine for biosynthesis and catabolism. Research deficits exist in economically competitive production technologies, construction of composite materials, and the ecological aspects of chemically modified chitosan.

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INTRODUCTION

Chitin is, after cellulose, the second most abundant polysaccharide on earth. It consists of β-1,4-linked glucosamine (GlcN) with a high degree of *N*-acetylation. The M_R of natural chitin is estimated to be in the range of 1–2 $\times$ 10^6 daltons. When the polysaccharide contains lower proportions of *N*-acetyl groups (i.e., GlcNAc units), it is named chitosan. The criteria for distinguishing between chitin and chitosan are the solubilities of the polymers in dilute aqueous acid: chitin is insoluble while chitosan forms viscous solutions. As a rule of thumb, the degree of *N*-acetylation (DA) of chitosan is ≤ 40. Chemical derivatives of chitin or chitosan are named by using the kind of functional group introduced as a prefix (e.g., carboxymethyl chitosan) or as a suffix (e.g., chitosan sulfate). A comprehensive treatise of general "chitinology" is available in Muzzarelli's unsurpassed classical textbook [1], while a more recent book deals more specifically with chitin chemistry [2]. The growing interest in chitin and chitosan is reflected by a series of International Chitin Conferences and the published proceedings [3–5].

OCCURRENCE AND RESOURCES

Chitin occurs as a structural component of the exoskeleton of insects and crustacea as well as in the cell wall of yeasts and fungi where its relative amounts are in the range of 30 to 60%. According to earlier estimates, the resources of chitin in marine organisms are in the order of 10^6 to 10^7 tons. The annual production of chitin in zooplankton alone is estimated to be several billion tons. Actually, as Yu et al. have stated in a pictorial formulation, there is a constant "rain" of chitin on the ocean floor [6]. Chitosan occurs naturally in several fungi, especially *Mucor* species.

FUNCTIONS IN NATURE

Chitin serves as a fibrous strengthening element in biological composite materials. Thus, it is nearly always associated with proteins which function as the matrix for enforcing reactions such as phenolic tanning in the exoskeleton of insects [7] and/or mineralization in the shell of crustaceae, some flies, and nacre (mother of pearl) [8].

BIOSYNTHESIS

The biosynthesis of chitin takes place in the membrane-bound protein complex chitin synthase. Chain elongation occurs by sequential transfer of GlcNAc from UDP-GlcNAc to the nonreducing end of the growing polymer. In contrast to the situation in arthropods, a detailed picture of chitin biosynthesis has been established in the yeast *Saccharomyces cerevisiae* [9]. A detailed description of chitin biosynthesis is beyond the scope of this article, and the reader is referred to some recent reviews [9, 10].

BIODEGRADATION

The natural pathway of chitin metabolism includes enzyme-catalyzed hydrolysis by chitinases. There are various forms of these enzymes which are usually grouped into endo- and exo-enzymes (see Fig. 1). The chitinases (EC 3.2.1.14) cleave the polysaccharide and higher chitooligosaccharides in a random fashion to give *N,N′*-diacetylchitobiose and *N,N′,N″*-triacetylchitotriose as the final products. There are also microbial chitinases that cut off chitobiose from the nonreducing end of oligosaccharides [11]. Further degradation of chitobiose takes place by *N*-acetylglucosaminidases (EC 3.2.1.30), formerly named chitobiases. The nomenclature of chitinases has been critically discussed by Muzzarelli [12]. Recently, a chitodextrinase was identified in the marine bacterium *Vibrio furnissii* by Bassler et al. [13]. This enzyme does not hydrolyze chitin, chitobiose, or chitotriose, but cleaves readily the tetra-, penta-, and hexamer, yielding exclusively the tri- and disaccharides. Most interestingly, the bacteria show chemotaxis toward chitooligosaccharides.

Another pathway of chitin turnover occurs in fungi which deacetylate chitin to chitosan by the action of chitin deacetylase (EC 3.5.1.41) [14, 15]. Undoubtedly, this reaction plays an important role in the biosynthesis of chitosan. Chitosan itself is degraded by chitosanes (EC 3.2.1.99) that occur in microorganisms and, after elicitation by chitooligosaccharides, as phytoalexins in plants.

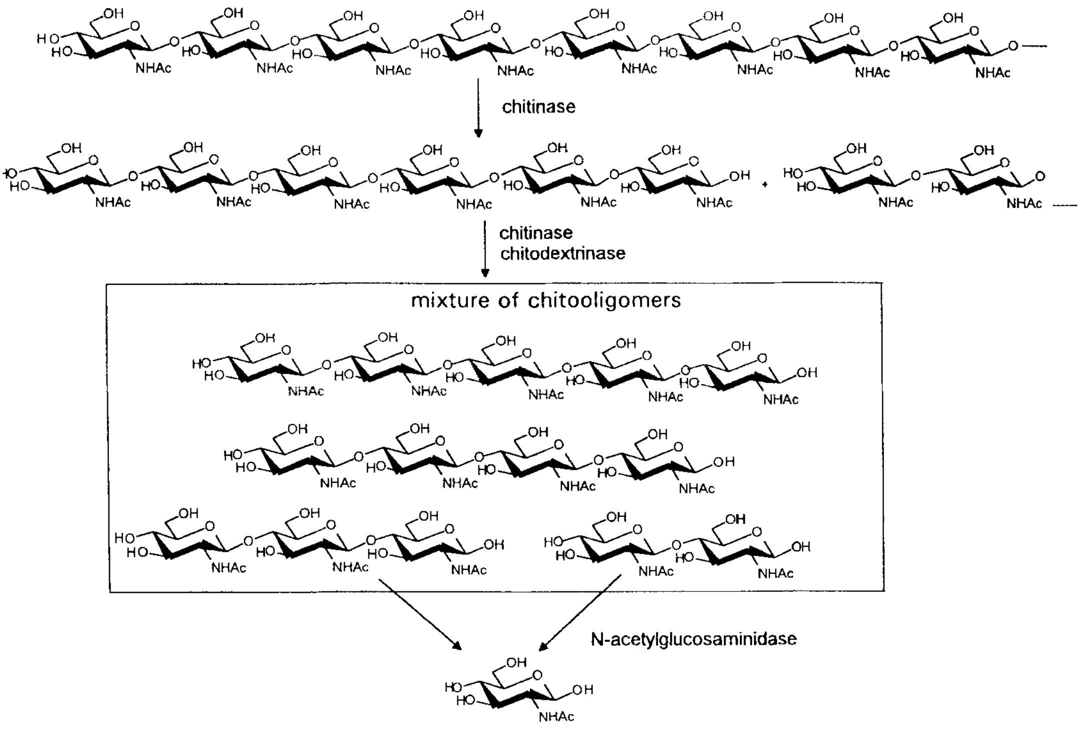

FIG. 1. Enzymatic degradation of chitin.

Chitin and chitosan are also degraded by various lysozymes (EC 3.2.1.17) which are widely distributed in plants and animals. A residual number of acetyl groups (DA $\geq$ 16) and the free hydroxy group at C-3 of the sugar units are required in order to observe reasonable rates of hydrolysis [16].

PRODUCTION OF CHITIN AND CHITOSAN

Production of chitin by chemical synthesis is technically not possible though small quantities of defined chitooligomers are principally accessible by appropriate procedures. Also, biotechnological production of chitin is presently not economically attractive. The primary sources of chitin are therefore the tremendous amounts of waste crab and krill shells from the fishing industry, while chitosan is usually produced by chemical deacetylation of chitin, though fermentation processes are also possible. In order to obtain rather crude chitin, crab shells are decalcified at ambient temperature by means of dilute aqueous hydrochloric acid, followed by extensive washing with water and deproteination with dilute sodium hydroxide. Pigments, such as carotenoids, may be extracted with appropriate organic solvents. Further purification is achieved by adding an ice-cold solution of chitin in 12 N hydrochloric acid slowly to a vigorously stirred large volume of water. This procedure may be repeated several times. For chitosan production, crude chitin is deacetylated with 40–50% sodium hydroxide at 110–115°C. All procedures involving acid or heat lead to a more or less extensive depolymerization which must be controlled by temperature, reaction times, and proper reagent compositions. Commercial preparations have M_r values between 10^4 and 10^5 daltons, though higher molecular weight materials are available also. A comprehensive and critical evaluation of chitin and chitosan processing was given by Muzzarelli [1].

The procedures employed in chitin/chitosan production require relatively harmless chemicals. The acetyl groups are recovered in the form of sodium acetate. Calcium carbonate, which is another major component of crab shells, is converted to calcium oxide and sodium carbonate. In addition, pigments such as astaxanthin may be recovered as high value side products. Thus, from the ecological view, chitin and chitosan production bears fewer problems than the production of cellulose. The market value of chitin is in the order of DM 10 to DM 300 per kg, depending on the product specification. High purity, pyrogen-free chitosan for medical applications may cost as much as DM 70,000 per kg.

MATERIALS PROPERTIES

The quaternary structure of chitin is similar to the structure of cellulose. The chitin of insect and crustacean cuticle occurs in the form of microfibrils typically 10–25 nm in diameter and 2–3 μm in length. Three natural modifications are known which differ in the orientation of the polysaccharide chains within the microfibrillae, namely, α- (antiparallel), β- (two parallel, one antiparallel), and γ- (random orientation) chitin. The most abundant form is α-chitin. Young's modulus of elasticity of chitin fibrils of locust tendon (E = 70–90 GPa) is comparable with that of gold.

CHEMICAL PROPERTIES OF CHITIN AND CHITOSAN

Chitin may be converted by use of proper reagents into a number of *O*-alkyl and *O*-acyl derivatives, as has been summarized in a recent review by Hirano [17] (see Fig. 2). Chitosan behaves as a moderately basic ($pK_a = 6.3$; cf. GlcN: $pK_a = 7.47$) cationic polyelectrolyte forming salts with acids. This is definitely an advantage in comparison with cellulose which, in order to achieve ion-exchange properties, has to be chemically converted into the appropriate amino group-containing derivatives. In addition, the presence of the primary amino group in chitosan offers further possibilities for modifications such as *N*-acylation, *N*-alkylation, and *N*-alkylidenation (see Fig. 2). Controlled degradation of chitin either by enzymatic or by acid-catalyzed hydrolysis gives chito-oligosaccharides which, besides possessing interesting biological activities, are valuable starting materials for the synthesis of oligosaccharides and other derivatives [18].

USES OF CHITIN AND CHITOSAN

The worldwide research activities going on in the chitin field are reflected by the number of patents, including patent applications, and other publications. Table 1 gives a survey of the results of a search in *Chemical Abstracts* on chitin-related sources which were recorded in the year 1993. Though this gives only a very rough picture which does not tell anything about the subjects of research, it is immediately clear that most work on chitin and chitosan is performed in Japan. Actually, this correlates with the annual production rate of presently ca. 3000 tons of chitin in Japan. The major uses of chitin and chitosan are in the food and cosmetics industries (S. Hirano, personal communication, May 1993). The rationale for applications in those areas are the formidable gel-forming properties as well as the moistening and stiffening effect of chitosan and certain derivatives, such as hydroxyalkylchitosans which are essentially nontoxic. Other applications which are based on the binding and flocculation of proteins and on the chelation of various heavy metal ions (see Table 2) are realized in the treatment of wastewaters. A summary of chitin and chitosan uses in Japan is given in Table 3 [19].

POTENTIAL FOR FURTHER USES

The fascinating properties of chitin, chitosan, and derivatives have initiated numerous research activities on the industrial as well as on the academic level, which result in an ever-increasing amount of new potential applications. It is impossible here to give a comprehensive treatment of the subject, and reference is given to the monographs [1, 2] and conference proceedings [3–5], as well as compilation of data published recently [20]. Just a few highlights will be mentioned.

Membranes formed from chitosan are less permeable for oxygen, nitrogen, and carbon dioxide than cellulose acetate membranes. This, in combination with mechanical strength, offers superior wrapping and packing materials in the food industry. Chitosan can be used as a film-forming product for fruit and seed conservation. The procedure is rather simple: the seeds or fruits are dipped into a 2–3%

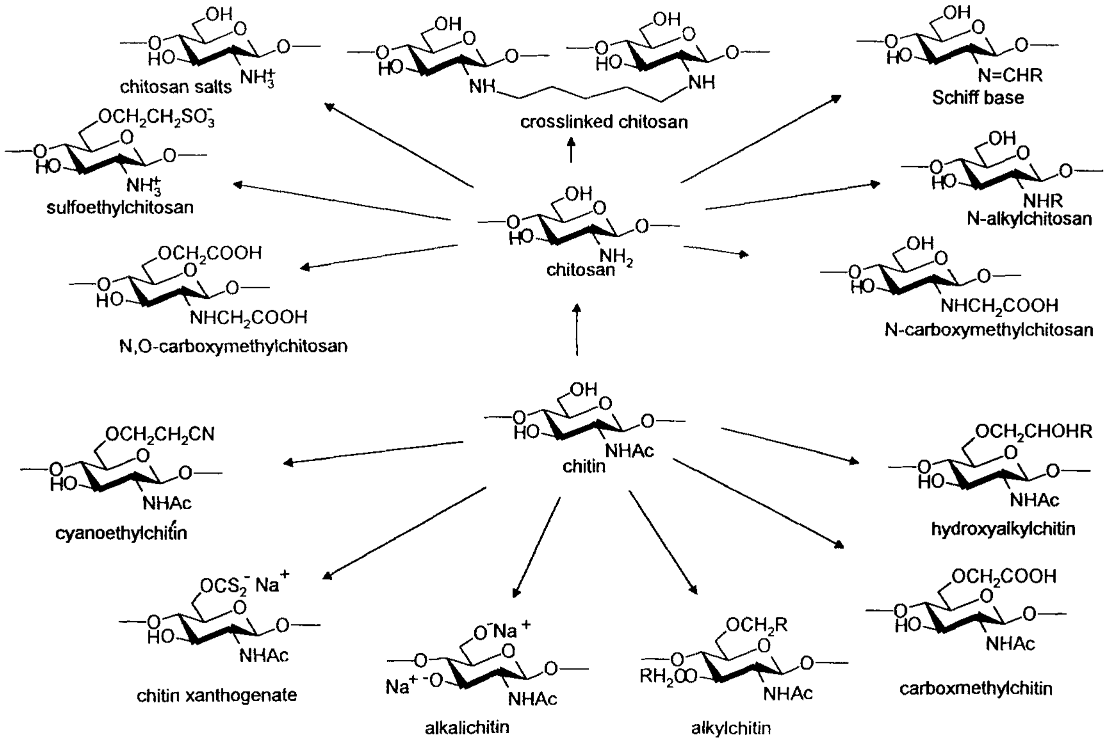

FIG. 2. Chemical derivatization of chitin and chitosan.

TABLE 1. Worldwide Research Activities 1993 in the Fields of Chitin and Chitosan as Reflected by the Number of Publications According to *Chemical Abstracts* Citations

Country	Patents	Other publications	Total
Japan	126	88	214
USA	25	59	84
UK	4	19	23
Germany	7	15	22
France	9	11	20
Canada	2	13	15
People's Republic of China	2	13	15
Russia	2	12	14
Italy	5	8	13
India		9	9
South Korea		7	7
Israel	2	4	6
Belgium		5	5
Netherlands		5	5
Australia	1	3	4
Cuba		4	4
Poland		4	4
Spain		4	4
Switzerland	2	2	4
Denmark		3	3
Greece		3	3
Sweden	1	2	3
Turkey		3	3
Brazil		2	2
Singapore		2	2
Slovenia		2	2
Argentina		1	1
Chile		1	1
Czech Republic		1	1
Egypt		1	1
Indonesia		1	1
New Zealand		1	1
Norway		1	1
Romania		1	1
Slovakia		1	1
Total	190	314	501

TABLE 2. Compilation of Data from Various References on the Binding Capacities of Polymers for Metal Ions (meq·g^{-1})[a]

Polymer	Method[b]	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}	Mn^{2+}	Ni^{2+}	Cu^{2+}	Cd^{2+}	Pb^{2+}	Zn^{2+}	Hg^{2+}	Co^{2+}	Cr^{2+}	Fe^{2+}	Ag^{+}	Au^{3+}	Pt^{4+}	Pd^{2+}
Chitosan 45%DA	A	0.3	0.8	1.5	1.1	1.1	3.5	5.3	6.5		5.5								
Chitosan 97%DA	A	0.5	0.4	0.6	0.8	0.5	2.3	4.8	4.9		3.2								
Chitosan	B					1.44	3.15	3.12	2.78	3.97	3.70	5.60	2.47	0.46	1.18	3.26	5.84	4.52	6.28
p-Aminostyrene	B						0.02	1.31	0.10	0.17	0.52	5.70	0.07	0.03	0.05	1.98	5.75	3.82	0.87
6-*O*-CM-chitin[c]	C	2.08	2.50	2.12	2.15	2.22	2.22		2.42	2.13									
3,6-Di-*O*-CM-chitin[d]	C	4.0	3,3[a]	2.8	10.1↓	.34	4.1↓	3.1↓	9.2↓	8.5↓									

[a] ↓: Gel formation or precipitation.

[b] Method: A: 0.04 M metal ion, pH 7.4, 20 h, 30°C; B: 1 g polymer, 0.02–0.4 mM metal chloride or nitrate, 24 h; C: not specified.

[c] 6-*O*-CM-chitin: 6-O-carboxymethylchitin.

[d] 3,6-Di-*O*-CM-chitin: 3,6-di-*O*-carboxymethylchitin.

[e] Ca^{2+} can be desorbed by means of EDTA.

TABLE 3. Practical Applications of Chitin-Derived Products in Japan (1988) [19]

Area	Chitin-based product	Application
Medicine	Chitin	Wound dressings
	Chitin fiber	Wound sutures
	Chitosan-collagen composite	Artificial skin
Cosmetics	Chitosan	Creams and other skin-care products
	Hydroxyalkyl chitosan	
	Liquid Chitin	Hair stiffeners
Technical applications	Chitin membranes	Loudspeakers
	Depolymerized chitosan	Adsorption of endotoxins and nucleic acids
	Schiff bases	Matrix for electron microscopy
Environmental	Chitosan and chitosan salts	Flocculant for purification of protein-containing wastewaters
Agriculture	Chitosan	Compostation accelerator
Food technology	Chitosan	Food additives
Biotechnology	Chitosan	Porous particles for bioreactors Immobilization of cells and enzymes
Chemistry	Chitosan	Carrier for catalysts
	Alkali chitin	Intermediate for the synthesis of chitin derivatives

solution of chitosan lactate. The conserving effect is due in part to the antimicrobial properties of the polysaccharide.

The presence of hydroxy and amino groups of chitosan allows for a wide variety of chemical modifications including reactions with crosslinking agents. Thus, porous beads are made from crosslinked chitosan that are excellent carrier supports for the immobilization of enzymes and cells in biotechnology. Porous beads are also required for chromatographic stationary phases. A product, Chitopearl, is commercially available in Japan. It has been used for the separation of proteins, peptides, and oligosaccharides.

Numerous medical applications have been suggested. Since chitin and chitosan can be degraded by lysozyme, they are useful as drug carriers and slow release formulations. Chitooligosaccharides exert antimicrobial and macrophage activating properties which are manifested in remarkable wound-healing acceleration by chitosan-based wound dressings. In fact, a chitosan-based product is registered and produced in Poland for applications in veterinary medicine.

Glucosamine, as the most abundant and cheapest amino sugar, is an excellent source from the chiral pool that can easily be used as starting material for the preparation of high value fine chemicals and enantiopure building blocks, such as amino alcohols, aldehydes, and acids, which thus are ultimatively derived from chitin [21]. The application of chitin as a source for the production of precious natural products has not yet been fully explored.

The design of chitin-based composite materials is still in its infancy. Most of the work done so far has focused on chitin/chitosan composites with cellulose. Chitosan membranes can be subjected to cold stretching, and fibers may be spun into a coagulation bath. Reacetylation yields chitin membranes and fibers. Numerous composite materials could principally be constructed by imitation of various natural biomaterials. This will certainly be one of many highly attractive research areas in the future.

RECYCLING

Any process of recycling should avoid consuming thermal energy from fossil resources for the degradation or transformation of materials, in particular when they have been generated previously at the cost of such resources. Recycling in nature always occurs in metabolic processes at moderate temperatures, converting one form of chemical energy into another, maintaining a strictly regulated balance between heat production in catabolic processes and high energy intermediates for biosynthetic requirements in anabolism. In the life cycle of arthropods, most of the huge amounts of chitin are recycled by enzymatic digestion during the molting process while the remainders are utilized after individual death by microorganisms for catabolism to GlcNAc-6-phosphate, fructose-6-phosphate, acetate, and ammonia and thus, ultimately, combustion to carbon dioxide and water.

Procedures for the recycling of chitosan membranes, wrappings, etc. have not yet been investigated. Since, in general, it is probably neither of economical nor of ecological advantage to recover intact chitin from composite materials, recycling of chitin or chitosan itself in an approach similar to that pursued with paper or with conventional plastics, i.e., shredding and remolding, appears impractical. Thus, the production of chitin will continue to rely on waste from seafood processing while the disposal of technical waste products can reasonably be envisaged to follow the way of microbial degradation, i.e., composting.

Rather little is known about the biodegradation of composite materials made from chitin and chemically modified chitosan. A recent study allows the conclusion that partially *N*-acylated derivatives of chitosan, *O*-carboxymethyl, and *O*-glycolylchitosans are degraded by the chitosanase of *Bacillus pumilus* [22]. Furthermore, there are a variety of fungi capable of using polyphenolic compounds as a source of carbon in peroxidative pathways. Thus, the chances for environmentally safe disposal of chitinous waste materials are excellent. However, this topic clearly needs further detailed research.

CONCLUSIONS AND FUTURE PROSPECTS

The majority of the applications are still in the developing stage. Major obstacles for large-scale technical applications of high quality chitin/chitosan are the high prices of the polymers and the difficulties in preparing uniformly reproducible charges in bulk quantities from various marine organisms. Issues for future research are:

- Development of economically competitive production processes
- Development of technical processes for the preparation of chitin derivatives and composite materials
- Investigations of the biodegradation of chemically modified chitosan

The situation of a rapidly rising demand for chitin and chitin based products may arise in the foreseeable future. Care must be taken in the exploration of natural resources, and concern about ecological questions could arise once chitin is produced from crabs and other marine organisms that are caught for the purpose of chitin production only. Therefore, the planning of an eventually large-scale utilization of chitin must also include thoughts about a possible decrease of biodiversity and shifts in the ecological balance in the marine environment.

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