

Preparation and Properties of Glucosamine and Carboxymethylchitin from Shrimp Shell

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ABSTRACT: Export of processed and frozen shrimp products is the backbone of seafood industries. But, shrimp processing industries give rise to a large amount of waste materials. These waste products generate serious pollution hazards. A wise method to minimize environmental threats from prawn processing waste is to convert them in to useful products. Shrimp shell waste can be economically converted to chitin, a mucopolysaccharide. This marine polysaccharide and its derivatives hold a major part in our lives as medicines, cosmetics, textiles, paper, food and other branches of industry because of their unique nature in properties such

as low toxicity, biocompatibility, hydrophobicity, etc. Hydrolysis of chitin yields a value added product, Glucosamine. Carboxymethylchitin is another derivative of chitin, prepared by the carboxymethylation reaction. This review article is an attempt to give an idea about the preparation, properties, and applications of the derivatives of chitin viz., glucosamine and carboxymethylchitin. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 107: 280–285, 2008

Key words: chitin; carboxymethylchitin; biopolymers; polysaccharides; nanotechnology

INTRODUCTION

Seafood holds a very unique position in our daily menu. It is peculiar with high nutritional value and easy digestibility. But major curse of seafood industry is the large amount of waste materials, which are highly putrescible and produce serious health hazards. Nowadays, environmental regulations are very strict; so dumping of wastes questions the sustainability of seafood processing plants. A better way to minimize the pollution is the conversion of these waste materials into useful products. Since marine organisms are the sources of many useful bioactive substances,¹ a lot of ecofriendly technologies have now come into practice to derive beneficial by-products from processing wastes. Chitin is a value added product made from shrimp shell. Glucosamine and carboxymethylchitin are prepared from chitin.

CHITIN

Chitin (Fig. 1)² is a mucopolysaccharide. It is the second most abundant biopolymer next to cellulose. Chitin was first isolated by Braconnot in 1811 from mushrooms and was named “fungine.” A material identical to fungine was found in insects in 1821 by Odier and he named it as “chitine.”³

Chitin is a colourless, hard substance found in the shells of cephalopods and in the internal structures of other invertebrates. Major sources of chitin are shellfish, krill, clams, oysters, squid, and insects.⁴ Chitin is found in the cell walls of some fungi, molds, and yeasts also.⁵ Zygometes also contain chitin.⁶ Fresh water bryozoans are able to produce colonies whose envelope is made up of chitin and protein in a noncalcified form allowing easy extraction of chitin.⁷ Some sources of chitin are given in Table I.³

Chitin is found not only in animals, but also found as cell wall material in plants. Amongst several sources, the exoskeleton of crustaceans consists of 15–20% chitin by dry weight. Chitin found in nature is a renewable bioresource. A gigantic amount of chitin is processed as solid waste from seafood processing industry.^{8,9} Chitin represents 14–27% and 13–15% of the dry weight of shrimp and crab processing wastes, respectively.¹⁰ Proximate composition of shrimp shell and crab shell were given in Table II.¹¹

Chemically chitin is a linear polymer of *N*-acetyl D-glucosamine.¹² Its three-dimensional α -helical configuration is stabilized by intramolecular hydrogen bonding¹³ and is biodegradable in behavior. The natural degradation of chitin presents an important feature, not only in the global recycling of carbon and nitrogen sources, but also in the production of useful chemical reagents.¹⁴ Chemically, chitin is identical to cellulose; but secondary hydroxyl group on the alpha carbon atom of the cellulose molecule is substituted with acetoamide groups. The enzyme chitinase can efficiently degrade chitin.¹⁵ A gram-nega-

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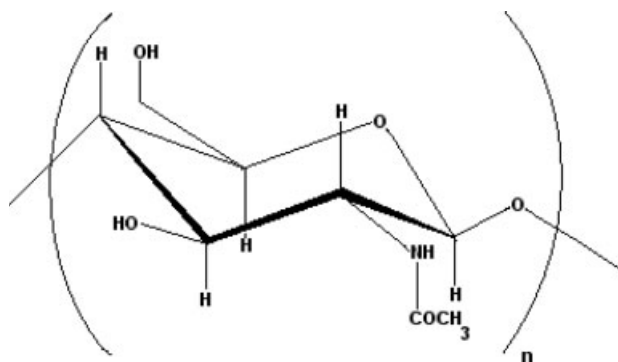


Figure 1 Structure of chitin.²

tive soil bacterium *Serratia marcescens* is also capable of degrading chitin.¹⁶ When this bacterium is growing in chitin medium, chitin binding protein, CBP21, is the major proteins secreted along with the enzyme chitinase.¹⁷

In nature chitin occurs in two major polymorphic forms: α -chitin and β -chitin. α -chitin is characterized by antiparallel and β -chitin is by parallel arrangement of polymer chains.¹⁸ Chitin obtained from prawn shell is α -chitin. β -chitin can be observed in squid pen. These two forms vary in the orientation of the molecular structure.

Chitin synthetases play an important role in cell wall biosynthesis. These enzymes are located in the plasma membrane fraction of cell homogenates or protoplast lysates where the enzyme is accessible to substrates and effectors from cytoplasm. As an integral protein spanning the membrane it produces the growing chitin chain.¹⁹ Chitin synthetases are not only the regulator of chitin synthesis. The formation of glucosamine from fructose 6-phosphate by transamination is also a point of control.²⁰ Chitosomes, the intracellular microvesicles, probably serve as conveyors of chitin synthetases zymogen from its point of synthesis to the cell surface where chitin microfibril assembly takes place. Trypsin or specific proteases are activating factors.²¹ To renew their exoskeletons, arthropods and insects possess enzyme systems that

enable them to recycle most of the chitin in their old integument and build a new one. This process occurs suddenly and rapidly. Enzymes in the moulting fluid can degrade chitin to *N*-acetyl glucosamine. The moulting hormone ecdysone is involved in the control of the synthesis and catabolism of chitin.¹⁹

Chitin is hydrophobic and is insoluble in water and in most of the organic solvents.²² Chitin is a polymer with versatile applications. It is applicable to different fields like chromatography, paper, textiles photography, food and nutrition, agriculture, pharmaceutical, etc.²³ Research has shown that chitin is nontoxic and nonallergenic, so the body does not reject this compound as foreign invader.²⁴ Polymers suitable for making both hard and soft contact lenses can be made from chitin. Since it is highly permeable to oxygen, chitin lens could be worn for long periods.²⁵ Chitin was found effective against ethanol-HCl induced peptic ulcer in rats.²⁶ Low solubility in aqueous solutions hinders its use in textile industry.²⁷ Chitin grabbed more commercial interest because of its high percentage of nitrogen (6.89%) content compared to synthetically substituted cellulose (1.25%).²⁸ Enzymatically degraded chitin can serve as a matrix for the controlled release of drugs, especially in the eye.²⁵ Chitin can be modified to obtain controlled amphiphilic polysaccharides, which may be either cationic (chitosan) or anionic (carboxymethylchitin).²⁹

GLUCOSAMINE

Glucosamine is a nitrogen containing sugar found in most of the tissues in our body. Unlike other forms of sugar in the body, amino sugars are components of carbohydrates that are incorporated into the structure of body tissues rather than being used as a source of energy. Glucosamine is the natural component of glycoproteins found in connective tissues and gastrointestinal mucosal membranes.³⁰ This monosaccharide is involved in the formation of nails, tendons, skin, eyes, bones, ligaments, and heart valves. It also plays a role in the mucus secretions of the respiratory and urinary tracts. It is incorporated in the biosynthesis of glycosaminoglycans and proteoglycans, which are essential for the extracellular

TABLE I
Annual Availability (Global) of Chitinaceous Materials

Source	Quantity harvested (10 ³ tonnes)	Chitinous waste (10 ³ tonnes)	Chitin potential (10 ³ tonnes)
Shell fish	1700	468	39
Krill (potential landing)	18,200	3640	56
Clam/oysters	1390	521	22
Squid	660	99	1
Fungi	790	790	32
Total	22,740	5118	150

TABLE II
Proximate Composition of Shrimp Shell and Crab

Parameter	Shrimp shell (%)	Crab (%)
Moisture	75–80	70
Ash (dry basis)	30–35	45–50
Protein (dry basis)	35–40	30–35
Chitin (dry basis)	15–20	13–15
Fat (dry basis)	3–5	1.0–1.5

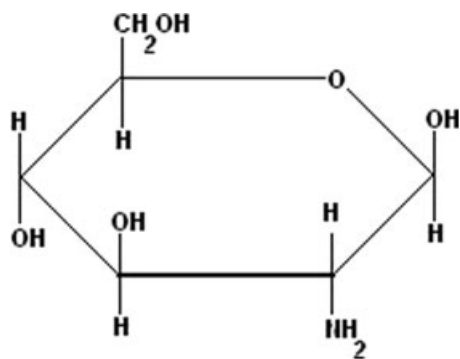


Figure 2 Structure of β -D-glucosamine.³⁷

matrix of connective tissues. Glucosamine is a nutritional supplement and therefore the authority of the Food and Drug Administration to regulate its use is now severely limited.³¹ There have been relatively few trials of glucosamine use in humans and those that have been published are mostly studies that examined the efficacy of glucosamine in the relief of joint pain.³² Glucosamine is available as an over-the-counter oral supplement, has few advert side effects and is relatively inexpensive. Glucosamine is a precursor of the disaccharide unit of glycosaminoglycans which are the building blocks of the articular cartilage, the proteoglycans.^{33,34} However, the ability to synthesize glucosamine in the body decreases with age. This in turn incapacitates the generation of proteoglycan and it is known that this incapacitation results in senile osteoarthritis.³⁵ Therefore, glucosamine caught interest as a useful substance for the prevention and treatment of osteoarthritis. Several clinical studies have been reported that glucosamine works better in reducing the symptoms of osteoarthritis.³⁶

Exoskeleton of marine organisms is the main source of glucosamine. It is also found in hyaluronic acid, a compound responsible for the lubricating and shock-absorbing properties of synovial fluid. Glucosamine is synthesized in chondrocytes in the body from glucose and glutamine. Commercial source of glucosamine is chitin.

Glucosamine is an aminomonosaccharide, i.e., an amino group is attached to a glucose ring (Fig. 2).³⁷ Thus comes the name glucose-amine. Chemically glucosamine is 2-amino-2-deoxy- α -D-glucose.

Preparation

Glucosamine is a monosaccharide generated by the hydrolysis of chitin with concentrated acids under drastic conditions.³⁸ Chitin can be hydrolysed to glucosamine by adding concentrated hydrochloric acid and warming until the solution no longer gives opa-

lescence on dilution with water. The excess acid can be distilled off under vacuum. The crude glucosamine is diluted with water and clarified with activated charcoal. The solution is filtered and evaporated under vacuum. The crude glucosamine coming as the residue can be separated from the mother liquor by adding alcohol and recrystallised.

Properties

Glucosamine is a colourless and odourless substance with sweet taste. It is crystalline in nature. It is having acidic pH, ranging from 3.5–4.5 and has density of 0.75 g/mL. Specific rotation of glucosamine is 70° and is readily soluble in water. When heated above 80°C , it undergoes Maillard reaction and its colour gets faded. On further heating, glucosamine will be charred. Glucosamine has anti-inflammatory and antioxidant actions.³⁰ It blocks the generation of superoxide free radicals by macrophages. Glucosamine is well tolerated. No severe side effects were reported for it.³⁶ Glucosamine is hexosamine component of keratan sulfate and heparin sulfate.³⁹ Glucosamine can increase proteoglycan synthesis,⁴⁰ which is a fundamental component found in articular cartilage. Proteoglycans synthesized in the presence of glucosamine had smaller glycosaminoglycan chains with a corresponding decrease in lipoprotein retention. This property makes it useful against atherosclerosis.⁴¹ Glucosamine inhibits the cartilage-destructive enzyme collagenase. Glucosamine helps in the synthesis of cartilage by increasing key components of cartilage such as glycosaminoglycans. Various reports confirmed that diabetes patients could also consume Glucosamine, which will not increase blood glucose level.⁴² Glucosamine appears to undergo a significant first-pass effect in the liver, which metabolizes a significant proportion of the dose to CO_2 , water, and urea.⁴³

Applications

Setnikar et al.^{43–45} have published several reports on the pharmacokinetics of glucosamine in rats, dogs, and humans. In both humans and rats, oral administration of radio labeled glucosamine results in absorption of more than 90% of the ingested dose.⁴¹

Glucosamine is found largely in cartilage and plays an important role in its health and resiliency. As age increases, we tend to lose some of the glucosamine and other substances in cartilage. This can lead to thinning of cartilage and the onset and progression of osteoarthritis. According to Braham,⁴⁶ glucosamine supplementation can provide some degree of pain relief and improved function in

persons who experience regular knee pain, which may be caused by prior cartilage injury or osteoarthritis. Since glucosamine can increase the synthesis of proteoglycans and glucosaminoglycans, which are necessary ingredients of connective tissue, it is proven to be effective against osteoarthritis.^{47,36} Canapp et al.⁴⁸ reported that glucosamine was found beneficial against acute synovitis. Glucosamine was proved to be effective against toxic liver damage because of its antioxidant and membrane stabilizing properties.⁴⁹ Glucosamine at concentration of certain range could kill tumor cells without influencing normal cells.⁵⁰ Therefore, it is postulated that combination of glucosamine with membrane-active drugs may have the potential to kill tumor cells, especially neuro-oncology.⁵¹

Every year about 20,000 people die from using nonsteroidal anti-inflammatory drugs (NSAIDs). The combination of glucosamine and NSAIDs may reduce the doses needed for anti-inflammatory activity as well as the side effects associated with these NSAID drugs.⁵² Dettmer⁵³ examined the effects of simultaneous administration of glucosamine and chondroitin sulfate on osteoarthritis. This study demonstrated greater effectiveness of this combination therapy. In combination with chondroitin sulfate, it can build blocks for cartilage, upregulate chondrocyte and reduce the extent of cartilage degradation.⁵⁴ Santhosh et al.⁵⁵ reported the antiulcerogenic property of glucosamine in experimental rats.

CARBOXYMETHYLCHITIN

Carboxymethylchitin is a water-soluble derivative of chitin.⁵⁶ Its property of solubility in water makes it unique for different applications. On carboxymethylation reaction, chitin yields carboxymethylchitin.

During carboxymethylation, the hydroxyl group of chitin is substituted by carboxymethyl group. Carboxymethylchitin is a polymer of carboxymethyl *N*-acetyl D-glucosamine with β (1–4) linkage (Fig. 3).⁵⁷

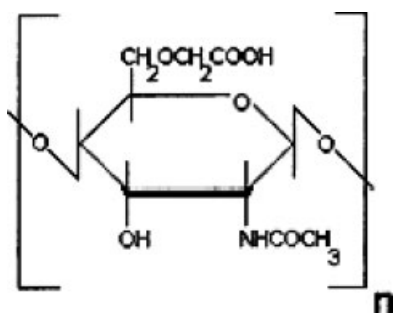


Figure 3 Structure of carboxymethylchitin.⁵⁷

TABLE III
Yield of Carboxymethylchitin Prepared at Different Alkali Concentrations and Temperature

Percentage of NaOH in alkali chitin	Yield in percentage	
	Uncontrolled temperature	Controlled temperature
45	36.7	37.4
50	49.8	44.7
55	56.5	52.0
60	69.6	65.8

Preparation⁵⁷

Chitin is the commercial source of carboxymethylchitin. Chitin was mixed thoroughly with sodium hydroxide in a ratio of 1:4 and stored for 1 h in ice. Afterwards, it was kept at -20°C for 12 h. This is alkali chitin. Alkali chitin was mixed with 200 mL isopropanol and then monochloroacetic acid was added at short intervals and stirred well till the whole mixture become neutral. This neutral viscous solution was dissolved in plenty of water and sufficient amount of acetone was added to this aqueous solution. Then, carboxymethylchitin was precipitated. The precipitated mass was then redissolved in distilled water. This solution was purified by using a dialysis membrane and freeze dried to get good quality carboxymethylchitin

Properties

Carboxymethylchitin is a polymer having molecular weight in the range of $1.07 \times 10^6 - 4.8 \times 10^6$ Da. Its viscosity comes around 2000 cp and degree of deacetylation is of about 45%. Sini et al.⁵⁷ reported that the strength of NaOH and processing temperature, during preparation, affects the yield of carboxymethylchitin (Table III)

Carboxymethylchitin is hygroscopic in nature. It is a colourless substance. It is readily soluble in water and insoluble in organic solvents. Ragnhild et al.⁵⁸ showed that the biodegradability of this polymer depended strongly on the degree of deacetylation and the substitution site. The biodegradability properties of carboxymethylchitin could be controlled by treatment involving vacuum-heating and gamma irradiation.⁵⁹

Applications

Carboxymethylchitin is having profound versatile applications. Carboxymethylchitin is popularly used in cosmetic products as smoothener, moisturizer, cleaner for face skin conditioning, and cell activator.⁶⁰ Carboxymethylchitin is extensively used in wound dressing.⁶¹ For wound dressing, this polymer

must be crosslinked to prolong its dimensional integrity during use. Tokura et al.⁶² used carboxymethylchitin as a drug carrier of sustained release for the subcutaneous injection. One important characteristic feature of carboxymethylchitin is that it is soluble not only in acid media but at any pH range.⁶³ This unique property makes carboxymethylchitin different from other derivatives of chitin. When chitosan is made into account, it is soluble in acidic pH only. Solubility of carboxymethylchitin at any pH makes it advantageous to use in food products and cosmetics. Carboxymethylchitin is used to preserve fruits also.⁶⁴ Uda et al.⁶⁵ have reported that a composite of hydroxyapatite and carboxymethylchitin can be used as an injectable material for facial augmentation. Sodium salts of carboxymethylchitin have been found usable as an absorbent for removing copper from aqueous solutions. An interesting application of carboxymethylchitin was reported in the preparation of artificial red blood cells.⁶⁶ Macrophages are activated to various extents by chitin derivatives. Accumulation of carboxymethyl chitin takes place in granulocytes and macrophages. These polysaccharides activate complement in analogy to zymosan.⁶⁷ Lazarova et al.⁶⁸ have reported that carboxymethylchitin–glucan enriched diet exhibits protective effect against oxidative DNA damage. Carboxymethylchitin has the property to increase the anti-inflammatory activity of the enzyme, super oxide dismutase.⁶⁹ According to Shi et al.,⁷⁰ iron-alginate-carboxymethylchitin bead could be a suitable polymeric carrier for site-specific protein drug delivery in the intestine. Biocompatible nanoparticles with an average diameter of around 200 nm were formed by mixing hydrolysates of chitosan and carboxymethylcellulose,⁷¹ which is very much similar in structure and properties to carboxymethylchitin. So, carboxymethylchitin offers a promising future in the area of nanotechnology.

CONCLUSION

Nowadays, the importance of biodegradable compounds is increasing in the emerging consciousness of over polluted world. Here comes the significance of the compounds like chitin, glucosamine, and carboxymethyl chitin. Since these compounds are prepared from shell waste, the threat of environmental pollution due to the dump of waste is minimized and they are biodegradable also. These compounds are closer to nature so side effects will be less for their pharmaceutical applications. Still a major part of their applicability is unrevealed. Since the outer shell of the crustaceans is very hard to break, which is mainly chitin, research should be done to make fibers (threads) of chitin. It can be a natural substi-

tute for polyesters like nylon. The source of chitin is also very cheap, shell waste. Studies should be conducted for the application of carboxymethylchitin in nanotechnology.

In summary chitin, carboxymethylchitin, and glucosamine give intelligent contributions to the world of science. Their cheap source and biocompatibility enhances their peculiarity. Anyway, these biomolecules of today can become the necessity of tomorrow.

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