CHAPTER I

INTRODUCTION

1.1 Chitin chemistry

Chitin is the second most abundant natural biopolymer in the world, behind only cellulose. Chitin has an inherent property of being environmentally friendly and easily degradable. It is also the most abundant naturally occurring polysaccharide that contains amino sugars, which possess many physical, chemical and biological properties different from cellulose such as its solubility in acidic aqueous solution and its binding abilities to metal ions and dyes. This abundance, combined with the specific chemistry of chitin and its derivative chitosan, make them interesting for the array of potential applications.

Chitin falls in the category of carbohydrate with fiber structure similar to that of cellulose of plants, except that the hydroxyl groups at C_2 positions are replaced by the acetylamide groups **(Figure 1.1)**. Not every unit of the naturally occurring chitin is acetylated; about 5-20% are deacetylated.
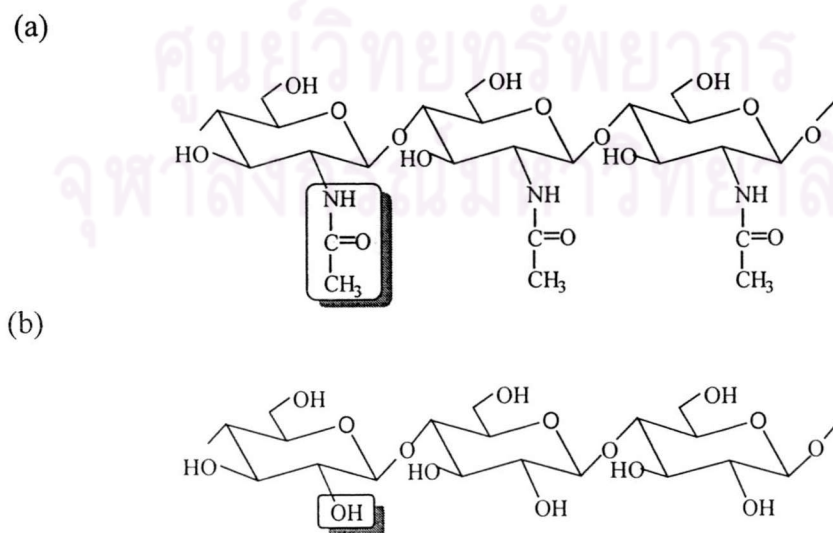
(a)

(b)

Figure 1.1 Chemical structures of (a) chitin and (b) cellulose

Some interesting characteristics of chitin are 1) biodegradable 2) biocompatible with organs, tissues and cells of animals and plants; 3) non-toxic in oral and implant administrations; 4) able to be processed into several casting products including flakes, fine powders, beads, membranes, sponges, cottons, fibers and gels; and 5) modifiable chemically and enzymatically.[1]

Chitin occurs as a component of exoskeleton of crustacean like shrimps, crabs, squid, silk worms, pearl shells, insects, fungal cells, microfauna, and plankton. It is found in association with proteins and minerals such as calcium carbonate. The different sources of chitin differ somewhat in their bulk structures and percentage of acetylation.

1.2 The structure of chitin

Chemically, chitin is a polymer formed primarily of repeating units of: beta (1-4) 2-acetamido-2-deoxy-D-glucose (or *N*-acetyl-D-glucosamine). Chitin can exist in three polymeric forms in nature. They are the alpha-, beta- and gamma-chitin, which differ only in the arrangement of the chains within the crystalline regions. The alpha-chitin consists of anti-parallel chains, while in beta-chitin the chains are parallel **(Figures 1.2 and 1.3)**. As for the gamma-chitin, the chains are randomly mixed in both parallel and anti-parallel orientations. The alpha-chitin, which is the most stable of the three, is found in the arthropod cuticle and is frequently associated with sclerotised protein or inorganic materials or both. The beta-chitin and gamma-chitin on the other hand are often found where flexibility and toughness are both required, i.e. beta-chitin is found in squid pen while gamma-chitin is found in squid's stomach lining.[2] However, the β-chitin can be converted to the α-chitin by treatment with anhydrous formic acid or strong nitric acid but no known means to date by which this transformation can be reversed.[3-4] The infrared spectra of α-chitin and β-chitin are also essentially similar.
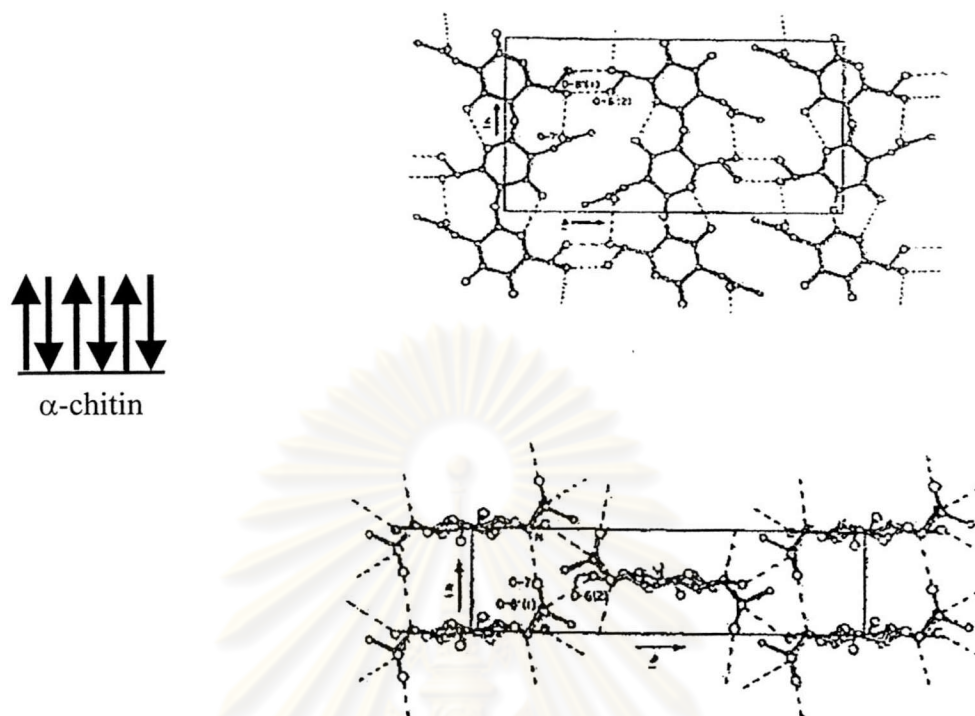
Figure 1.2 Diagrammatic illustration of antiparallel arrangement and X-ray crystal structure displaying hydrogen bond linkage between C=O and NH groups of α-chitin.
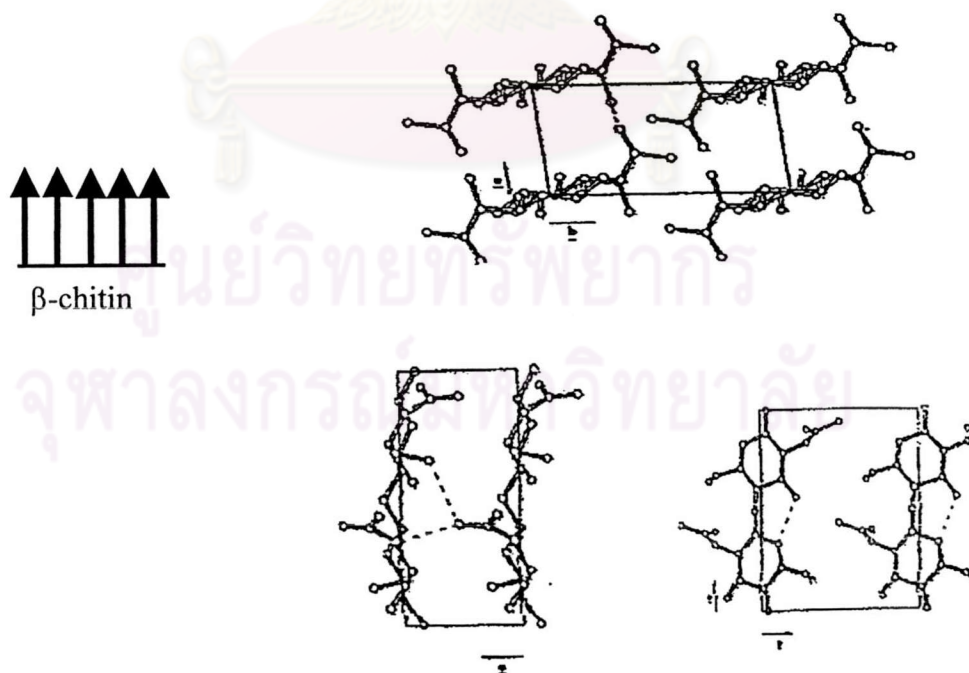

Figure 1.3 Diagrammatic illustration of parallel arrangement and X-ray crystal structure displaying hydrogen bond linkage between C=O and NH groups of β-chitin.

1.3 Manufacture of chitin

Even though chitin is widely distributed in nature, it is never found in its pure form. Chitin in its natural state is tightly associated with proteins, lipids, pigments and calcium deposits.[5] Thus it needs to be purified before it is of any commercial use. Currently, the purification of chitin consists of two main steps:

- Demineralisation: removal of minerals with dilute acid or chelating agents
- Deproteination: protein separation with dilute alkali or proteolytic enzymes

These steps can be carried out in any order. However, if protein recovery is of interest, then the deproteination step should be carried out first. The pH of the protein solution is adjusted to about 4.0 and the precipitate is separated and dried. By this way, protein yield and quality is maximized.[6] Following the demineralisation and deproteination steps, the product may be decolourised by removing the pigments with acetone or hydrogen peroxide. This step is not essential as it depends on the specification of the required end product.

1.4 Applications of chitin

Chitin is a by-product or a waste from crab, shrimp and squid processing industries. However, isolation and preparation of chitin from other marine invertebrate shells have taken place.[7,8] Chitin and chitosan offer wide range of applications, including clarification and purification of water and beverages, applications in pharmaceuticals and cosmetics, as well as agriculture, food and biotechnological uses.[9,10] Recent efforts for the use of chitin and chitosan have intensified since efficient utilization of marine biomass resources has become an environmental priority. Early applications of chitin and chitosan include a treatment of wastewater and heavy metal adsorption in industry, immobilization of enzyme and cells, resin for chomatography, functional membrane in biotechnology, seed coating and animal feed in agriculture, artificial skin, absorbable surgical suture, controlled releasing material for pharmaceutical agents, and wound healing accelerator in the medical field. However, chitin and chitosan have been developed as new physiological materials lately since they possess antitumor activity by immuno-

enhancing antibacterial activity, hypocholesterolemic activity, and antihypertensive action.[9]

Although chitin and chitosan are known to have very interesting physiological properties, but there is doubt concerning their level of absorption in human intestine, their high molecular weights and highly viscous nature may restrict their in-vivo uses. Because most animal intestines, especially human gastrointestinal tract, do not possess enzyme such as chitinase and chitosanase which can directly degrade the β-glucosidic linkage in chitin and chitosan. Recently, studies have attracted interest in converting chitin and chitosan to their monomer and oligomers (**Figure 1.4**). The monomers and oligomers of chitin and chitosan have low viscosity due to their small molecular weights and short-chain lengths that allows them to be readily soluble in neutral aqueous solution and absorbed in the in vivo system.

1.5 The use of monomers and oligomers of chitin and chitosan

Unlike cellulose, chitin, chitosan and their subunits have many physiological activities. These activities have led to progressively increased utilization of these materials in food and pharmaceutical fields for human health and in chemistry as biologically important synthesis building blocks (**Table 1.1**). N,N'-diacetylchitobiose ($(GlcNAc)_2$) has been widely used as a starting material for synthesis of biologically active compounds and an important building block for synthesis of various complicated oligo- and polysaccharides. Furthermore, $(GlcNAc)_2$ is the core disaccharide of N-linked glycoproteins. The chitobiose produced was subjected to chemical modifications to give novel disaccharide derivatives of 2-acetamido-2-deoxy-D-allopyranose moieties that are potential intermediates for the synthesis of an enzyme inhibitor. Following on from recent progress in the biochemical field, this compound is expensive, difficult to prepare and isolate before have become accessible as raw materials. The preparation of N,N'-diacetylchitobiose thus far has been through chemical, microbial, or enzymatic chitin degradation.

Hirano and Nagao[11] studied relationships between the degree of polymerization (DP) of chitosan and the degree of pathogen inhibition. They showed that chitosan oligomers (DP 2-8) as well as partially hydrolyzed chitosan with a low molecular weight possessed stronger growth inhibition than the high molecular weight

chitosan against several phytopathogens including *Fusaruim oxyporum*, *Phomopsis fukushi*, and *Alternaria alternata*.

Chitin, chitosan, and their oligomers have been reported to exhibit elicitor activities toward several plants, and have been widely used as elicitors for the induction of secondary products in plant cell cultures.[12,13] Chitin oligomers were active as elicitors for defending mechanism of higher plants, whereas chitosan oligomers had almost no eliciting activity.[14,15]

Suzuki demonstrated that chitin and chitosan oligomers inhibited the growth of tumor cells by immuno-enhancing effect.[16] Suzuki *et al.* also revealed the chitin oligomers from $(GlcNAc)_4$ to $(GlcNAc)_7$ displayed strong attracting response to peritoneal exudate cells in BALB/c mice, whereas chitosan oligomers from $(GlcN)_2$ to $(GlcN)_6$ did not show this effect.[17] Tokoro *et al.* showed that both $(GlcN)_6$ and $(GlcNAc)_6$ had growth-inhibitory effect against Meth-A solid tumor transplanted into BALB/c mice.[18]

On the effects of water-soluble chitin and chitosan oligomers, Suzuki *et al.* demonstrated that chitin hexamer, $(GlcNAc)_6$, possessed a strong candidacidal activity.[19] Tokoro *et al.* found that $(GlcNAc)_6$ exerted strong growth-inhibitory effect on *Listeria monocytogenes* by elevating the function of cellular immunity.[20]

Tsukada *et al.* reported a significant antimetastic effect for $(GlcNAc)_6$ in mice bearing Lewis lung carcinoma.[21] Suzuki *et al.* analyzed the change of the spleen cells from tumor-bearing mice administered with chitooligosaccharide such as $(GlcNAc)_6$ to unravel the tumor inhibition mechanism and cell growth by immuno-enhancing effects of the oligomers.[22] It was demonstrated that increase of cytotoxic T lymphocytes activity by accelerating the differentiation of helper T cell was remarkable and paralleled a decrease of suppressor T cell activity.

Shikhman *et al.* reported that glucosamine and its derivatives, including *N*-acetylglucosamine, are some of the most commonly used drugs to treat osteoarthritis.[23] However, the mechanisms of their antiarthritic activities are still poorly understood.

Hiroshi synthesized amphiphilic chitooligosaccharides, having antitumor activity, by using $(GlcNAc)_2$ as a stating material. The researcher expected those chitooligosaccharide derivative would aggregate in water and form a micelle, which improve the biological activity.[24]
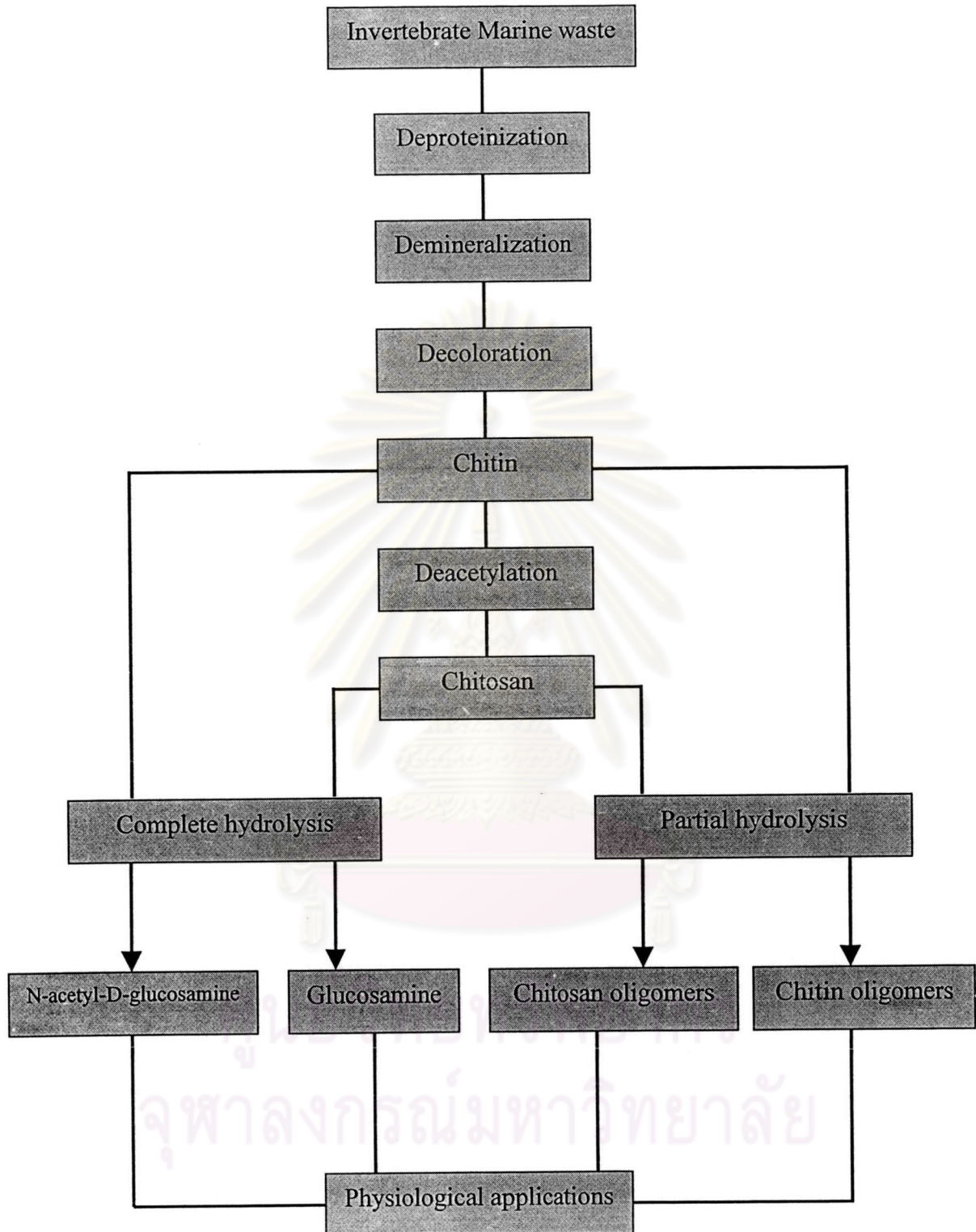
Figure 1.4 Simplified flowsheet for preparation of chitin and chitosan, their monomers and oligomers from invertebrate marine's waste.

Table 1.1 Applications of chitin, chitosan, their monomers and oligomers

Field	Chitin and chitosan	Monomer and oligomers
Food	- Antimicrobial agents - Preservative agents - Edible film	- Antimicrobial agents - Preservative agents
Pharmaceutical	- Antibacterial infection - Antitumor agents - Immunopotentialting agents - Carrier for drug delivery system	- Antibacterial infection - Antitumor agents - Immunopotentialting agents
Medical	- Accelerator for wound healing - Artificial skin - Fiber for absorbable sutures	- Osteoarthritis and inflamma- tory - Bowel disease treatment
Nutritional	- Dietary fiber - Hypocholesterolemic agents - Antihypertensive agents	- Hypocholesterolemic agents - Calcium absorption accerela- tor in vitro
Biotechnological	- Carrier for immobilized enzymes and cells - Porous beads for bioreactors - Resin for chromatography - Membrane materials	
Agricultural	- Seed coating preparation - Activator of plant cells	- Activator of plant cells - Plant growth

Other	- Coagulant for wastewater
	  treatment
	- Protein recovery preparation in
	  food processing plants
	- Removal of heavy metal from
	  wastewater
	- Cosmetics materials
		- Chemistry building blocks
		- Cosmetics materials

Takahashi *et al.* have synthesized an azapseudodisaccharides related to allosamidin by using (GlcNAc)₂ as starting material. These compounds were shown to be chitinase inhibitors which may be used as insecticides and antifungal agents.[25]

Richardson *et al.* reported the potential of low molecular weight chitosans as a DNA delivery system. The low molecular weight chitosans were neither toxic nor hemolytic, and could complex DNA and protect against nuclease degradation compared with the high molecular weight chitosan.[26]

1.6 Hydrolysis of chitin

N-acetyl-D-glucosamine is the smallest repeating unit of chitin. Chitooligosaccharides are the oligomers of β-(1→4) linked *N*-acetyl-D-glucosamine. There are two hydrolytic methods, chemical hydrolysis and enzymatic hydrolysis, used for preparation of *N*-acetyl-D-glucosamine and chitooligosaccharides from chitin.

1.6.1 Chemical hydrolysis

Chemical method for the preparation of GlcNAc, GlcN, and chitooligo-saccharides mostly deals with acid hydrolysis.[27-29] Recently, the series of chitooligosaccharides have become commercially available. They are usually prepared by hydrolysis of chitin with concentrated hydrochloric acid, followed by extensive column chromatographic fractionation.[27] The conventional procedure for their isolation is as follows: 1) acid hydrolysis, 2) neutralization, 3) demineralization, 4) charcoal-celite column fractionation, 5) HPLC fractionation, and 6) lyophilization[28]

Rupley used concentrated hydrochloric acid to digest chitin for preparation a substrate for lysozyme assaying.[27] Moreover, Horowitz *et al.* explained that acid hydrolysis of chitosan with concentrated HCl also led to the production of chitosan oligomers with low degree of polymerization (DP) (monomer to trimer) in quantitative yields.[33] However, such a simple method, using only concentrated hydrochloric acid associates with some inherent problems such as cost for purification of the products, environmental concerns, and a low yield of high oligomer product with many by-products. Acetolysis, fluorolysis, fluorohydrolysis, and hydrolysis with sonolysis have thus been studied to alleviate these problems (**Figure 1.5**).

Inaba *et al.* used acetolysis of chitin to synthesize a substrate for the assay of lysozyme.[30] In addition, Kurita *et al.* suggested squid β-chitin as a starting material for simple acetolysis giving rise to the formation of *N*-acetyl chitooligosaccharide peracetates in high yields with good reproducibility.[31]

Defaye *et al.* noted that fluorohydrolysis of chitin in anhydrous hydrogen fluoride (HF) led to chitin oligomers in almost quantitative yield and conditions can be conveniently monitored in order to optimize the preparation of specific oligomers ranging from 2 to 9 residues.[32] However, major products of chitin oligomers obtained are mainly dimer to tetramer and chitin oligomer isomers (β-(1→6)-linked 2-acetamino-2-deoxy-D-glucosyl oligosaccharide) exclusively formed when solutions of chitin were kept in HF for over 10 hrs at room temperature.

Takahashi *et al.* reported a production of chitin oligomers by a combination method of mild acid degradation and sonolysis, which is able to degrade chitin without dependence on the temperature of the bulk solution and hydrolyze chitin by hydrochloric acid under ultrasound irradiation.[28]
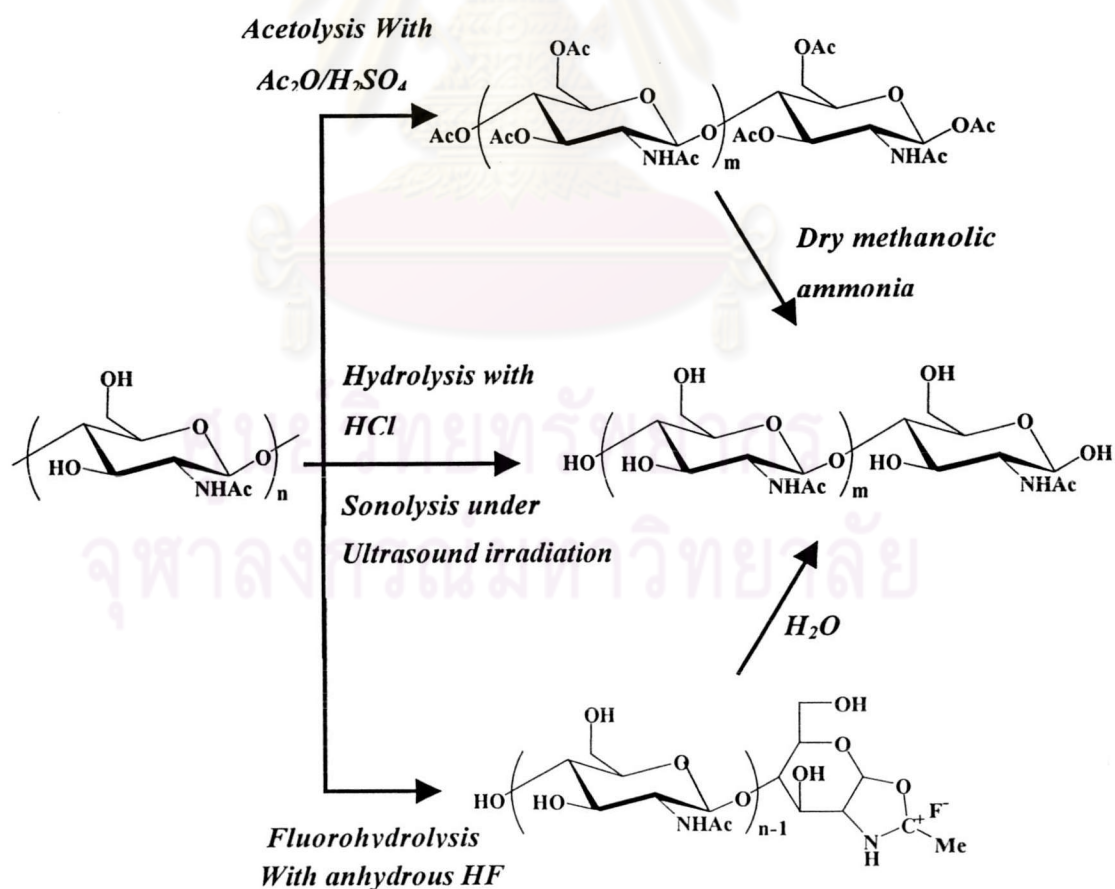

Figure 1.5 Reactions for acid hydrolysis of chitin.

1.6.2 Enzymatic hydrolysis

In contrast to chemical hydrolysis, enzymatic hydrolysis of chitin has several benefits to produce monomers and oligomers with milder reaction condition. Uchida *et al.* explained that the enzymatic hydrolysis was a useful method for the preparation of the oligomers from chitin and chitosan because the yield of specific products was usually greater in the enzymatic hydrolysis than in the acid hydrolysis.[34]

Chitin is hydrolyzed at the β(1-4) glycosidic bond by both chitinase and lysozyme to N-acetyl-D-glucosamine (GlcNAc) **(Figure 1.6)**. There are two types of chitinase[35] : endo-chitinase (E.C.3.2.1.14) which produces the *N*-acetylchitooligosac charides containing *N*-acetylglucosamine at the reducing end and exo-chitinase (or chitobiase, or *β*-N-acetylhexosaminidase) (E.C.3.2.1.30) which hydrolyzes the chitin oligosaccharide from the nonreducing end to release a monomeric *N*-acetylglucosamine.[36,37]
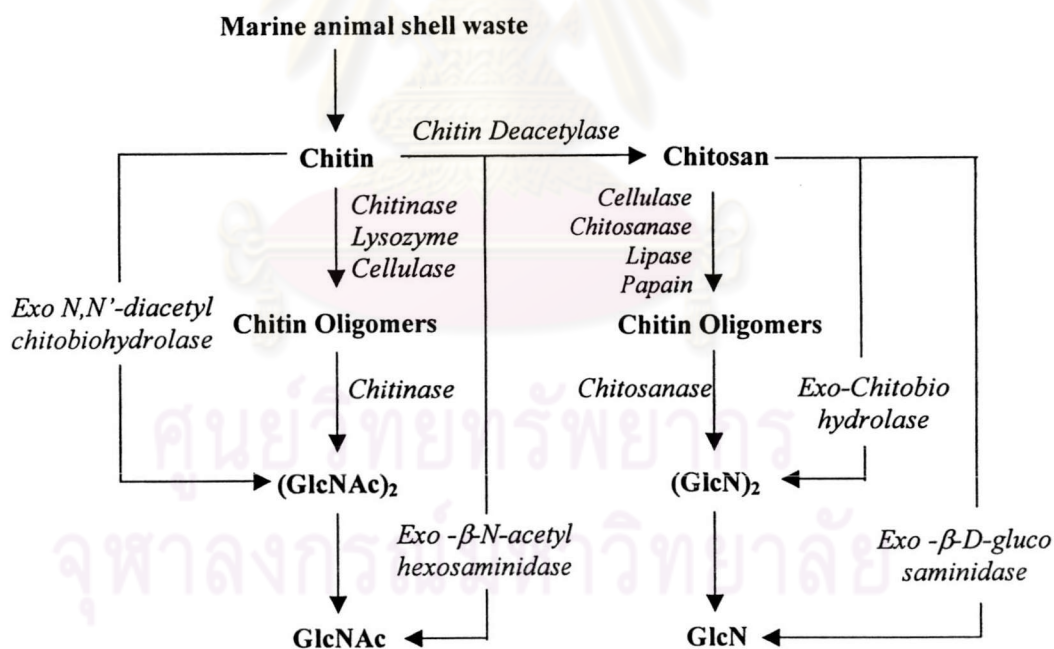
Figure 1.6 Pathway for the conversion of chitin and chitosan into their oligomers by enzymatic means.

Takiguchi and Shimahara reported a prodution of only (GlcNAc)$_2$ from chitin with an enzyme from thermophilic bacterium.[38] Takayanagi *et al.* reported that four kinds of thermostable chitinases isolated from the cell-free culture broth of *Bacillus licheniformis* X-7u produced (GlcNAc)$_2$ and GlcNAc.[39]

Aiba also suggested that, in the case of degradation of chitin by chitinases, hydrolyzed sites cannot be regulated by the enzyme.[40]

Recent studies on enzymatic transglycosylation have revealed production of higher oligomers, such as hexamer and heptamer form lower oligomers. Kobayashi *et al.* prepared *N,N'*-diacetylchitobiose using the reversed action of chitinase by combining a sugar oxazoline derivative as a glycosyl donor and *N*-acetyl-D-glucosamine as a glycosyl acceptor in the presence of chitinase (from *Bacillus* sp.) (**Figure 1.7**).[41]
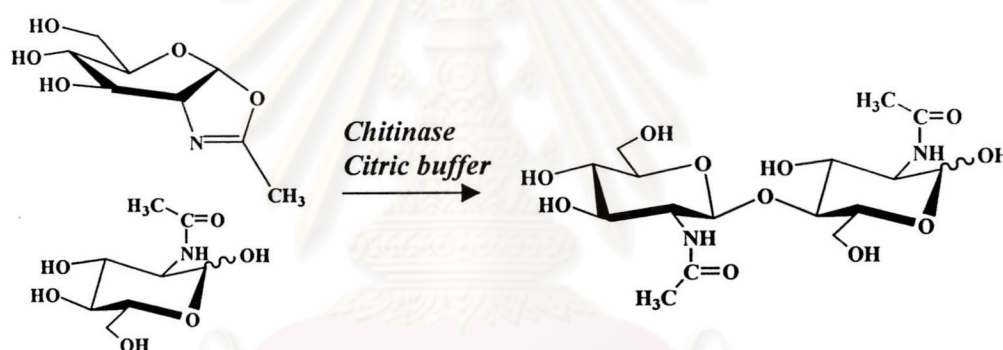

Figure 1.7 Preparation of (GlcNAc)$_2$ by enzymatic transglycosylation.

Although a number of chitinases and chitosanases have been isolated from microorganisms over the past two decades, they are still very expensive to be utilized in the industrial process. Several commercial enzymes have been examined for their potential usage in the preparation of GlcNAc and chitooligosaccharides by enzymatic hydrolysis of chitin and chitosan with a low production cost. Aiba and Muraki used low-cost enzymes such as lipase, cellulase and hemicellulase and found that, in the case of hemicellulase, the yield of hexamer was more than 20% when chitosans with 9-22% deacetylated were used.[42]

Recently, there are approaches of using the commercially available crude enzymes without purification for preparation the monomer and oligomers of chitin and chitosan. Sashiwa *et al.* reported that crude enzymes had some advantages to

produce the GlcNAc owing to their low cost and their inclusion of both endo- and exo-type chitinases.[43] These researchers can hydrolyze β-chitin and produced the GlcNAc with high yield (76%) for 8 days when used crude enzyme from Cellulase *Tricoderma viride*. Sukwattanasinitt *et al.* studied the utilization of commercial non-chitinase enzyms form fungi to prepare GlcNAc. They found that 64% of GlcNAc was obtained within only 4 days with fewer enzymes used by combination of two enzymes, that had high chitinase and β-*N*-acetylhexosaminidase activity.[44] Sashiwa *et al.* also attempted to digest the α-chitin with crude enzyme from *Aeromonas hydrophila* H-2330. The selective and effective production of GlcNAc was achieved by obtaining of 77% yield and clean reaction without by-product.[45] In addition, Pichyangkura *et al.* used crude chitinase from *Serratia sp.* TU09 and *Bacillus lichenniformis* SK-1 to digest the α- and β-chitin powder.[46] The results from these suggested that certain enzymes could hydrolyze crystalline chitin to give GlcNAc in high yield (>70%).

In the development process for efficient enzymatic hydrolysis of chitin, an immobilized enzyme was employed for a continuous production of oligosaccharides. Matsuoka *et al.* used a dialysis technique in a preparation of *N,N'*-diacetylchitobiose by continuous enzymatic degradation of colliodal chitin with chitinase from *Streptomyces griseus* and the method had potential to be used for industrial production.[47]

1.7 Chitinase

Chitinase (EC 3.2.1.14) is a glycosyl hydrolase that catalyzes the hydrolytic degradation of chitin. Chitinases are found in a wide variety of organisms that possess chitin as well as bacteria, plants, and vertebrates. The roles of chitinases in these organisms are diversed.[48] Invertebrates require chitinases for partial degradation of old exoskeletons. Fungi produce chitinases to modify chitin, which is used as an important cell wall component. Bacteria produce chitinases to digest chitin and utilize it as carbon and energy source. Production of chitinases by higher plants is a part of the defense mechanisms against fungal pathogens.[49]

Based on their amino acid sequences and hydrolytic mechanism, chitinases are grouped into two distinct glycosyl hydrolase families, families 18 and 19.[50] Family 18

includes chitinases from bacteria, fungi, viruses, and animals and chitinases from class III and V of higher plants. The crystal structures of these chitinases, e.g., chitinase B from *Serratia marcescens*[51], hevamine from *Heveabrasiliensis*[52], endo-β-N-acetyl-glucosaminidase F_1 from *Flavobacterium meningosepticum*[53], and endo-β-N-acetyl-glucosaminidase H from *Streptomyces plicatus*[54], reveal a α/β 8 barrel fold that is common to family 18 enzymes.

Family 19 chitinases are bilobal structures with an ancient core structure of α-helices and three stranded β-sheets. These chitinases include not only chitinases from plants in classes I, II, and IV, but also a bacterial chitinase, *Streptomyces griseus* HUT 6307 chitinase C.[55] The crystal structure of the family 19 chitinases show a completely different fold, which remotely resembles that of lysozyme.[56]

The differences of these types of chitinases are the specificity of glycosidic linkage recognition to degrade chitin including the different mechanism of catalytic hydrolysis.

1.8 Substrate binding mechanism of chitinolytic enzyme

Hen egg white lysozyme has well-known binding subsites, so called A, B, C, D, E, and F, and the glycosidic bond cleavage takes place between sites D and E. According to the subsite nomenclature proposed by Davies *et al.*, the binding subsites can be written as (-4)(-3)(-2)(-1)(+1)(+2). The subsite structure was estimated from model building of the lysozyme-$(GlcNAc)_6$ complex based on the crystal structure of the complex with $(GlcNAc)_3$, and can be confirmed from experimental time-courses of oligosaccharide degradation and product formation obtained by high performance liquid chromatography (HPLC).[57]

The binding mode of $(GlcNAc)_6$ to family 19 chitinase from barley seeds was estimated by experimental time-course of $(GlcNAc)_n$ (n = 4, 5, and 6) degradation by the enzyme.[58] The chitinase hydrolyzed $(GlcNAc)_6$ producing $(GlcNAc)_3$ + $(GlcNAc)_3$ and $(GlcNAc)_2$ + $(GlcNAc)_4$. The amounts of $(GlcNAc)_3$ produced was twice of those of $(GlcNAc)_2$ and $(GlcNAc)_4$, indicating that the splitting frequency into $(GlcNAc)_3$ + $(GlcNAc)_3$. They reported that the biding subsite model was (-3)(-2)(-1)(+1)(+2)(+3) for family 19 chitinase.

For family 18 chitinases, the entire substrate binding cleft was first revealed by superposition of the structure of *H. brasiliensis* chitinase complexed with $(GlcNAc)_4$ and that of *S. marcescence* chitinase A complexed with $(GlcNAc)_2$.[59] Brameld and Goddard have done the molecular dynamics simulations of $(GlcNAc)_6$ binding to *S. marcescence* chitinase A. Both works indicated that the binding clefts are represented by (-4)(-3)(-2)(-1)(+1)(+2) in family 18 chitinases.[60]

1.9 *Serratia* sp.[61]

Serratia sp. is a gram-negative bacterium that is currently attracting considerable attention for its extraordinary versatility as a plant pathogen, saprophyte, biocontrol agent, bioremediation agent and human pathogen. Formerly known as *Pseudomonas cepacia*, this bacterium was first described in 1950 as the cause of sour skin of onions by Cornell University plant pathologist Walter Burkholder. *P. cepacia* was later renamed *Serratia sp.* and transferred to the beta subdivision of the proteobacteria. *Serratia sp.* is naturally abundant in soil, water and on plant surfaces. It is distinctive in its ability to metabolize a broad range of organic compounds as carbon and energy source, an attribute which has spurred the development of *Serratia sp.* for use in bioremediation of soil and ground water contaminated with chlorinated hydrocarbons and herbicides. *Serratia sp.* has also been the focus of considerable research by plant pathologists who have shown it to be an effective biocontrol agent against soil borne, foliar and post-harvest diseases. Many strain of *Serratia sp.* produce one or more antibiotics active against a broad range of plant pathogenic fungi. These antibiotics appear, in many cases, to be important for disease suppression. Biocontrol with *Serratia sp.* can be an effective substitute for chemical pesticides. However some strains can cause fatal lung infection of individuals with cystic fibrosis (CF), an inherited disease that impairs lung functions and makes them susceptible for opportunistic bacteria infections.

Serratia sp. was found to produce high chitinase activity. Chitinase gene from *Serratia sp.* TU09 was cloned into *E. coli*. The molecular weight obtained from SDS-PAGE with chitinase activity straining was about 60 kDa. This enzyme was called Chi 60. The structure of chitinase Chi 60 was similar to *Serratia marcescens* (**Figure 1.8**)

(a)
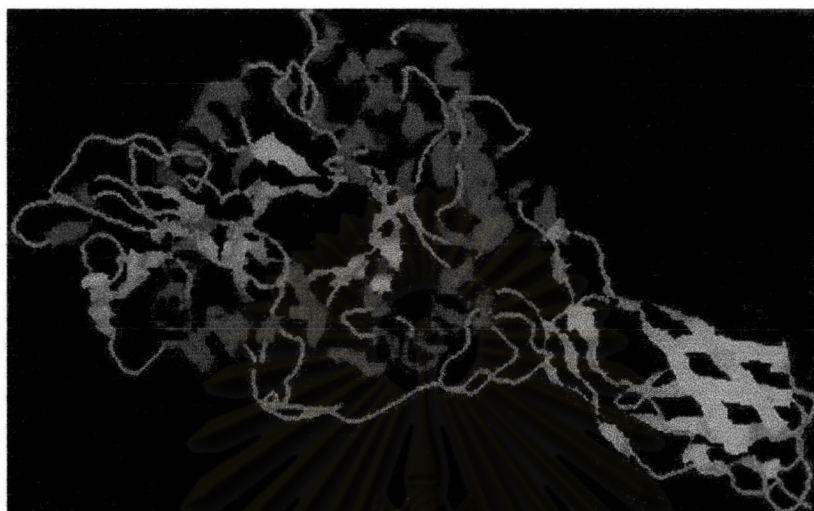
(b)

Figure 1.8 Structure of chitinase Chi 60, **(a)** Structure of *Serratia marcescens* ChiA displayed by Rasmol 2.6 and **(b)** Theoretical model of *Serratia sp.* Chi 60 accomplished by Swiss-Model Protein Modeling (SWISS-MODEL version 36.0002).[61]

1.10 *Acremonium* sp.

Colonies are usually slow growing, often compact and moist at first, becoming powdery, suede-like or floccose with age, and may be white, grey, pink, rose or orange in color. Hyphae are fine and hyaline and produce mostly simple awl-shaped erect phialides. Conidia are usually one-celled (ameroconidia), hyaline or pigmented, globose to cylindrical, and mostly aggregated in slimy heads at the apex of each phialide.

This genus is distinguished from hyaline isolates of *Phialophora* by the absence or very limited development of a collarette on the phialide and the predominant formation of well differentiated, awl-shaped phialides with a basal septum. Microconidial *Fusarium* isolates may be confused with *Acremonium*, but they usually grow faster and have colonies with a characteristic fluffy appearance.

For identification, potato dextrose agar and cornmeal agar are the most suitable media to use and exposure to daylight is recommended to maximize culture color characteristics.

The genus *Acremonium* currently contains 100 species, of which most are saprophytic, being isolated from dead organic debris, hay, food stuffs and soil. *Acremonium* sp. is most commonly found in the south island. Mode of dissemination was wet spore, insect/water droplet and wind (old growth). Allergen: Type I allergies (hay fever, asthma) and type III hypersensitivity pneumonitis: Humidifier lung. A number of species are recognized as opportunistic pathogens of man and animals, causing mycetoma, keratitis, onychomycosis, and hyalohyphomycosis. Other rare infections reported in immunodeficient patients, and in persons with wound injuries. Most species of Acremonium do not grow at 37 $^{\circ}$C, these include *A. falciforme, A. kiliense* and *A. recifei, A. alabamensis, A. potroni, A. roseo-griseum* and *A. strictum.* However, many reports only identify *Acremonium* species to genus level. Clinical manifestations of hyalohyphomycosis caused by *Acremonium;* include arthritis, osteomyelitis, peritonitis, endocarditis, pneumonia, cerebritis and subcutaneous infection.

It could grow indoors by widespread which requires very wet conditions. In industrial, it used to produces cephalosporins, an important class of antibiotics. Formerly this genus called Cephalosporium.

1.11 Aims of thesis

This thesis focuses on potential use of crude chitinolytic enzyme from cellulase *Acremonium cellulolyticus* and *Serratia sp.* cloned in the preparation of *N,N'*-diacetylchitobiose. The optimum hydrolytic conditions for both enzymes were investigated. A suitable protocol for preparation of *N,N'*-diacetylchitobiose from chitin will also be described.