CHAPTER VI
FUNCTIONALIZATION OF CHITIN WHISKER WITH POLY(ETHYLENE GLYCOL)

Abstract

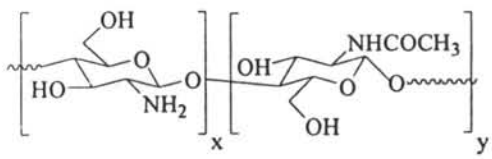
The conjugation reaction of chitin whisker in colloidal water solution is originally proposed. An intermediate carboxylated chitin whisker provides the carboxyl group to conjugate with polyethylene glycol amine in mild condition by using the carbodiimide water soluble conjugating agent. The compound obtained not only maintains its whisker morphology and the colloidal state in water but also shows redispersion ability in choloform after lyophilization. The present work demonstrates that we can derivatize chitin in a simple but effective condition if we use chitin whisker nanoparticles as a starting material instead of chitin powder or flakes.

Keywords: Chitin, Whisker, Poly(ethylene glycol), PEG, Carboxylation

Introduction

Chitin-chitosan is the second most naturally abundant copolysaccharide next to cellulose consisting of β-(1-4)-2-acetamido-2-deoxy-β-D-glucose and β-(1-4)-2-amino-2-deoxy-β-D-glucose (Scheme 6.1) obtained from the shells of crustaceans, the cuticles of insects, the cell-walls of fungi and yeasts. Chitin-chitosan has received much development as a precursor to value-added biopolymers due to its specific properties including bioactivity[1], biocompatibility[2], biodegradability[3], and nontoxicity[4] and the potential for physical and chemical modification.[5-6]

Scheme 6.1



Considering the chemical structure of chitin based copolymer (% degree of deacetylation for less than 70%)[7], chitin has its own limitation about the inertness, either the solubility or the reactivity due to the strong inter- and intramolecular hydrogen bonding via acetamide and hydroxyl groups. The ordinary chitin is not soluble and even swelled in water or most organic solvents. The specific solvents such as *N,N*–dimethylacetamide (DMAc) containing 5-10% LiCl, hexafluoroacetone, and hexafluoro-2-propanol are reported and accepted as the pathway to physical or chemical modification.[8] In the past, various chemical modifications of chitin were succeeded to develop the solubility and reactivity, such as acylation[9], deacetylationy[10], alkylation[11], tosylation[12], and carboxymethylation.[13]

Chitin is an attractive biopolymer not only as an abundant natural resource but also as a potential functional material, thus, the development of chitin to become a value-added products has been received much attention for decades.

Recently, Nair et al.[14] proposed an alternative method to improve the solubility and reactivity of chitin by treating chitin flakes in acid to obtain the chitin whisker. The chitin whisker is an aqueous suspension of elongated microcrystals with high aspect ratio and high reactivity comparing to the chitin starting material. The functionalization of chitin whisker is expected for the high reactivity and mild reaction conditions comparing to that of chitin. Nair et al.[15] also proposed the chemical modification of lyophilized chitin whisker with phenyl isocyanate, alkenyl succinic anhydride, and 3-isopropenyl-α,α′-dimethylbenzyl isocyanate in order to enhance the filler-matrix interaction between chitin whisker and rubber.

It should be noted that poly(ethylene glycol) (PEG) is a high soluble in water and in most organic solvents polymer widely used in chemical, pharmaceutical, biomedical, and industrial applications resulting from its low cost, and attractive properties, such as biocompatibility, non-toxicity, non-immunogenic, non-antigenic, metal complexing ability, and ease of chemical modification.[16] For chitin-chitosan, many researches proposed about modifications of chitosan with PEG not only to improve the solubility but also to develop the value-added products.[17-19] The chitosan-PEG derivatives were applied as a biomaterial in various applications, especially biomedical and pharmaceutical applications[17-19], such as drug carrier[20] whereas the direct modifications of chitin with PEG are rarely reported. Sugimoto et

al.[21] proposed indirect modification of water-soluble chitin by converting chitosan-PEG hybrid to chitin-PEG hybrid via the acetylation with acetic anhydride.

Recently, our group has succeeded in modifying chitin flakes to whisker based on the method reported by Nair et al.[14] Here, we demonstrate the functionalization of chitin whisker with PEG to propose how we can achieve nano-size chitin with an effective surface modification.

Experimental Section

Chemicals. Chitin flakes from shrimp shells were provided by Seafresh Chitosan (Lab) Company Limited, Bangkok, Thailand. Sodium hydroxide, sodium bromide, hydrochloric acid, 2,2,6,6-tetramethyl-1-piperidinyloxyradical (TEMPO), *N*-hydroxysuccinimide (NHS), and sodium hypochlorite solution were supplied from Wako, Japan. Acetic acid was purchased from Kanto Chemical Co., Inc., Japan. 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) was the products from Dojindo, Japan. Sodium chloride was obtained from Matsunaga Chemical Industrial Co., Ltd., Japan. Hexafluoro-2-propanol (HFP) was a product of Central Glass Co., Ltd., Japan. Sodium trifluoroacetate (CF_3CO_2Na) was purchased from TCI-EP, Japan. Methoxypolyethylene glycol amine (PEG) molecular weight 5000 was supplied from Fluka Chemika, Switzerland. *N,N*-dimethylformamide (DMF) dimethylsulfoxide (DMSO), toluene, chloroform, methanol, and ethanol were purchased from Lab-Scan, Ireland. All chemicals were analytical grade and used without further purification.

Instruments and Equipment. Qualitative and quantitative Fourier transform infrared spectra were obtained from a Thermo Nicolet Nexus 670 with 32 scans at a resolution of 2 cm^{-1}. A frequency range of 4000-400 cm^{-1} was observed using a deuterated triglycinesulfate detector with a specific detectivity, D*, of 1×10^9 cm $Hz^{½}$ w^{-1}. Powder X-ray diffraction (XRD) patterns were recorded over 2°-60° 2θ by a RIGAKU RINT 2000 using CuKα as an X-ray source equipped with Ni filter with operating conditions of 40 kV and 30 mA. TG-DTA thermogravimetric analyses were carried out using a Perkin Elmer Pyris Diamond with N_2 flow rate of 20 mL/min and a heating rate of 10 °C/min starting from 30 to 650 °C. The

morphology investigation of whisker was analyzed by a JEOL JEM-1230 transmission electron microscopy (TEM) at 80 kV. Molecular weight of chitin flake and chitin whisker were measured by a Toso HLC-8220 GPC, a gel permeation chromatography (GPC), equipped with KI detector and a Toso TSK-gel super H-RC and HM-N column operating at 40 °C. HFP containing 10 mM CF_3CO_2Na was used as an eluent and polymethyl methacrylate as a standard and the eluent was 0.2 mL/min.

Procedures.

Chitin whisker, 2. Chitin flakes, **1** (1.00 g), were treated in 3N hydrochloric acid (100 mL), stirred at 105 °C for 3 h and centrifuged to collect the samples. The residues were collected and treated with hydrochloric for other three times. In final, the residues were dialyzed in distilled water until neutral to give chitin whisker, **2** (Scheme 6.2), for 85.73% yield.

FT-IR (KBr, cm^{-1}): 1661, 1624, and 1557 cm^{-1} (amide I and amide II).

Carboxylated chitin whisker, 3. To the suspension of **2** (1% wt/v), TEMPO (0.1 g, 0.64 mmol), NaBr (1.0 g, 0.017 mol) and hypochlorite solution (11.0 mL, 1.5 moles equivalent to pyranose rings) were added and the reaction was carried out at room temperature for 4 hour. The solution obtained was adjusted to pH 10-11 and centrifuged to collect the particles. The particles were further washed thoroughly with 1.0 N NaCl and followed by 0.1 N HCl. The product obtained was dialyzed in distilled water to give **3** (Scheme 6.2), for 55.33 yield%.

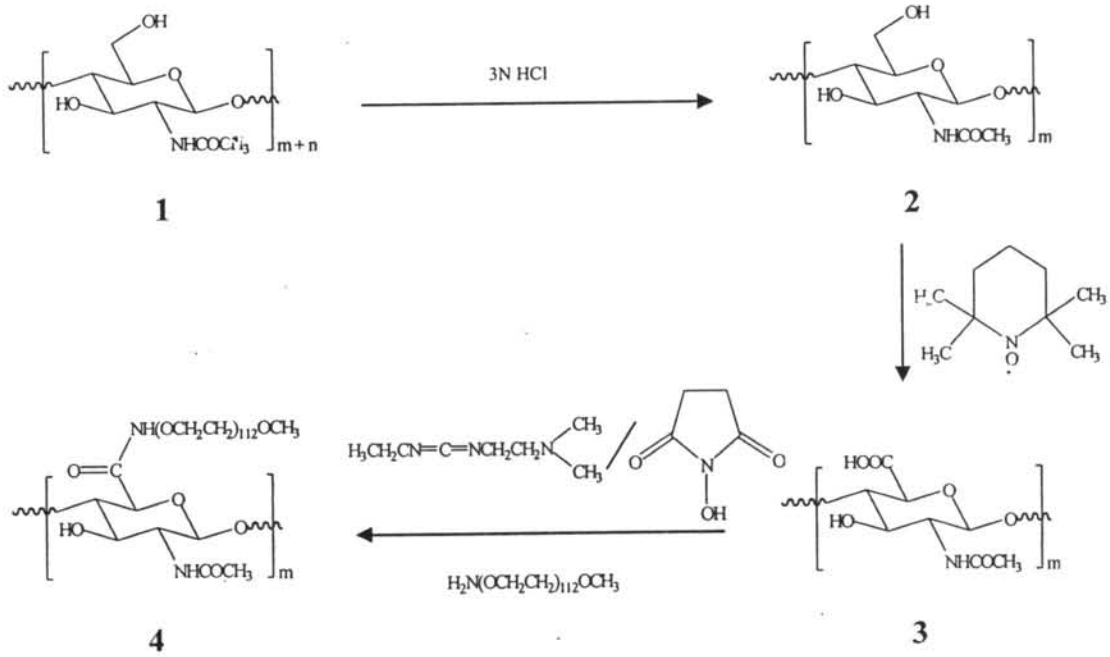
FT-IR (KBr, cm^{-1}): 1735 cm^{-1} (-COOH).

Chitin whisker-PEG, 4. Compound **3** (35.0 mL, 0.2% solid content) was mixed with EDC (1.24 g, 1.5 moles equivalent to pyranose ring) and NHS (0.14 g, 1.5 moles equivalent to pyranose ring). The pH of the mixture was adjusted to 7.5-8.0 and stirred at room temperature for 20 min. Methoxypolyethylene glycol amine (8.2 g, 2 moles equivalent to pyranose ring) was added and the reaction was carried out at room temperature under pH 7.5-8.0 for 12 h. After the pH was adjusted to 1, the product was dialyzed in distilled water and freeze-dried to yield **4** (Scheme 6.2), for 2.29 yield%.

FT-IR (KBr, cm^{-1}): 2889, 1468, 1342, 1150, 1117, 963, and 842 cm^{-1}

($-CH_2-$).

Scheme 6.2



Results and Discussion

Chemical Structure Analysis.

In previous, Nair et. al.[14] reported the preparation of chitin whiskers from crab shells by acid treatment. Here, the preparation chitin whisker form shrimp shells was challenged by modifying the procedures as proposed by Nair et al. The adding anti-bacterial reagent and storing in aqueous at pH 4 as reported by Nair et al. were unnecessary in our case since the crude whisker was immediately carboxylated after dialysis. The well-dispersed colloidal chitin solution (Figure 6.1) was achieved under the reaction time for 3 h at 105 °C. The FT-IR spectrum of **2** (Figure 6.2 (a)) demonstrates a typical type of chitin with the characteristic peaks at 1661, 1624, and 1557 cm^{-1} (amide I, and amide II), implying the success of acid hydrolysis for chain degradation.

Figure 6.1 Appearance of **2** in water.

The carboxylation was carried out as similar to the method proposed by Araki et al.[22] The carboxylation of chitin whisker was expected to occur at hydroxyl group (C-6). The FT-IR spectrum of **3** (Figure 6.2 (b)) shows the characteristic peaks of carboxyl group at 1735 cm^{-1} implying the successful carboxylation.

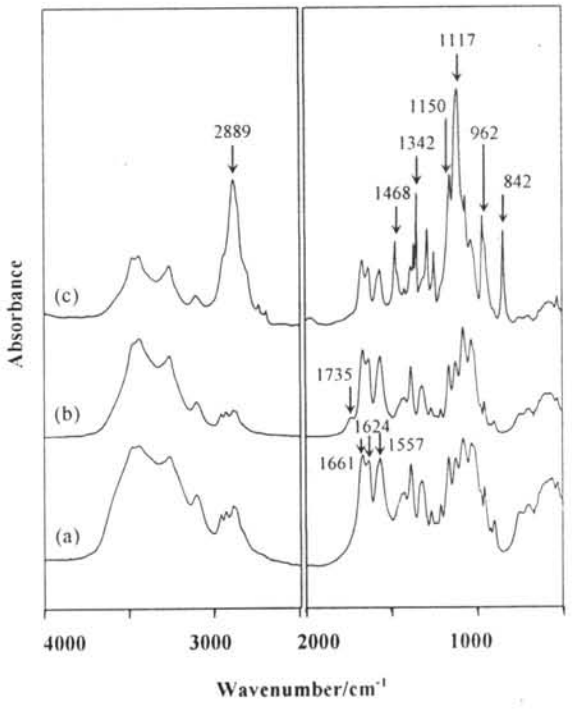


Figure 6.2 FT-IR spectra of: (a) **2**, (b) **3**, and (c) **4**.

In the final step, the chemical modification of chitin whisker with a single terminally aminated polyethylenglycol was achieved using a water-soluble carbodiimide, 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), as a coupling agent due to its high reactivity with carboxylic acids.[23] Compound **4** shows the new peaks at 842 and 962 (-CH_2- bending), 1117 and 1150 (-C-O-C-), 1342 and 1468 (-CH_2-O-, CH_2 stretching), and 2889 cm^{-1} (-CH_2- stretching) confirming a successful PEG conjugation. The disappearance of COOH peak at 1735 cm^{-1} also supported the successful reaction (Figure 6.2 (c)).

Molecular Weight Determination.

GPC was applied to determine the molecular weight reduction by using hexafluoro-2-propaol (HFP)[24] as solvent for dissolving chitin flakes and chitin whisker. GPC resulted the molecular weight of chitin for 62838 which is 35 times lower than that of chitin flakes confirming the successful depolymerization of chitin flakes.

Morphological Studies.

The observation of the change in XRD pattern is a practical way to evaluate the effect of depolymerization on the packing structure. In general, chitin whisker shows the two significant peaks at 9° 2θ and 19° 2θ (Figure 6.3 (a)). After carboxylation, the WAXD pattern of **3** (Figure 6.3 (b)) shows the same positions and intensity as those of **2**, implying that the carboxylation slighty affected the packing structure of **2.** This result is also supported by the thermogravimetry results (see Thermal Stability). Generally, methoxypolyethylene glycol amine (PEG) shows two significant sharp peaks at 19° 2θ and 23° 2θ.[22] Compound **4** shows two sharp peaks at 19° 2θ and 23° 2θ which is similar to those of PEG implying the existence of PEG including its packing structure along with that of chitin. The WAXD pattern of **4** at 9° 2θ also reveals the same position as those of **2**, and **3**; however, the peaks are broader (Figure 6.3 (c)). This might be due to the packing structure was destroyed after introducing PEG onto chitin whisker.

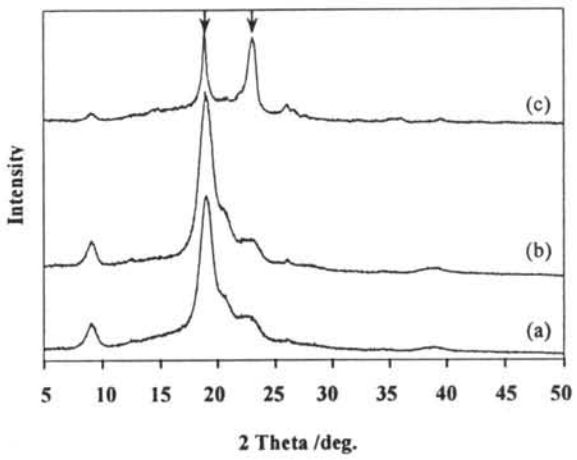


Figure 6.3 X-ray diffractograms of: (a) **2**, (b) **3**, and (c) **4**.

The observation from transmission electron microscopy (TEM) gives us the information to determine the precise structure and size before and after either depolymerization or functionalization. Figure 6.4 (a) shows TEM micrograph of **2** in rod-like shape which has a broad distribution in size. Compound **2** shows a length ranging from 200 to 560 nm and a width ranging from 18 to 40 nm (Figure 6.4 (a)). The average aspect ratio is, thus, around 18. The dimension is similar to that of chitin whisker obtained from crab shell as reported by Nair et al.[14] Compound **3** (Figure 6.4 (b)) maintains a rod-like morphology and shows a good dispersion in aqueous which might come from an electrostatic repulsion due to the carboxyl group. This result is relevant to that of the work reported by Araki et al.[22] Figure 6.4 (c) shows that after PEG introduction the rod-like morphology is still remained. However, **4** shows bundle-like-aggregated morphology implying hydrophilic-hydrophilic interaction belonging to long PEG chain. Araki et al.[22] demonstrated that the PEG-grafted-cellulose gave the microcrystal and dispersed in water comparable to the cellulose microcrystal.

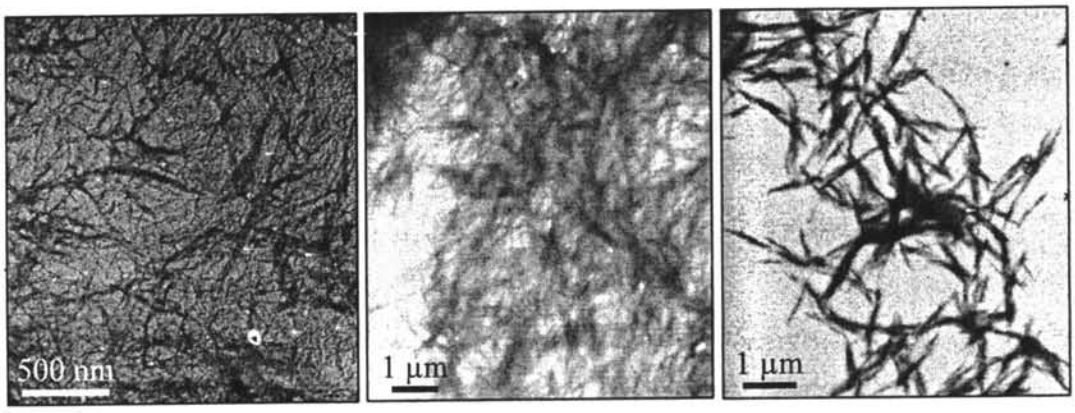


Figure 6.4 Transmission electron microscopy (TEM) micrographs of: (a) **2**, (b) **3**, and (c) **4**.

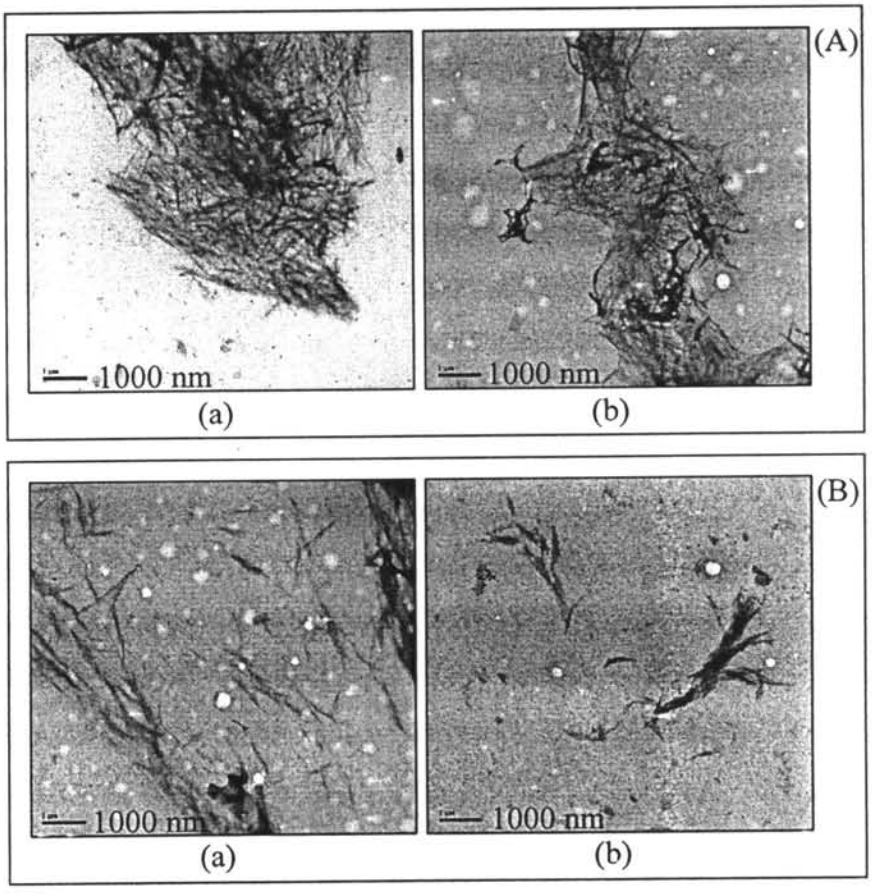


Figure 6.5 (A) Transmission electron microscopy (TEM) micrographs of **2** after lyophilization and dispersion in: (a) deionized water and (b) chloroform, and (B) of **4** after lyophilization and dispersion in: (a) deionized water and (b) chloroform.

It should be pointed out that the dispersion ability of **2** in water including common organic solvents was decreased after lyophilization. This might be the result from the recovery of hydrogen bond belonging to chitin chain after lyophilization. Here, after lyophilization, the redispersion ability of **2** and **4** in purified deionized water and solvents, i.e. methanol, ethanol, DMSO, DMF, toluene, and chloroform, were observed. The results revealed that **4** in lyophilized form showed the well-dispersion in chloroform as compared to **2**. The morphologies of lyophilized **2** and **4** in deionized water and chloroform were also confirmed by TEM. TEM micrographs clarify that the rod-like fibril of lyophilized **4** are separated from each others either in water or chloroform (Figure 6.5(B) ((a) and (b))) whereas those of **2** are in agglomerated form (Figure 6.5(A) ((a) and (b))). This might be due to that the PEG chains obstruct the repacking structure of chitin whisker including the fact that PEG has high affinity with water and chloroform as compared to chitin whisker.

Thermal Stability.

Thermogravimetry analysis was used to evaluate thermal stability of the derivatives obtained. Compound **3** shows T_d at 386 °C whereas **3** shows the degradation temperature at 383 °C (Table 6.1), implying the carboxylation leads to the small decrease of the thermal stability. This might be due to the decrease of inter and intramolecular hydrogen bonding after the introduction of the carboxyl group. After introduction of PEG onto chitin whisker, compound **4** shows the degradation temperature at 402 °C which is in between that of chitin whiskers and PEG (406 °C) comfirming a successful reaction of **4**.

Table 6.1 Degradation temperature (T_d) and ash content of **2**, **3**, and **4**

Compound	T_d (°C)	Ash content (%)
2	386	23.61
3	383	19.70
4	402	3.59
PEG	406	3.59

Conclusions

Chitin whisker grafted poly(ethylene glycol) was successfully prepared by a simple water-based reaction using water-soluble carbodiimide as a coupling agent. The compound obtained not only showed a stable colloidal solution but also maintained a rod-like morphology in a bundlelike aggregated form as observed by TEM. After lyophilization, the chitin derivative showed well-redispersion ability in chloroform as compared to the starting chitin whisker.

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