論 文 の 内 容 の 要 旨

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論文題目　　Studies on Preparation and Structural Characterization of Nano-Dispersed Chitins
(ナノ分散キチンの調製と構造解析に関する研究)

Chitins are present in the outer shells of crustaceans such as crab and shrimp, the cuticles of insects and the cell walls of some fungi, coexisting with proteins and certain minerals. More than 100 million tons of chitins are biosynthesized every year. Since a large amount of crab and shrimp shells are produced as food waste, further utilization of chitin as functionalized materials or commodities is highly required. The importance of chitin as a sustainable resource is increasing owing to not only its abundance but also unique structure and properties.

Isolated chitins are linear and crystalline hetero-polysaccharides consisting of *N*-acetylanhydroglucosamine and anhydroglucosamine units with various ratios linked by (1→4)-β-glycoside bonds. Followed by cellulose, chitin is one of abundant structural polysaccharides, which physically support living bodies, forming hierarchical structures, that increase in size from simple molecules and highly crystalline fibrils at the nanometer level to composites at the micron level upward in sea-animals, insects and fungi and so on. The lateral dimensions of the crystalline fibrils of chitins range from 2.5 to 25 nm, depending on

their biological origins. Thus, chitins intrinsically have potential to be converted to crystalline nano-fibers or nano-whiskers by so-called downsizing processing. If chitins can be individualized at nano level without impairing the original high crystallinities or high molecular weights, they would be used as new functional and bio-based nano-materials with biodegradability, reproducibility, biocompatibility, and other advantages.

Preparation of nano-dispersed chitins by TEMPO-mediated oxidation

TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical)-mediated oxidation was applied to crab shell α-chitins and tubeworm β-chitins. When the TEMPO-oxidized α- and β-chitins were subjected to ultrasonication in water, the slurries were converted to highly viscous and translucent gels, which consisted of mostly individualized chitin nano-whiskers and nano-fibers, respectively. In these cases, the position-selective formation of dissociated C6 carboxylate groups on the chitin nano-crystallite surfaces by TEMPO-mediated oxidation is the necessary point to convert original chitins to individualized nano-whiskers or nano-fibers. Electrostatic repulsions and/or osmotic effects between anionically-charged chitin microfibrils are likely to be the key driving force for the nano-conversion. Therefore, the mechanism to prepare chitin nano-fibers/whiskers is intrinsically the same as that for preparation of cellulose nanofibers by TEMPO-mediated oxidation of native celluloses reported by Saito et al. (*Biomacromolecules*, **8**, 2485 (2007)).

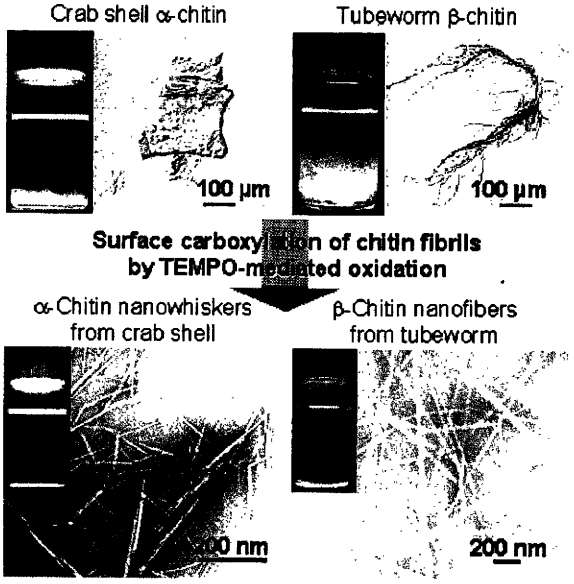

Figure 1: Nano-fibrillation of crab shell α-chitin and tubeworm β-chitin by TEMPO-mediated oxidation followed by mechanical disintegration in water.

Preparation of nano-dispersed chitins by mechanical treatment under acid conditions

Based on the mechanism of the TEMPO-mediated oxidation method to prepare cellulose and chitin nano-fibers, cationization of chitin microfibrils were studied. When squid pen β-chitin was disintegrated in water at pH 3-4 for several minutes by ultrasonication, highly viscous and transparent gels were obtained. The TEM (Transmission electron microscope) observations revealed that the gels consisted of nanofibers 3-4 nm in cross sectional width and at least a few microns in length. Cationization or protonation of the C2 amino groups present on the crystalline fibril surfaces under acid conditions is likely to be one of the most significant and necessary conditions for the nanofiber conversion. Thus, nanofibers can be directly obtained from squid pen β-chitin at pH 3-4 without any chemical modification.

However, the cationization/individualization was applicable only to squid pen β-chitin, *i.e.* the nano-fiber conversion by the simple ultrasonication is characteristic for squid pen β-chitin. Hence, the simple disintegration method could not convert a-chitin to nano-fibers or nano-whiskers, although α-chitin is present more abundantly than β-chitin in nature and easy to be collected as food wastes.

The relatively low degree of *N*-acetylation (DNAc) and low crystallinity of squid pen β-chitin, which are different from α-chitins, are probably necessary conditions for the nano-fiber conversion. Thus, partial deacetylation was applied to α-chitins under heterogeneous solid/liquid conditions.

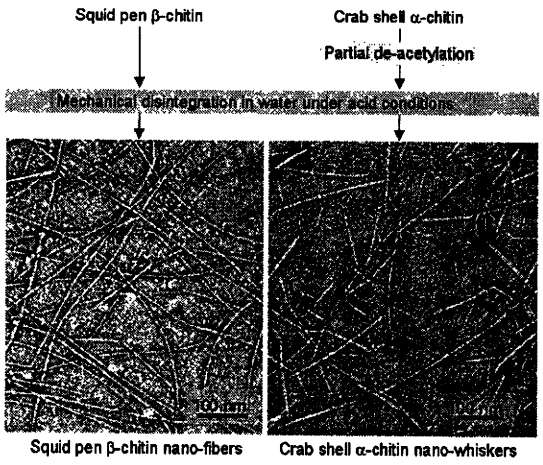

Figure 2: Nano-fibrillation of partially de-acetylated crab shell α-chitin and squid pen β-chitin by simple mechanical treatment under acid conditions.

When partially de-acetylated α-chitin was disintegrated in water at pH 3-4 by magnetic stirring for several days, highly viscous and transparent gels consisting of mostly individual rod-like nano-whiskers 6-7 nm and 100-500 nm in width and length respectively, were obtained. This is the first finding to obtain α-chitin nano-whiskers having widths quite similar to crystal sizes determined by X-ray diffraction method. Thus, complete individualization of chitin fibrils can be achieved by the de-acetylation and the following disintegration in water at pH 3-4. Some long and individual nano-fibers with length up to microns were observed in the TEM images. Conversion of C2 acetylamide groups present on the crystallite surfaces of α-chitin to corresponding amino groups by heterogeneous de-acetylation and the following cationization/protonation under acid conditions is necessary for nano-fibrillation of α-chitin, providing strong inter-fibrillar electrostatic repulsions.

Characterization of chitins nano-fibers and nano-whiskers

α-Chitin nano-whisker. Two methods were used to prepare α-chitin nano-whiskers; one is TEMPO-mediated oxidation followed by mechanical disintegration in water (TEMPO-oxidized α-chitins), the other is heterogeneous de-acetylation followed by mechanical agitation in water under acid conditions (partially de-acetylated α-chitins). Properties of thus prepared α-chitin nano-whiskers were compared with those of nano-whiskers prepared by conventional acid hydrolysis (hydrolyzed α-chitins). The water dispersion of de-acetylated α-chitins showed somewhat different rheological properties, and had the highest light transmittance and the highest viscositys, indicating that de-acetylated α-chitins had the highest degree of individualization.

Squid pen β-chitin nanofiber films and aerogels. Due to high aspect ratios of individualized squid pen β-chitin nanofibers (3-4 nm in width and at least a few microns in length with aspect ratios more than 250), highly functional films or aerogels were expected to be obtained from β-chitin nanofibers. When squid pen β-chitin nanofiber/acidic water dispersions were filtrated on membranes, films with high light-transmittance and tensile strength were obtained Moreover, sponge-like aerogels with specific porous structures could be acquired from squid pen β-chitin nanofibers.