Research paper

Flexibility of backbone fibrils in α-chitin crystals with different degree of acetylation

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ABSTRACT

Acetyl groups are backbone outreaches that enhance inter-fibril connection in chitin and chitosan fibril bundle. Removal of acetyl groups affects flexibility of chitosan fibril bundle, thereby affecting mechanical strength of chitosan-based products. Understandings of relationship between degree of acetylation and flexibility of chitin fibril bundle could lead to optimization of synthetic chitin materials. Here, the relationship is examined by performing molecular dynamics simulations. Coiling of chitin and chitosan fibril bundle with different degree of acetylation is observed and flexibility of fibrils is measured. Number and alignment of acetyl groups are found to be important factors determining the flexibility of chitin and chitosan fibril bundle. Structural instability can be caused by incompatible alignment of acetyl groups. Our findings on synthetic chitin-based materials indicate that adding a small amount of acetyl groups to chitosan can significantly enhance the integrity of fibril bundle.

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1. Introduction

Chitin and chitosan are cellulose-akin natural polymers containing N-acetyl groups and amino groups respectively. Chitosan molecule is glucosamine and it is derivable from chitin molecule, which is N-acetyl glucosamine. Natural chitin-related materials often involve both chitin and chitosan, where degree of acetylation (DA) indicates weight ratio of chitin. Chitin is one of the most abundant natural polymers in the world (Harish Prashanth & Tharanathan, 2007). Marine animals produce huge amount of chitin-based material, constituting as a widely available and economic resource for industrial usage (Arbia, Arbia, Adour, & Amrane, 2013). With materials derived from raw chitin resource, synthetic chitin-based products can be prepared and their good bio-compatibility facilitates extensive applications in biomedicine industry (Rinaudo, 2006). For example, chitin powders can be used as additives for water treatment, food as well as cosmetic products (Ravi Kumar, 2000; Varma, Deshpande, & Kennedy, 2004; Zeng, Wu, & Kennedy, 2008), chitin-based textiles are good wound dressing materials that are beneficial to heal wounds (Miraftab, Barnabas, Kennedy, & Masood, 2011; Smart, Miraftab, Kennedy, & Grocock, 2005), chitin substrate serves as an effective biological scaffold for tissue engineering and enzyme immobilization (Tang et al., 2010). To prepare those chitin–based products, extraction of chitin from raw material is an essential processing procedure, during which the raw material undergoes many purification steps and the degree of acetylation (DA) can be varied (Knill et al., 2005; Tharanathan & Kittur, 2003). Derivatives from raw chitin resources are featured with a variable range of DA from less than 10% to about 90%. DA influences chemical and physical properties such as solubility and conformational stability of chitin fibril (Cui, Yu, & Lau, 2016; Mazeau, Pérez, & Rinaudo, 2000). High-DA chitin is not soluble and exists in 3 kinds of crystalline structures including α-, β- and γ-chitin, among which the α-chitin is the major kind (Sikorski, Hori, & Wada, 2009). In these crystalline structures of chitin, acetyl groups are essential interactive sites that connect to adjacent chitin chains via hydrogen bonds as well as other non-bonded interactions (Petrov, Lymparakis, Friá, & Neugebauer, 2013). As DA decreases, chitin–chitosan mixture becomes soluble in dilute organic acids such as acetic acid. Highly de-acetylated chitosan (DA lower than 50%) is soluble in water (Lu, Song, Cao, Chen, & Yao, 2004). Chitin featured with higher than 90% DA is insoluble in either water or acids, probably due to strong crystallization. With a freeze-thaw method (Hu et al., 2007; Liu et al., 2010), the high-DA chitin can be dissolved and used to fabricate gel (Duan et al., 2013). A comparison between gels formed by chitin and chitosan shows that high-DA chitin fibrils provide reinforcement to mechanical strength (Ifuku et al., 2013). This is probably due to a
higher strength of chitin fibril and more interactive sites (acetyl groups) on subunits that compose macroscale polymeric material systems (Benjamin & Keten, 2016; Egan, Sinko, LeDuc, & Keten, 2015; Hamed, Ma, & Keten, 2015). The relationship between DA and tensile strength suggests a feasibility to tune mechanical properties of synthetic chitosan materials by controlling DA of raw materials.

Thermal movements make fibrils fluctuate. For a material making fibrils with different lengths, persistent length is defined as the length of the longest fibril which does not change its course (Gáspár & Csermely, 2012; Trachtenberg & Hammel, 2010). A flexible fibril tends to curve, while a persistent fibril is likely to maintain its straightness. Flexibility parameter is reciprocal to persistent length and it measures how flexible a fibril is when being bent. For example, a hair filament is more flexible than a steel bar, so flexibility parameter of hair is greater than that of steel bar. Flexibility of constituent fibrils plays a key role in determining mechanical performance of the fibril network, e.g., a soft spider net cannot support its shape while a steel-made net can maintain its structure. At the nanoscale, high tensile strength can be widely observed due to strong covalent bonds along backbone direction of single-chain fibrils, while high rigidity is rare. Cross-linking (Depalle, Qin, Shelbobine, & Buehler, 2015) between adjacent fibrils is an effective way to improve fibril rigidity (Liu et al., 2015; Liu et al., 2017). Through assembling single-chain fibrils into a bundle, improvement of rigidity and toughness can be achieved (Keten, Xu, & Buehler, 2011; Keten, Xu, Ihle, & Buehler, 2010; Qin & Buehler, 2012). Compared to individual fibril, fibril bundle is more rigid, e.g., collagen fibrils made of coiled peptides are more rigid than individual peptide filaments (Buehler, 2006; Chang & Buehler, 2014). For synthetic chitin products, which contain net-like structures made of nanoscale chitin fibril bundles, the flexibility of stems (i.e., the chitin fibril bundles) determines the corresponding mechanical properties. Studies on the flexibility of chitin and chitosan microfibril bundles can contribute to a comprehensive understanding on the mechanics of synthetic chitin products. From natural to synthetic chitin materials, extraction of chitin fibrils and reconstruction of material structure reflect a bottom-up concept. Following the bottom-up concept, molecular dynamics method (Buehler, 2008) that simulates the behaviors of atoms and molecules is a suitable investigation tool to explore the mechanics of material from a fundamental perspective. The molecular dynamics approach provides reasonable evaluations of material mechanics at the nanoscale (Demir & Walsh, 2016; Tam & Lau, 2014, 2015) as well as the microscale (Tam, Zhou, Yu, Qiu, & Lau, 2017; Yu, Zhou, & Lau, 2016; Zhou, Tam-h., Yu, & Lau, 2015). On chitin-related material systems, molecular dynamics studies demonstrate that atomistic models of chitin can reproduce structural and mechanical properties of chitin fibrils with an acceptable accuracy (Brown & Walsh, 2016; Jin, Feng, & Xu, 2013; Yu & Lau, 2015b). Structural dynamics of individual chitin and chitosan fibril has been investigated by molecular dynamics simulations (Franca, Lins, Freitas, & Straatsma, 2008; Strelcova et al., 2016). However, investigations on chitin and chitosan fibril bundles with different degree of acetylation are limited in the existing literature.

The objective of the present study is to examine the flexibility of fibril bundle containing chitin and chitosan with different degree of acetylation. As chitin fibrils are often nanoscale polymers, molecular dynamics simulation can serve as a useful investigating tool. Chitin and chitosan fibril bundle models with different DA are constructed. By performing molecular dynamics simulations, behaviors of the fibril bundle models are recorded. Flexibility parameter and persistent length are obtained to evaluate the bending properties of the modeled fibril bundles. Relationship between the flexibility and DA is investigated. Two types of alignment patterns of acetyl groups are identified. A bio-inspired design based on our work on chitin crystallinity is suggested.

2. Methods

2.1. Model construction

Chemical formula of chitin molecule is shown in Fig. 1a with acetyl groups outlined in red rectangles. Chitin polysaccharide consists of antiparallel chains as shown in Fig. 1b. Atomic model is designed following description of chitin fibrils in biological materials (Ehrlich, 2010; Lehane, 1997). It has been estimated that natural chitin fibrils (in insect cuticles) are featured with a cross section of around 3-nm diameter (Lehane, 1997; Merzendorfer & Zimoch, 2003). The molecular structure is built by duplicating orthogonal α-chitin unit cell. The cross section is circular, implying that it should be composed of orthogonal blocks aligning in a hexagonal manner, i.e., 3 layers including 5 blocks at the top, 6 at the middle and 5 at the bottom. This 5-6-5 alignment resembles a circular cross section with 3-nm diameter. Also, it has been inferred that length of chitin fibril should be around 100 nm. We have constructed models with 30-nm as well as 100-nm length and it turns out that the phenomena are comparable. Here, we choose to report results based on snapshots of the 30-nm models for a clear demonstration of fibrils morphology. Modeled fibril bundles are constructed by replicating α-chitin unit cells. The model contains 16 fibrils, the length is around 30 nm and cross section area is roughly $3 \times 3 \, \text{nm}^2$. Viewing the cross section of a chitin fibril bundle model shown in Fig. 1c, 16 chains and 32 acetyl sites are observable. According to symmetry, those chains are classified into 6 categories including core fibrils C1, C2 and shell fibrils S1 to S4, as shown in schematic diagram in Fig. 1d. An atomistic model of chitin fibril bundle with $3 \times 3 \times 30 \, \text{nm}^2$ size is obtained and its snapshot is shown in Fig. 2a. Eight levels of DA are assigned to the fibril bundles. Based on fully
acetylated situation (chitin), acetyl groups are gradually removed to achieve different DA. Because deacetylation occurs when acetyl groups meet alkali (or other chemicals in ambient environment that promote deacetylation), the acetyl group in the outermost layer is most likely to be exposed to alkaline environment and tends to detach. For fully acetylated case, DA is 32/32, which is equal to 100%. Next, outermost acetyl groups are removed, DA decreases to 22/32, equal to 68.75%. With acetyl groups being gradually removed, 8 models with DA equal to 32/32, 22/32, 18/32, 14/32, 10/32, 06/32, 02/32 and 00/32 are obtained, as shown by schematic diagrams in Fig. 2b. An analogy to the 32/32 chitin fibril bundle is drawn in Fig. 3, where acetyl groups and hydroxyl groups are regarded as outreach sites of chitin molecule. In a chitin polysaccharide, those functional groups form rugged surfaces and lead to interconnected pattern between adjacent chitin chains.

2.2. Simulation details

Molecular dynamics simulations are performed by using LAMMPS (Large-scale Atomic- Molecular Massively Parallel Simulator) code (Plimpton, 1995), which is an universal platform to implement molecular dynamics algorithms with rigorous physics. CHARMM36 all-atom force field for carbohydrate (Guvench et al., 2011) is employed to govern interaction between atoms in chitin and chitosan molecules. The CHARMM force field is derived from systematically quantum mechanical (QM) calculations and has been successfully used to govern molecular interactions of biological molecules including chitin nanocrystal (Cui et al., 2016; Jin et al., 2013; Yu, Xu, & Lau, 2014). Results from CHARMM all-atom simulations are in good agreement with ab initio calculations and experimental observations in terms of α-chitin unit cell size and elastic modulus (Petrov et al., 2013; Yu & Lau, 2015a). The correctly modeled structure and mechanical properties evidence the rationale to conduct further analysis on structural dynamics of chitin fibrils. Here, parameters are set with reference to those previous molecular dynamics studies on chitin nanocrystals for equilibration. The $3 \times 3 \times 30 \text{ nm}^3$ fibril bundle is placed in an $8 \times 8 \times 35 \text{ nm}^3$ simulation box. Periodic boundary condition is employed in all three directions. The simulation box is large enough that the interaction between adjacent periodic images is eliminated. Equilibration runs in an NVT ensemble with temperature controlled by a Nose–Hoover thermostat at 300 K. Time step is
set to 1 fs and simulation runs for 1 ns until 1000 frames of snapshots are collected. In each trajectory, root-mean-square deviation (RMSD) shows fluctuations with decreasing amplitudes. The observations are similar to previously reported equilibration process of chitin nanocrystals (Strelcova et al., 2016; Yu & Lau, 2015a). At the end of the 1-ns simulation, vibration of the modeled fibril bundles becomes small and equilibrated configurations can be obtained. Flexibility parameter is calculated based on the recorded trajectory. Morphologies of fibril bundles at the last frame are analyzed.

2.3. Calculation of flexibility parameter

For chitin and chitosan fibril bundles, flexibility is related to degree of acetylation (DA). In this study, flexibility parameter of fibril is calculated and plotted as a function of DA. Theoretically, contour length $L$, end-to-end distance $R$ and flexibility parameter $\lambda$ of the chitin fibril are related in Eq. (1) (Takebayashi, Morita, & Oosawa, 1977).

$$R_{\text{theo}}(\lambda, L) = \sqrt{\left( \frac{2}{\lambda^2} \right) [\lambda L - 1 + \exp(-\lambda L)]}$$  \hspace{1cm} (1)

The end-to-end distance $R$ and contour length $L$ are measurable from molecular dynamics trajectories. The unit is micrometer, $\mu$m, in the present study. With known parameters $L$ and $R$, a minimization process in Eq. (2) is conducted.

$$\min_{\text{frame}=1} \sum_{\text{frame}=1}^{1000} [R_{\text{theo}}(\lambda, L) - R_{\text{measured}}]^2$$  \hspace{1cm} (2)

The flexibility parameter is the most probable value of $\lambda$, its unit is $1/\text{length} (\mu\text{m}^{-1})$ in this study. Here, fibril bundle is composed of 16 fibrils and flexibility parameter of each fibril is calculated. According to observed configuration of the fibril bundles, coiling and buckling are observed. The flexibility parameters of outer and inner fibrils reflect degree of coiling and buckling respectively.

3. Results and discussion

3.1. Coiling and buckling of the fibril bundle

Two phenomena are visible in recorded trajectories, including coiling of outer fibrils and buckling of entire fibril bundle. As acetyl groups being gradually removed, inter-fibril connections in chitosan fibril bundles become weak and buckling is gradually observable. Snapshots of fibril bundle models at the last frame are captured and presented in Fig. 4.

The coiling behavior is obvious in cases where DA is equal to 14/32 and 06/32, where outer-layer fibrils spins around central fibrils along backbone direction. This coiling behavior is also observed in high-DA cases at early period of equilibration, similar with structural dynamics reported previously (Strelcova et al., 2016). After equilibration, those high-DA fibril bundles return to a steady state as demonstrated in 32/32, 22/32 and 18/32 cases. The buckling of fibril bundle becomes observable as DA decreases and is highly obvious in 02/32 and 00/32 cases, where the entire fibril bundle is curved. As aforementioned, 16 fibrils are classified into 6 categories as marked by different colors. C1 and C2 are regarded as core fibrils because they are surrounded by outer fibrils. In turn, S1, S2, S3 and S4 are regarded as shell fibrils. Flexibility of shell fibrils reflects the degree of coiling and core fibril behaviors can reflect the bundle buckling. Flexibility parameters of those fibrils are calculated and presented in Sections 3.2 and 3.3.

3.2. Behavior of core fibrils

Core fibrils are located at inner layer, surrounded by shell fibrils. Behaviors of the core fibril can reflect buckling of the entire fibril bundle. Flexibility parameters of core fibrils C1 and C2 are plotted against DA in Fig. 5.

Reviewing Fig. 4, it is observable that high-DA fibril bundles (32/32, 22/32 and 18/32) are stable and low-DA models (02/32 and 00/32) exhibit buckling. A general trend is that as DA decreases, flexibility parameter increases, indicating that fibril bundle with fewer acetyl groups becomes more flexible. However, the trend is not monotonic. Coiling is visible in mid-DA models (14/32 and 06/32). Two peak values are visible in Fig. 5b. In 14/32 and 06/32 cases, fibril bundle exhibits intense coiling. The coiling of shell fibril brings impact to core fibril and makes the core fibril behave more flexible. According to the flexibility parameters of C1 and C2, it is found that persistent length of core fibril in chitin fibril bundles ranges from 0.45 $\mu$m to 2.35 $\mu$m depending on different DA. Persistent length of individual chitin and chitosan fibril is around 10 nm (Mazeau et al., 2000), much lower than the fibril bundle. This comparison shows that to assemble fibrils in bundle is effective to obtain high rigidity, similar to assemblage of protein filaments in collagen material systems (Keten et al., 2011).

3.3. Behavior of shell fibrils

Coiling behaviors are reflected by flexibility parameters of shell fibrils. By comparing snapshots of the fibrils in Figs. 4 and 6 b, it is found that in the fibril–coiling cases, structural dynamics of both shell and core fibrils become intense.

Flexibility parameters of shell fibrils S1, S2, S3 and S4 are calculated and plotted in Fig. 6. It is shown that the shell fibril at the outer layer behave more dynamic than core fibril and exhibit higher flexibility parameter. Among these shell fibrils, S2 (in blue) exists at the central position and is relatively a stable one, while S4 (in red) is an outermost fibril that exhibits intensely high flexibility. Peak values in 14/32 and 06/32 cases correspond to severe coiling shown by snapshots in Fig. 4. High flexibility parameter in 00/32 case corresponds to curved shape of the fibril bundle. Persistent lengths of the shell fibrils are shorter than those of the core fibrils.

3.4. Alignment of acetyl groups

Peak values of shell fibrils’ flexibility parameter in DA = 14/32 and 06/32 cases imply that alignment of acetyl groups might affect coiling of the entire fibril bundle. In Fig. 7, cross sections of fibril bundle in 14/32, 10/32 and 06/32 cases are shown and analyzed in the schematic diagrams. The 14/32 and 06/32 cases are fibril bundles exhibiting intense coiling and the 10/32 fibril bundle shows stable behavior. The difference in structures between these two types of fibril bundles lies in the alignment of acetyl groups. In 14/32 and 06/32 cases, the acetyl groups align in a pattern similar to a capitalized phi, ““ (the middle part extends), where the central fibrils contain additional dangling acetyl groups (outlined by red circles). In the 10/32 case, a capitalized “H” character (the middle part is enclosed) can describe alignment of the 3-layer acetyl groups, where dangling acetyl groups (outlined in blue circles) belong to outer-layer fibrils. In the ““ pattern, adjacent chitosan fibrils that are attracted by dangling acetyl groups tend to move in circular direction and this movement initiates coiling of fibril bundle. In the “H” pattern, surrounding chitosan fibrils are equally attracted by dangling acetyl groups and coiling would not be initiated.

These findings indicate that chitosan fibrils are not compatible with chitin fibrils when embedded in crystalline structure, i.e., substituting chitin by chitosan might significantly reduce the stability
Fig. 4. Snapshots of 8 models after equilibration. High-DA fibril bundles (32/32, 22/32, and 18/32) are stable. Coiling is visible in mid-DA models (14/32 and 06/32). Buckling is visible in low-DA models (02/32 and 00/32). The 10/32 case exhibits no coiling and a bit of buckling.

Fig. 5. a Schematic diagram of fibril cross section and location of core fibrils. b Flexibility parameter of core fibrils (C1 and C2) is plotted. The trend is that as DA decreases, fibril bundle becomes soft and flexible. In 14/32 and 06/32 cases, local peak values are observed, indicating that fibril bundle in these two cases are flexible than that in 10/32 case. In 00/32 case, chitosan fibril bundle with no acetyl group exhibits highest flexibility, suggesting that acetyl groups contribute to stabilization of the chitin fibril bundle.

Fig. 6. a Schematic diagram of fibril cross section and definition of shell fibrils. b Flexibility parameters are plotted. The outermost fibril S4 exhibits high flexibility and the relatively inner fibril S2 is stable. In 14/32 and 06/32 cases, obvious local peak values are found. This abnormality can be ascribed to special alignment of acetyl groups that leads to intense coiling. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
if an “Φ” configuration is occasionally generated. On the other hand, adding a minor amount of chitin fibril into pure chitosan systems may improve the mechanical performance significantly. Moreover, it is found that acetyl groups at the surface are insignificant to fibril flexibility, while the internal acetyl groups play critical roles in maintaining structural stability. The findings can be related to the extraction of chitosan from natural resources and shows that there exists a critical DA of extracted chitosan. If high-DA chitosan (when only the surface-layer acetyl groups are removed) is obtained, crystallinity of the extracted fibrils can be preserved because fibrils are mechanically stable. On the contrary, if the extracted chitosan has a low DA (when internal acetyl groups are removed), the crystallinity is broken due to coiling or buckling of every strand of the fibril bundle. It is envisioned that the mechanical performance of bio-inspired material will be improved if our findings in terms of chitin crystallinity is considered in the design stage.

4. Conclusion

By performing molecular dynamics simulations, the effect of DA on flexibility of chitin fibril bundle is examined. It is found that both the number and the alignment of acetyl groups influence the fibril flexibility in chitin bundle. On one hand, chitin fibril bundle with fewer acetyl groups (lower degree of acetylation) becomes more flexible. On the other hand, there are some singular cases, where locally lower-DA bundle can behave more rigid due to some special alignments of acetyl groups. Overall, both the number and the alignment of acetyl groups are determining factors for the flexibility of chitin fibril bundle. The general trend is that as acetyl groups become fewer, chitin fibril bundles become more flexible. Meanwhile, there are special alignment patterns of acetyl groups including “Φ” and “H” patterns. In the “Φ” pattern, dangling acetyl groups existing in the core fibrils can initiate the coiling of shell fibrils. In the “H” pattern, dangling groups in shell fibrils limit the motion of core fibrils and the coiling is not initiated. Our findings show that there is an effect of DA on the stability of α-chitin crystal. In addition, our understandings about the extraction of chitosan fibrils from natural chitin materials are further enriched. The persistent length measured in this study is a useful reference for developing coarse-grained chitin models that are applicable to perform relatively large-scale (around 1 micrometer) simulations.

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