Biomimicking the Formation of Nacre/Shell: One Step Forward

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Abstract. Chitin is biocompatible and biodegradable, with antimicrobial activity and low immunogenicity, and the second most abundant biopolymer after cellulose. It is one of the necessary components of the formation of nacre/shell. So harnessing behavior of chitin not only would lead to mimics of these structures but also to potential applications in academic research and industry. A peptide induces a unique chitin-based gel formation. Then, in the presence of CaCO3 a flexible structure was obtained. This structure and complex has many potential applications for different applications such as development of bioinspired instruments, or complexes, nanotechnology etc. It is early step toward mimicking the structure of nacre/shell.

Keywords: Bioinspired, nanotechnology, mimicking, nacre/shell, peptide, chitin.

1 Introduction

Chitin is biocompatible and biodegradable, with antimicrobial activity and low immunogenicity, and the second most abundant biopolymer after cellulose. It is one of the necessary components of the formation of nacre/shell [1], [3]. So harnessing behavior of chitin not only would lead to mimics of these structures but also to potential applications in academic research and industry [2].

2 Method

2.1 Phage displaying peptide that can bind specifically to chitin was obtained by affinity selection from a phage display combinatorial peptide library. The selected peptide was chemically synthesized, dissolved in water and a 0.7 mM solution was prepared. Chito-hexose was prepared at 5 mM for gel-formation analysis. Peptide and chitohexose, 100 µl each, were mixed and incubated at room temperature for 2 hrs before sampling for transmission electron microscopy (TEM) analysis, and at 42°C for 15 hrs for scanning electron microscopy (SEM) analysis, respectively.

2.2 Then, a 0.7 mM aqueous solution of the selected peptide was prepared. 100 µL of 5 mg/mL CaCO3 was added to 100 µl of 5 mM chitohexose followed by addition of

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100 µl of the synthesized peptide solution. This mixture was incubated at 42˚C with shaking (350 rpm) for 15 hrs. The formed material was washed and used for scanning electron microscopy (SEM) photography.

3 Results

3.1 The selected 12-amino acid peptide contains charged residues. It could induce a unique pattern of gel-formation as shown in Figures 1 and 2.

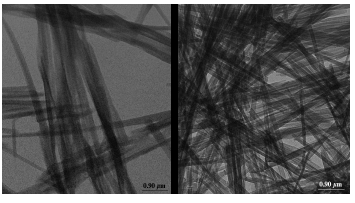

Fig. 1. TEM photographs of peptide-chitohexaose vs control, after 2 hrs incubation at room temperature. Left: Control (contains only chitohexamer), right: peptide-chitohexose.

3.2 Then, a rubber-like material was obtained (Fig 3 and 4). The peptide itself is 12 residues long with charged residues at both ends and hydrophobic residues through the middle part.

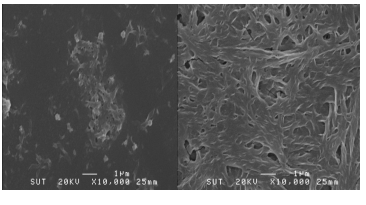

Fig. 2. SEM photographs after 15 hrs incubation at 42 ˚C. Left: Control contains only chitohexose vs Right: Chitohexose with the peptide.

In the presence of chitin alone $CaCO_3$ forms rosette-like aggregates (Figure 3 Left), a form which is lost in the presence of the peptide (Figure 3 Right).

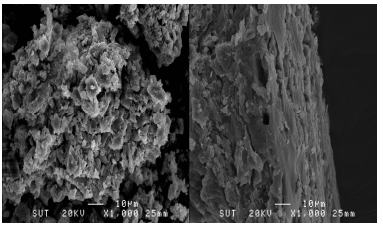

Fig. 3. SEM photographs after 15 hrs incubation at 42˚C with shaking (350 rpm). Left: $CaCO_3$-chitohexamer; Right: $CaCO_3$-chitohexamer-peptide.

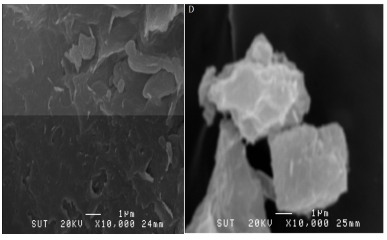

Fig. 4. SEM photographs of, Left (A), Figure 3B at higher magnification and, right (B), crystals of $CaCO_3$ formed in the presence of the peptide

4 Discussions and Conclusion

4.1. Results indicate that a specific chitin binding peptide obtained from applying phage display technology can induce a unique chitin-based gel formation. After incubating a 1:7 molar ratio of peptide and chitohexose at 42˚C for 15 hrs, a porous structure with nano-size holes could be formed. These results suggest that a short charged peptide is able to bind to chitohexose and change its behavior toward a well-ordered shape.

4.2. This structure (Figs. 3 and 4) shows high flexibility with rubber-like characteristics. CaCO3 forms typical rhombohedral crystals, which may indicate that the peptide interrupts the interaction between chitin and CaCO3 crystals allowing them to grow differently. The results of this research have potential applications in several fields such as bio-nanotechnology, drug delivery, implantable devices, etc. More research needs to be done to answer the following questions; how can mineralization be harnessed in nano- and/or micro-level, or basically, how to direct the spatiotemporal deposition of macromolecules in solution to produce structures with different functions for different applications such as development of bioinspired instruments, or complexes.

References

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