STUDY OF SILK FIBROIN PROTEIN REFOLDING AND ITS SELF ASSEMBLY PROCESS

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by

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Submitted

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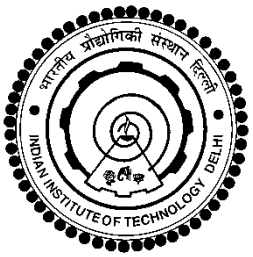

INDIAN INSTITUTE OF TECHNOLOGY DELHI

APRIL 2018

Dedicated to

My Parents & Husband

Mr. D. N. Dubey & Mrs. Pratibha Dubey

&

Dr. Saurabh Gautam

An experiment is a question which science poses to Nature, and a measurement is the recording of Nature's answer.

MAX PLANCK

CERTIFICATE

This is to certify that the thesis entitled **"STUDY OF SILK FIBROIN PROTEIN REFOLDING AND ITS SELF ASSEMBLY PROCESS"** being submitted by Ms. **Priyanka Dubey** to **Indian Institute of Technology Delhi** for the award of the degree of **"DOCTOR OF PHILOSOPHY",** is a record of the authentic research work carried out by her under my supervision and guidance. She has fulfilled all the requirements for submission of this thesis, which to the best of my knowledge has reached the required standard.

The material contained in this thesis has not been submitted in part or full to any other University or Institute for the award of any other degree.

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Priyanka Dubey

ABSTRACT

Controlling the mechanism of self assembly in proteins has emerged as an interesting and important tool for various biomedical applications. Silk fibroin has been used to form a variety of biomaterials for various biomedical applications. Silk fibroin self assembly consists of gradual conformational transition from silk I (non crystalline) to stacked β-sheet crystal rich (silk II) structure. The fabrication of unique silk-based biomaterials with tunable/controlled secondary conformation can be achieved by modulating the self assembly process of silk fibroin. Self assembly in silk fibroin may result in different secondary structural conformations, which may further lead to different immune responses. Thus, controlling the self assembly process of silk fibroin may have a direct influence on the immune responses.

In the present thesis different methodologies has been applied for modulating the self assembly process of silk fibroin namely the effect of calcium ions and the macromolecular crowding agents. The thesis also presents a mechanistic point of view of such secondary structural modulation of silk fibroin. Silk fibroin with calcium ions resulted in a predominantly α-helical intermediate conformation, which was absent under controlled conditions (i.e. fibroin without calcium ions). Further, the molecular modelling of the N-terminal region of fibroin with calcium ions showed that negatively charged glutamate and aspartate amino acids play a key role in the electrostatic interaction with positively charged calcium ions. Furthermore, we used silk fibroin as a model protein for aggregation to modulate the self assembly process and its inhibition using combination of different molecules. It is worthwhile to note that protein aggregation has been implicated in a number of human pathological disorders including numerous neurodegenerative diseases. We explored the propensity of silk fibroin protein to undergo amyloid-like nano-fibrilization and

its prevention using optimized concentrations of curcumin and β-cyclodextrin combination. The results demonstrated that fibroin treated with ethanol, without adding the optimized combination, showed the formation of nano-fibrils. However, addition of optimized combination of curcumin and β-cyclodextrin kept the silk fibroin in random coil native conformation. Based on our findings we hypothesized that the benzene rings of curcumin interact with the Tyrosine, Tryptophan and Phenylalanine aromatic residues present in the significant proportion (~8%) in silk fibroin *via* hydrophobic interactions. Whereas, β-cyclodextrin also interacted with the non-polar residues which are core components for nucleation dependent protein aggregation.

Macromolecular crowding is a one of the major factors that governs the protein folding/self assembly and aggregation kinetics inside the cells. Therefore, another approach to modulate the self assembly process of silk fibroin used was the introduction of a macromolecular crowder in the silk fibroin solution. Our data revealed that macromolecular crowders PEG (PEG 200 and PEG 6000) and dextran (dextran 6000 and dextran 70000) may induce a conformational transition in silk fibroin under defined parameters. Also the increase in the effectual concentration of macromolecular crowders results in the improved thermodynamic activity. Based on these findings we further investigated the role of macromolecular crowders and silk fibroin on human fibroblast, wherein we found that culturing of cells in the crowded microenvironment may lead to enhanced extracellular matrix as compared to control (media without crowders and silk fibroin).

सार

प्रोटीन में स्वयं असेंबली की व्यवस्था को नियंत्रित करना विभिन्न जैव चिकित्सा अनुप्रयोगों के लिए एक दिलचस्प और महत्वपूर्ण उपकरण के रूप में उभरा है। विभिन्न बायोमेडिकल अनुप्रयोगों के लिए विभिन्न प्रकार के जैव पदार्थ बनाने के लिए रेशम फाइब्रॉइन का उपयोग किया गया है। रेशम फाइब्रॉइन स्वयं असेंबली में रेशम मैं (गैर क्रिस्टलीय) β-शीट क्रिस्टल समृद्ध (रेशम द्वितीय) संरचना से क्रमिक गठनात्मक ट्रांजीशन होते हैं। रेशम फाइब्राइन की स्वयं असेंबली प्रक्रिया को विनियमित करके ट्यूनेबल / नियंत्रित माध्यमिक संरचना के साथ अद्वितीय रेशम-आधारित बायोमैटिरियल्स का निर्माण किया जा सकता है। रेशम फाइब्राइन में स्वयं असेंबली का परिणाम विभिन्न माध्यमिक संरचनात्मक रूपों में हो सकता है, जो अलग-अलग प्रतिरक्षा प्रतिक्रियाओं को आगे बढ़ा सकता है। इस प्रकार, रेशम फाइब्राइन की स्वयं असेंबली प्रक्रिया को नियंत्रित करने से प्रतिरक्षा प्रतिक्रियाओं पर सीधा प्रभाव पड़ सकता है।

वर्तमान थीसिस में रेशम फाइब्राइन की स्वयं असेंबली प्रक्रिया को अर्थात् कैल्शियम आयनों और मैक्रोमॉलिक्यूलर समूहन वाले एजेंटों के प्रभाव के लिए अलग-अलग तरीके से प्रयोग किया गया है। थीसिस रेशम फाइब्राइन के ऐसे माध्यमिक संरचनात्मक मॉडुल के बारे में एक यंत्रवत् बिंदु भी प्रस्तुत करता है। कैल्शियम आयनों के साथ रेशम फाइब्रॉइन मुख्यतः α- हेलिकल इंटरमीडिएट कन्फोर्मशन मौजूद पाया गया। इसके अलावा, कैल्शियम आयनों के साथ फाइब्रॉएन के एन टर्मिनल क्षेत्र के आणविक मॉडलिंग से पता चला है कि नकारात्मक रूप से चार्ज किए गए ग्लूटामेट और एस्पार्टेट अमीनो एसिड, सकारात्मक रूप से चार्ज कैल्शियम आयनों के साथ इलेक्ट्रोस्टैटिक इंटरैक्शन में महत्वपूर्ण भूमिका निभाते हैं। इसके अलावा, हम अलग-अलग अणुओं के संयोजन का उपयोग करके स्वयं असेंबली प्रक्रिया को नियंत्रित करने के लिए एकत्रीकरण के लिए एक मॉडल प्रोटीन के रूप में रेशम फाइब्राइन का उपयोग कर सकते है। यह ध्यान देने योग्य है कि प्रोटीन एकत्रीकरण

कई न्यूरोडीजेनेरेटिव रोगों सहित कई मानव रोग का कारण है। हमने रेशम फाइब्राइन प्रोटीन की प्रवृत्ति को पता लगाया है कि अमाइलॉइड जैसे नैनो- फिब्रिलेशन और इसकी रोकथाम करक्यूमिन और β- साइक्लोडेक्सट्रिन संयोजन की अनुकूलित सांद्रता के उपयोग से की जा सकती हैं उपयोग कर रहा है। परिणामों से पता चला हैं कि अनुकूलित संयोजन के बिना, इथेनॉल के साथ फाइब्रॉइन ने नेनो-फाइब्रिल का गठन दिखाया। हालांकि, करक्यूमिन और बीओ-साइक्लोडेक्सट्रिन के अनुकूलित संयोजन के बग़ैर यादृच्छिक कुंडल मूल रचना में रेशम फाइब्रॉइन पाया गया। हमारे निष्कर्षों के आधार पर हमने अनुमान लगाया है कि करक्यूमिन के बेंजीन के छल्ले हाइड्रोफोबिक इंटरैक्शन के माध्यम से रेशम फाइब्राइन में महत्वपूर्ण अनुपात (~ 8%) में मौजूद ट्रायोसिन, ट्रिप्टोफैन और फेनोइलैलाइन ऐरोमैटिक अवशेषों के साथ इंटरैक्ट करते हैं। जबकि, β- साइक्लोडेक्सट्रिन गैर-ध्रुवीय अवशेषों के साथ भी इंटरैक्ट कर रहा है जो कि न्यूक्लियेशन आश्रित प्रोटीन एकत्रीकरण के मुख्य घटक हैं। मैक्रोमॉलिक्यूलर समूहन एक महत्वपूर्ण कारक है जो कि कोशिकाओं के अंदर प्रोटीन की स्वयं असेंबली प्रक्रिया और बलगति विज्ञान को नियंत्रित करती है। इसलिए, रेशम फाइब्रेइन के स्वयं असेंबली प्रक्रिया को व्यवस्थित करने के लिए यह एक अन्य तरीका है, रेशम फाइब्रॉइन घोल में मैक्रोमॉलिक्यूलर एजेंट को डालना । हमारे डेटा से पता चला है कि मैक्रोमॉलिक्यूलर समूहन वाले पीईजी (पीईजी 200 और पीईजी 6000) और डेक्सट्रान (डेक्सट्रान 6000 और डेक्सट्रान 70000) परिभाषित पैरामीटर्स के तहत रेशम फाइब्राइन में एक गठनात्मक ट्रांजीशन पैदा कर सकते हैं। इन निष्कर्षों के आधार पर हमने मानव फाइब्रोब्लास्ट पर मैक्रोमॉलिक्यूलर समूहन और रेशम फाइब्रॉइन की भूमिका की जांच की, जिसमें हमने पाया कि कोशिकाओं के संवर्धन को समूहन वाले सूक्ष्म - वातावरण में बाह्य मैट्रिक्स में वृद्धि की जा सकती है।

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