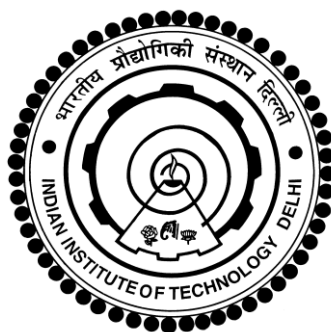


**STUDY OF SILK FIBROIN PROTEIN
REFOLDING AND ITS SELF ASSEMBLY
PROCESS**

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**DEPARTMENT OF TEXTILE TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

APRIL 2018

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by

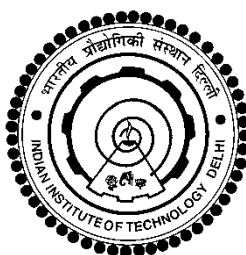
PRIYANKA DUBEY

Department of Textile Technology

Submitted

In fulfilment of the requirements of the degree of Doctor of Philosophy

to the



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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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 में (गैर क्रिस्टलीय) β -शीट क्रिस्टल समृद्ध (रेशम द्वितीय) संरचना से क्रमिक गठनात्मक ट्रांजीशन होते हैं। रेशम फाइब्रॉइन की स्वयं असेंबली प्रक्रिया को विनियमित करके ट्यूनेबल / नियंत्रित माध्यमिक संरचना के साथ अद्वितीय रेशम-आधारित बायोमैटेरियल्स का निर्माण किया जा सकता है। रेशम फाइब्रॉइन 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 फाइब्रॉइन की स्वयं असेंबली प्रक्रिया को नियंत्रित करने से प्रतिरक्षा प्रतिक्रियाओं पर सीधा प्रभाव पड़ सकता है।

वर्तमान थीसिस में रेशम फाइब्रॉइन 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 फाइब्रॉइन 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 कन्फॉर्मेशन मौजूद पाया गया। इसके अलावा, कैल्शियम आयनों के साथ फाइब्रॉइन 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 फाइब्रॉइन का उपयोग कर सकते हैं। यह ध्यान देने योग्य है कि प्रोटीन एकत्रीकरण

कई न्यूरोडीजेनेरेटिव रोगों सहित कई मानव रोग का कारण है। हमने रेशम फाइब्रॉइन 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 है कि अनुकूलित संयोजन के बिना, इथेनॉल के साथ फाइब्रॉइन ने नैनो-फाइब्रिल का गठन दिखाया। हालांकि, करक्यूमिन और बीओ-साइक्लोडेक्सट्रिन के अनुकूलित संयोजन के बगैर 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 फाइब्रॉइन में महत्वपूर्ण अनुपात ($\sim 8\%$) 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 फाइब्रॉइन 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 फाइब्रॉइन 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।

CONTENTS

<i>CERTIFICATE</i>	i
<i>ACKNOWLEDGEMENTS</i>	ii
<i>ABSTRACT</i>	iv
<i>CONTENTS</i>	vi
<i>LIST OF FIGURES</i>	ix
<i>LIST OF TABLES</i>	xv
Chapter 1: Introduction	1-9
1. Introduction	2
1.1 Objectives and thesis organization	4
1.2 References	7
Chapter 2: Review of Literature	10-46
2.1 Silk fibroin isolation methodology	10
2.1.2 Various methods for dissolution of silk fibroin fibers	11
2.2 Self assembly/protein folding of silk fibroin	12
2.3 Different methods for inducing self assembly in silk fibroin	14
2.4 Different applications of modulating the self assembly process of silk fibroin.	16
2.4.1. To fabricate/develop a unique biomaterial for various biomedical applications	17
2.4.2. Role of self assembly in modulating immune responses	18
2.4.3. Role of self assembly in pathological disorders	19
2.5. Present research problem in the context of this thesis	21
2.5.1. Use of calcium ion to modulate the self assembly process of silk fibroin in order to develop a unique biomaterial with desired secondary conformation.	22

2.5.2. Effect of macromolecular crowding on protein aggregation/self assembly of silk fibroin	24
2.5.3. Role of β -cyclodextrin (β -CD) and curcumin on self assembly and aggregation inhibition of Silk Fibroin	27
2.6 References	30
Chapter 3: Modulation of self-assembly process of Fibroin: an insight for regulating the conformation of silk biomaterials	47-78
3.1 Introduction	48
3.2 Methods and Materials	50
3.3 Results	53
3.4 Discussion	64
3.5 References	72
Chapter 4: Synergistic Effect of Curcumin and β-cyclodextrin on Inhibition of Silk Fibroin Self Assembly	79-117
4.1 Introduction	80
4.2 Methods and Materials	83
4.3 Results	86
4.4 Discussion	101
4.5 References	110
Chapter 5: Effect of macromolecular crowders on self assembly process of Silk Fibroin	118-145
5.1 Introduction	119
5.2 Methods and Materials	121
5.3 Results	124
5.4 Discussion	137
5.5 References	140

Chapter 6: Effect of macromolecular crowders and silk fibroin on the behaviour of human fibroblast cells	146-162
6.1 Introduction	147
6.2 Methods and Materials	148
6.3 Results	151
6.4 Discussion	158
6.5 References	162
Chapter 7: Conclusion and Future aspects	165
7.1 Conclusion	166
7.2 Future directions	169
Author's Biography	171-174

LIST OF FIGURES

Fig 2.1	The antiparallel β -sheet structure of silk fibroin. The ribbon representation (pink) is the N-terminal region of <i>Bombyx mori</i> fibroin (PDB ID: 3UA0) visualized using PyMOL software.
Fig 2.2	Chemical structures of used synthetic crowders to mimic the intracellular environment.
Fig 3.1	ThT fluorescence intensity of fibroin was measured at 485 nm with the excitation wavelength at 430 nm. ThT fluorescence of fibroin was recorded from 0 to 200 min under three different incubation conditions: (◆) at 37°C with shaking at 150 rpm, (●) at 37°C without shaking and (♣) at 150 rpm shaking at 25°C.
Fig 3.2	ThT fluorescence intensity of (a) Fibroin incubated for 100 min at 37°C with shaking at 150 rpm with different concentration of CaCl_2 . (b) Fibroin Incubated with optimized concentration of CaCl_2 at 37°C with shaking at 150 rpm. (c) Fibroin incubated with 80% methanol at 37°C with shaking at 150 rpm from 0 to 100 min.
Fig 3.3	Turbidity analysis: (■) Fibroin, (●) Fibroin with optimized concentration of CaCl_2 and (▲) Fibroin with 80% methanol. Absorbance recorded at 600 nm with respect to time. All samples were incubated at 37°C with shaking at 150 rpm.
Fig 3.4	Dynamic light scattering: (a-c) Fibroin at 0 min (initial), 90 (intermediate) and 180 min (final) incubation (ii) (d-f) Fibroin with 100 μM CaCl_2 at 0 (initial), 40 (intermediate) and 80 min (final) incubation (iii) (g-h) Fibroin with 80 % methanol at 0 (initial) and final 100 min. All samples were incubated at 37°C with shaking at 150 rpm.

Fig 3.5	CD spectra were recorded at 25°C from 0 to 200 min at 190 to 250 nm wavelength. All samples were incubated at 37°C with shaking at 150 rpm. (a) Fibroin incubated without adding calcium and methanol. (b) Fibroin incubated with 100 μ M CaCl ₂ from 0 to 200 min (c) Fibroin incubated with 80% methanol from 0 to 100 min.
Fig 3.6	ATR-FTIR of (a-c) Fibroin at 0 (initial), 90 (intermediate) and 180 min (final) incubation (ii) (d-f) Fibroin with 100 μ M CaCl ₂ at 0 (initial), 40 (intermediate) and 80 min (final) incubation (iii) (g) Fibroin with 80 % methanol at 0 min. All samples were incubated at 37°C with shaking at 150 rpm.
Fig 3.7	Molecular modeling of N-terminal sequence of fibroin (PDB id: 3UA0) with CaCl ₂ to determine its binding sites using BION server.
Fig 3.8	Hypothesis of the mechanism involved during conformational transition of fibroin incubated with CaCl ₂ at 37°C with shaking at 150 rpm
Fig 3.9	Fibroin incubated with optimized concentration of CaCl ₂ at 37°C with shaking at 150 rpm showing intermediate α -helical secondary conformations. However, without calcium fibroin is not showing an intermediate α -helical conformation.
Fig 4.1	<i>In silico</i> analysis of fibroin with a) curcumin, b) β -cyclodextrin c) Complex of 1:1 curcumin and β -cyclodextrin and d) 1:2 curcumin and β -cyclodextrin using PatchDock docking server.
Fig 4.2	Congo red absorbance of a) 1 mg/ml fibroin solution incubated with different concentration of ethanol for 75 min at 25°C collected at 540 nm with regular time interval b) Silk fibroin with 20% ethanol in the presence of the CR dye under plane polarized light.
Fig 4.3	Aggregation inhibition of 1mg/ml fibroin incubated with 20% ethanol treated with a) 0-60 μ M of curcumin, b) 0-200 μ M of β -cyclodextrin (β -CD) monitored by

	congo red at 540 nm.
Fig 4.4	Aggregation inhibition of 1mg/ml fibroin solution incubated with 20% ethanol till 75 min by combination of 15 μ M curcumin and 30 μ M β -cyclodextrin at 540 nm detected by Congo red.
Fig 4.5	Turbidity of 1 mg/ml fibroin with 20% ethanol, fibroin with ethanol treated with combination of 15 μ M curcumin and 30 μ M β -cyclodextrin, only silk fibroin (control) was monitored at 600 nm using UV-visible spectrophotometer.
Fig 4.6	ATR-FTIR spectroscopy of a) 1 mg/ml fibroin solution (control) at 0 min b) with 20% ethanol at 75 min and c) fibroin with 20% ethanol treated with combination of curcumin and β -cyclodextrin at 75 min.
Fig 4.7	CD spectroscopy of a) 1 mg/ml fibroin solution at 0 min without ethanol showed random coil structure b) with 20% ethanol at 75 min showed β -sheet conformation and c) fibroin with 20% ethanol treated with combination of curcumin and β -CD at 75 min showed random coil conformation.
Fig 4.8	DLS of a) 1mg/ml fibroin solution (control) at 0 min, b) fibroin solution incubated with 20% ethanol at 75 min and c) fibroin with 20% ethanol treated with combination of curcumin and β -cyclodextrin at 75 min.
Fig 4.9	AFM of a) Only 1 mg/ml fibroin solution (control) at 0 min b) incubated with 20% ethanol at 75 min showed fibrillation c) fibroin with 20% ethanol with combination of curcumin and β -cyclodextrin at 75 min.
Fig 4.10	Congo red data of 5 mg/ml fibroin solution incubated with 20% ethanol at 75 min treated with different concentration of combination of curcumin and β -cyclodextrin.
Fig 4.11	β -sheet alignment of amyloid fibrils and silk fibroin
Fig 4.12	Fibroin self assembly inhibition in the presence of complex of curcumin and β -

	cyclodextrin.
Fig 4.13	Mechanism involved in the aggregation inhibition of in the presence of complex of curcumin and β -cyclodextrin.
Fig 5.1	Turbidity analysis of fibroin (10 mg/ml) incubated with different concentrations (50, 100 and 150 mg/ml) of PEG200 and PEG 6000 were collected at regular interval at 600 nm.
Fig 5.2	Turbidity analysis of fibroin (10mg/ml) incubated with different concentrations (50, 100 and 150 mg/ml) of Dextran 6000 and 70000 was collected at regular interval at 600 nm.
Fig 5.3	Aggregation kinetics of fibroin measured by Thioflavin T fluorescence. Aggregation kinetics of fibroin incubated with (a) PEG 200 (b) PEG 6000 (c) Dextran 6000 and (d) Dextran 70000. All spectra were recorded at three different concentration of crowders (50, 100 and 150 mg/ml) along with control (fibroin without crowder) at 25°C.
Fig 5.4	CD spectroscopy of 10 mg/ml fibroin incubated with different crowders at initial and final time point of all different concentration of the respective crowders.
Fig 5.5	ATR-FTIR of fibroin with 50, 100 and 150 mg/ml concentration of PEG-200 at initial (0 min) and their subsequent final time point.
Fig 5.6	ATR-FTIR of fibroin with 50, 100 and 150 mg/ml concentration of PEG-6000 at initial (0 min) and their subsequent final time point.
Fig 5.7	ATR-FTIR of fibroin with 50, 100 and 150 mg/ml concentration of Dex-6000 at initial (0 min) and their subsequent final time point.
Fig 5.8	ATR-FTIR of fibroin with 50, 100 and 150 mg/ml concentration of Dex-70000 at initial (0 min) and their subsequent final time point.
Fig 5.9	ATR-FTIR spectra of fibroin recorded at 35 hrs without any crowder.

Fig 5.10	AFM of fibroin incubated with PEG 200 at 0 min with a) 50 mg/ml b) 100 mg/ml c) 150 mg/ml. The final aggregation time point of fibroin with d) 50 mg/ml e) 100 mg/ml f) 150 mg/ml.
Fig 5.11	AFM of fibroin incubated with PEG 6000 at 0 min with a) 50 mg/ml b) 100 mg/ml c) 150 mg/ml. However, d-f are representing a final aggregation time point of fibroin with d) 50 mg/ml e) 100 mg/ml f) 150 mg/ml.
Fig 5.12	AFM of fibroin incubated with dextran 6000 at 0 min with a) 50 mg/ml b) 100 mg/ml c) 150 mg/ml. The final aggregation time point of fibroin with d) 50 mg/ml e) 100 mg/ml f) 150 mg/ml of dextran 6000.
Fig 5.13	AFM of fibroin incubated with dextran 70000 at 0 min with a) 50 mg/ml b) 100 mg/ml c) 150 mg/ml. However, d-f is demonstrating the final aggregation time point of fibroin with d) 50 mg/ml e) 100 mg/ml f) 150 mg/ml.
Fig 6.1	Immunostaining of Human fibroblast cell in the presence of different crowders at day 1 fibronectin -green) and DAPI-blue).
Fig 6.2	Immunostaining of Human fibroblast cell in the presence of different crowders at day 4 fibronectin -green) and DAPI-blue).
Fig 6.3	Immunostaining of Human fibroblast cell in the presence of different crowders at day 8 fibronectin -green) and DAPI-blue).
Fig 6.4	Collagen estimation of human fibroblast cells cultured in the presence of macromolecular crowders and SF at day 1, 4 and 8. The Total collagen content of all groups were normalized to their respective total DNA contents at day 1, 4 and 8.
Fig 6.5	Quantitative Real-time PCR studies to evaluate the fibronectin gene expression in fibroblast cells cultured in the presence of macromolecular crowders and SF at day 1, 4 and 8

Fig 6.6	Quantitative Real-time PCR studies to evaluate the collagen I gene expression in fibroblast cells cultured in the presence of macromolecular crowders and SF at day 1, 4 and 8
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LIST OF TABLES

Table 2.1	Summary of different dissolution methods of silk.
Table 2.2	Methodology inducing secondary conformation in silk fibroin.
Table 3.1	ATR-FTIR deconvolution data of the Amide I region ($1600\text{--}1700\text{ cm}^{-1}$) of (i) Fibroin (ii) Fibroin with $100\text{ }\mu\text{M}$ CaCl_2 (iii) Fibroin with 80 % methanol. All samples were incubated at 37°C with shaking at 150 rpm.
Table 3.2	Amino acids involved in interaction between N-terminal of fibroin protein and calcium ions using molecular modeling by BION server. Server predicted 36 amino acids which includes 14 aspartate, 8 glutamate with 5 threonine, 2 phenylalanine and one each of alanine, asparagine, arginine, serine, glycine, isoleucine and valine residues.
Table 4.1	Fibroin docked with curcumin.
Table 4.2	Fibroin docked with β -Cyclodextrin.
Table 4.3	Fibroin docked with 1:1 complex of Curcumin- β -Cyclodextrin.
Table 4.4	Fibroin docked with 1:2 complex of Curcumin- β -Cyclodextrin.
Table 4.5	ATR-FTIR of Fibroin at 0 min, Fibroin with 20% ethanol at 75 min and Fibroin with 20% ethanol treated with curcumin and β -cyclodextrin in the Amide I Region ($1600\text{--}1700\text{ cm}^{-1}$).