A COMPREHENSIVE STUDY OF TRANSFORMING GROWTH FACTOR BETA (TGFβ) 3: TOWARDS IMPROVED SOLUBILITY, REFOLDING MODULATION AND BINDING INTERACTIONS

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by

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Dedicated to my parents, aunt, husband and my beloved late grandmother

CERTIFICATE

This is to certify that the thesis titled "**A Comprehensive Study of Transforming Growth Factor (TGFβ) 3: Towards Improved Solubiity, Refolding Modualtion and Binding Interaction**" being submitted by **Ms. Amrita Dawn** to the Indian Institute of Technology Delhi for the award of degree of **Doctor of Philosophy** is a record of bonafide research work carried out by her.

Ms. Amrita Dawn has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard.

The results contained in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.

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Amrita Dawn

ABSTRACT

The thesis entitled **"A comprehensive study of Transforming growth factor beta (TGFβ) 3: towards improved solubility, refolding modulation and binding interactions"** presents a detailed and systematic study of the expression, purification, refolding and interaction.

Chapter 1 (Introduction) introduces the TGFβ family of cytokines with main focus on TGFβ3, structural and functional aspects of this protein and its potential as a biotherapeutic agent. It provides a brief description on the molecular and chemical chaperones, polyphenols as folding modulators and thermodynamics of protein-protein interactions.

Chapter 2 **(Materials and Methods)** describes procurement of chemicals and reagents, along with techniques used during the investigation specifically, AKTA based chromatography, SDS (reducing and non-reducing) and Native PAGE, Isothermal Titration Calorimetry (ITC), UV-VIS spectroscopy, Circular Dichroism (CD).

Chapter 3 [**Expression, purification and characterization of TGFβ3 and its two receptors; Type II (TβRII) &Type I (TβRI)]** presents in detail the multistep schemes of expression and purification of TGFβ3 and its two receptors (Type II and Type I), problems associated with them and certain optimization and characterization of the proteins.

In Chapter 4 (**Exploiting the folding modulators for a switch from misfolded to folded TGFβ3 during over-expression in *Escherichia coli*)** we carried out co-expression experiments of TGFβ3 with chaperones and demonstrated that by coordinated action of a combination of DnaK-DnaJ-GrpE and GroESL, it is possible to achieve native soluble conformation of TGFβ3 during over-expression in *E coli*. In addition, we have shown that presence of osmolytes in the form of sorbitol

or trehalose in the growth media had an appreciable impact on the solubility of TGFβ3. A synergistic approach of both molecular and chemical chaperones were also taken. The functionality of soluble TGFβ3 was confirmed by performing binding interactions with its cognate receptor TβRII. Our study delineates the fact that an effective combination of chaperones or optimum concentration of compatible osmolyte, can efficiently abrogate competing aggregation pathways and help attain the native conformation of a cysteine rich cytokine in a facile manner.

In Chapter 5 (**Natural polyphenols in combination with β-cyclodextrin can be effectively used to increase the yield of native TGFβ3 during refolding)** we have exploited polyphenols beyond their conventional roles of being aggregation alleviators. Several polyphenols (EGCG, baicalein, myricetin) either alone or in combination with the pseudo-chaperone beta cyclodextrin were added in the refolding buffer of TGFβ3 in an attempt to increase its refolding yield. With the help of non-reducing SDS PAGE and size exclusion chromatography, we showed that the presence of baicalein or EGCG along with β-CD indeed increase the relative yield of the native protein in a time dependent manner in comparison to the control with no additives. EGCG expedites the refolding process giving a maximum increase within twenty four hours while baicalein takes as long as forty eight hours to provide an optimum amount of refolded protein. Thus these small molecules provide a promising alternate route in increasing the yield of aggregation prone proteins during refolding.

Chapter 6 (**Effect of synthetic membranes on refolding of TGFβ3)** presents a study of the effects of membanes on refolding of TGFβ3. Surfaces (provided by AMT Inc; USA) with varying chemical and physical properties were tested to understand the effect of smooth or rough polymeric surfaces on protein folding. We demonstrated that presence of an uncoated or aminated hollow

fiber membranes (HFM) with open pores can greatly increase the relative yield of the native TGFβ3 after refolding in a dose dependent manner while coating the surfaces with polymers of siloxane having a closed pore structure could do so only to a marginal level (in comparison to control with no membrane).

In Chapter 7 (**Interaction of TGFβ3 with its receptors, TβRII and TβRI: a unique mechanism for protein-protein association)** we present an in-depth profiling of the binding mechanisms of TGFβ3 ligand with its type II and type I receptors (TβRII and TβRI) using isothermal titration calorimetry (ITC). Studies were carried out in acidic pH as it has great physiological relevance in terms of TGFβ3 activity. Our findings reveal an unusual entropy-driven binary complex formation between TGFβ3 and TβRII. Recruitment of TβRI to the aforementioned complex leading to a ternary unit, however is associated with a distinct shift towards enthalpy dominated thermodynamics. Binding studies of TGFβ3 and TβRII are also carried out at different temperature, pH, salt concentration to gain further insight on the thermodynamics of the interaction.

सार

थीसिस "**ट्रांसफॉर्मिंग ग्रोथ फैक्टर बीटा (TGFβ3) का एक व्यापक अध्ययन 3: बेहतर घुलनशीलता की ओर, मॉड्यूलेशन और बाइंडिंग इंटरैक्शन की ओर**" शीर्षक का एक विस्तृत अध्ययन अभिव्यक्ति, शुद्धिकरण, रीफॉल्डिंग और इंटरैक्शन का विस्तृत अध्ययन प्रस्तुत करता है।

अध्याय 1 (परिचय) TGFβ3पर मुख्य फोकस के साथ साइटोकिन्स के TGF (परिवार का परिचय देता है, इस प्रोटीन के संरचनात्मक और कार्यात्मक पहलुओं और एक जैव-चिकित्सीय एजेंट के रूप में इसकी क्षमता। यह एक संक्षिप्त विवरण प्रदान करता है आणविक और रासायनिक chaperones, तह modulators और प्रोटीन-प्रोटीन बातचीत के ऊष्मप्रवैगिकी के रूप में पॉलीफेनोल्स।

अध्याय 2 (सामग्री और तरीके) विशेष रूप से जांच के दौरान इस्तेमाल की जाने वाली तकनीकों के साथ-साथ AKTA आधारित क्रोमैटोग्राफी, एसडीएस (कम करने और गैर-कम करने) और मूल पृष्ठ, इसोथर्मल अनुमापन कैलोरिमित्री (ITC), यूवी-विज़ स्पेक्ट्रोस्कोपी के साथ रसायनों और अभिकर्मकों की खरीद का वर्णन करता है।, वृत्ताकार द्वैतवाद (सीडी)।

अध्याय 3 [TGFβ3और इसके दो रिसेप्टर्स की अभिव्यक्ति, शुद्धि और लक्षण वर्णन; टाइप II (TβRII) और टाइप I (TβRI)] TGF of3 और इसके दो रिसेप्टर्स (टाइप II और टाइप I) की अभिव्यक्ति और शुद्धिकरण की बहुस्तरीय योजनाओं, उनके साथ जुड़ी समस्याओं और निश्चित अनुकूलन और लक्षण वर्णन के बारे में विस्तार से प्रस्तुत करता है। प्रोटीन का।

अध्याय 4 में (एस्चेरिशिया कोली में ओवर-एक्सप्रेशन के दौरान मिसगॉल्ड से मुड़े हुए टीजीएफबी 3 पर स्विच के लिए फोल्डिंग मोडुलेटर का उपयोग करते हुए) हमने टीजीएफ 3 के सह-अभिव्यक्ति प्रयोगों को चपेरोन के साथ किया और यह प्रदर्शित किया कि DnaK-DnaJ-संयोजन के समन्वित कार्रवाई द्वारा। -

GrpE और GroESL, ई कोलाई में अति-अभिव्यक्ति के दौरान TGFβ3 के मूल घुलनशील विरूपण को प्राप्त करना संभव है। इसके अलावा, हमने सोर्बिटोल के रूप में ऑस्मोलिट्स की उपस्थिति को दिखाया है या ग्रोथ मीडिया में trehalose का TGFβ3 की घुलनशीलता पर एक सराहनीय प्रभाव था। आणविक और रासायनिक दोनों चापाकलों का सहक्रियात्मक दृष्टिकोण भी लिया गया। घुलनशील TGFβ3 की कार्यक्षमता को उसके संज्ञानात्मक रिसेप्टर TβRII के साथ बाध्यकारी बातचीत करके पुष्टि की गई थी। हमारा अध्ययन इस तथ्य को उजागर करता है कि चैपोरों का एक प्रभावी संयोजन या संगत ओस्मोलिएट का इष्टतम एकाग्रता, कुशलता से प्रतिस्पर्धी एकत्रीकरण मार्गों को निरस्त कर सकता है और एक सिस्टीन समृद्ध साइटोकिन के मूल विरूपण को एक सहज तरीके से प्राप्त करने में मदद कर सकता है।

अध्याय 5 में (β-cyclodextrin के साथ संयोजन में प्राकृतिक पॉलीफेनोल्स को प्रभावी ढंग से देशी (native) TGFβ3 की उपज को बढ़ाने के दौरान उपयोग किया जा सकता है) हमने पॉलीफेनोल्स को एकत्रीकरण अभियोक्ता होने की उनकी पारंपरिक भूमिकाओं से परे शोषण किया है। कई पॉलीफेनोल्स (EGCG, baicalein, myricetin) या तो अकेले या छद्म-चैपरोन बीटा साइक्लोडेक्स्ट्रिन के साथ संयोजन में TGFβ3 की रिफ़ंडिंग बफर में इसकी रिफंडिंग उपज बढ़ाने के प्रयास में जोड़े गए थे। एसडीएस पृष्ठ और आकार अपवर्जन क्रोमैटोग्राफी को कम न करने की मदद से, हमने दिखाया कि β-CD के साथ बालिकलिन या ईजीसीजी की उपस्थिति वास्तव में बिना किसी एडिटिव्स की तुलना में एक समय पर निर्भर तरीके से देशी प्रोटीन की सापेक्ष उपज को बढ़ाती है। । ईजीसीजी रिफ्लेडिंग प्रक्रिया को चौबीस घंटे के भीतर अधिकतम वृद्धि प्रदान करता है जबकि बायालिसिन को अधिकतम आठ घंटे लगते हैं, ताकि अधिकतम मात्रा में रिफॉल्ड प्रोटीन मिल सके। इस प्रकार ये छोटे अणु, रिफंडिंग के दौरान एकत्रीकरण प्रवण प्रोटीन की उपज को बढ़ाने के लिए एक आशाजनक वैकल्पिक मार्ग प्रदान करते हैं।

अध्याय 6 (TGFβ3 की रीफिलिंग पर सिंथेटिक झिल्लियों का प्रभाव) TGFβ3 की रीफिलिंग पर मेमबन्स के प्रभावों का एक अध्ययन प्रस्तुत करता है। विभिन्न रासायनिक और भौतिक गुणों के साथ सतहों (AMT

इंक; यूएसए द्वारा प्रदान) का परीक्षण प्रोटीन तह पर चिकनी या खुरदरी पॉलिमर सतहों के प्रभाव को समझने के लिए किया गया था। हमने प्रदर्शित किया कि खुले छिद्रों के साथ एक uncoated या संशोधित खोखले फाइबर झिल्ली (HFM) की उपस्थिति देशी TGFβ3 की सापेक्ष उपज को बहुत हद तक खुराक पर निर्भर तरीके से वापस करने के बाद बढ़ा सकती है, जबकि सिलोक्सेन के पॉलिमर के साथ सतहों को बंद छिद्र संरचना होने के कारण ऐसा केवल एक सीमांत स्तर तक (बिना किसी झिल्ली के नियंत्रण की तुलना में) कर सकता था।

अध्याय 7 में (अपने रिसेप्टर्स के साथ TGFβ3 की बातचीत, TβRII और TβRI: प्रोटीन-प्रोटीन संघ के लिए एक अनूठा तंत्र) हम अपने टाइप II और टाइप I रिसेप्टर्स में TGFβ3 लिगेंड के बंधन तंत्र की गहराई से रूपरेखा प्रस्तुत करते हैं। (TβRII और TβRI) इज़ोटेर्माल अनुमापन कैलेरीमेट्री (ITC) का उपयोग कर। अध्ययन अम्लीय पीएच में किए गए क्योंकि TGFβ3 गतिविधि के संदर्भ में इसकी महान शारीरिक प्रासंगिकता है। हमारे निष्कर्षों में TGFβ3 और TβRII के बीच एक असामान्य एन्ट्रापी-चालित बाइनरी कॉम्प्लेक्स फॉर्मेशन का पता चलता है। TβRI की भर्ती पूर्वोक्त परिसर में एक टेर्नेरी यूनिट के लिए होती है, जो कि थैलेपी डोमिनेटेड थर्मोडायनामिक्स की ओर एक अलग बदलाव के साथ जुड़ा हुआ है। TGFβ3 और TβRII के बंधन अध्ययन भी बातचीत के ऊष्मागतिकी पर अधिक जानकारी हासिल करने के लिए अलग-अलग तापमान, पीएच, नमक एकाग्रता पर किए जाते हैं।

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