

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

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**DEPARTMENT OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY DELHI
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INTERACTIONS**

by

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Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the



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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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ABSTRACT

The thesis entitled “**A comprehensive study of Transforming growth factor beta (TGF β) 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 TGF β family of cytokines with main focus on TGF β 3, 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 TGF β 3 and its two receptors; Type II (T β RII) & Type I (T β RI)**] presents in detail the multistep schemes of expression and purification of TGF β 3 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 TGF β 3 during over-expression in *Escherichia coli***) we carried out co-expression experiments of TGF β 3 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 TGF β 3 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 TGF β 3. A synergistic approach of both molecular and chemical chaperones were also taken. The functionality of soluble TGF β 3 was confirmed by performing binding interactions with its cognate receptor T β RII. 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 TGF β 3 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 TGF β 3 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 TGF β 3**) presents a study of the effects of membranes on refolding of TGF β 3. 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 TGF β 3 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 TGF β 3 with its receptors, T β RII and T β RI: a unique mechanism for protein-protein association**) we present an in-depth profiling of the binding mechanisms of TGF β 3 ligand with its type II and type I receptors (T β RII and T β RI) using isothermal titration calorimetry (ITC). Studies were carried out in acidic pH as it has great physiological relevance in terms of TGF β 3 activity. Our findings reveal an unusual entropy-driven binary complex formation between TGF β 3 and T β RII. Recruitment of T β RI to the aforementioned complex leading to a ternary unit, however is associated with a distinct shift towards enthalpy dominated thermodynamics. Binding studies of TGF β 3 and T β RII 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 (TGF β 3) 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) TGF β 3 पर 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 [TGF β 3 और इसके दो रिसेप्टर्स की अभिव्यक्ति, शुद्धि और लक्षण वर्णन; टाइप II (T β RII) और टाइप I (T β RI)] TGF α 3 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 मोड्युलेटर 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 TGF β 3 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 TGF β 3 की घुलनशीलता पर एक सहायनीय प्रभाव था। आणविक और रासायनिक दोनों चापाकलों का सहक्रियात्मक दृष्टिकोण भी लिया गया। घुलनशील TGF β 3 की कार्यक्षमता को उसके संज्ञानात्मक रिसेप्टर T β RII के साथ बाध्यकारी बातचीत करके पुष्टि की गई थी। हमारा अध्ययन इस तथ्य को उजागर करता है कि चैपरोन 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) TGF β 3 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 TGF β 3 की रिफ़ोल्डिंग बफर में इसकी रिफ़ोल्डिंग 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 रिफ़ोल्डिंग प्रक्रिया को चौबीस घंटे के भीतर अधिकतम वृद्धि प्रदान करता है जबकि बायालिसिन को अधिकतम आठ घंटे लगते हैं, ताकि अधिकतम मात्रा में रिफ़ोल्ड प्रोटीन मिल सके। इस प्रकार ये छोटे अणु, रिफ़ोल्डिंग के दौरान एकत्रीकरण प्रवण प्रोटीन की उपज को बढ़ाने के लिए एक आशाजनक वैकल्पिक मार्ग प्रदान करते हैं।

अध्याय 6 (TGF β 3 की रीफिलिंग पर सिंथेटिक झिल्लियों का प्रभाव) TGF β 3 की रीफिलिंग पर मेमब्रान्स 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 TGF β 3 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 TGF β 3 की बातचीत, T β RII और T β RI: प्रोटीन-प्रोटीन संघ के लिए एक अनूठा तंत्र) हम अपने टाइप II और टाइप I रिसेप्टर्स में TGF β 3 लिगेंड के बंधन तंत्र की गहराई से रूपरेखा प्रस्तुत करते हैं। (T β RII और T β RI) इज़ोटेर्मल अनुमापन कैलेरीमेट्री (ITC) का उपयोग कर। अध्ययन अम्लीय पीएच में किए गए क्योंकि TGF β 3 गतिविधि के संदर्भ में इसकी महान शारीरिक प्रासंगिकता है। हमारे निष्कर्षों में TGF β 3 और T β RII के बीच एक असामान्य एन्ट्रापी-चालित बाइनरी कॉम्प्लेक्स फॉर्मेशन का पता चलता है। T β RI की भर्ती पूर्वोक्त परिसर में एक टेनरी यूनिट के लिए होती है, जो कि थैलेपी डोमिनेटेड थर्मोडायनामिक्स की ओर एक अलग बदलाव के साथ जुड़ा हुआ है। TGF β 3 और T β RII 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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