

**STUDIES ON INTERACTION OF CD11bA DOMAIN OF β_2
(CD11b/CD18) INTEGRIN WITH METAL IONS AND SMALL
MOLECULES**

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INTEGRIN WITH METAL IONS AND SMALL MOLECULES

by

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CERTIFICATE

This is to certify that the thesis which is entitled as ‘**Studies on CD11bA domain of β_2 (CD11b/CD18) integrin with various metal ions and small molecules**’ being submitted by “**Mr. Vinay Kumar**” to the “Indian Institute of Technology Delhi” for the award of “**Doctor of Philosophy**” is a record of bonafide research work carried out by him. He has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis.

To the best of my knowledge, the results contained in this thesis have not been submitted in part or full to any other university or institute for award of any degree or diploma.

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*Whatever you are going through, just be positive. There is light somewhere. But you have to go on.
What matters is moving forward and not the speed.*

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ABSTRACT

Understanding the complexity and regulation of immune system in our body is a challenging task. Deregulation of these immune responses has been reported in several diseases as disparate as cancer, chronic inflammation, neurological disorder such as Parkinson's, and Alzheimer's. Regulation of these immune responses is complex and involves a number of factors. Integrins are trans-membrane receptor proteins involved in bidirectional signaling across the cell membrane. Leukocytic "CD11b/CD18" integrin proteins are involved in cell-cell or cell-ECM adhesion, and thus required for cells growth and functions. Apart from their role in organ development, they participate in immune responses against infection and autoimmune diseases. Therefore, they are potential therapeutic target for drug discovery in the treatment of autoimmune diseases such as multiple sclerosis and psoriasis.

This thesis entitled as '**Studies on CD11bA domain of β_2 (CD11b/CD18) integrin with various metal ions and small molecules**' consists of seven chapters.

The **Chapter-1 "Introduction"** has discussed some of the important features of integrin protein along with the outline of work proposed in the context of problems.

Chapter-2 of this thesis "**Materials and methodologies**" describes the expression and purification protocol used for various variants of integrin, with various biophysical techniques used for the study.

Chapter-3 of this thesis deals with the characterization of the CD11bA protein constructs in the absence or presence of various metal ions and agonist (LA-1) was done by using fluorescence and CD spectroscopy. The results of this chapter suggest that stability of the CD11bA protein can be regulated by activation of integrin (removal of isoleucine), metal ions, and certain ligands (LA-1).

Chapter-4 deals with energetics of binding CD11bA domain with metal ions in the absence or presence of agonist (LA-1). This chapter suggests that binding affinity of metal ions to CD11bA domain can be regulated by activation state of the integrin and LA-1, an agonist.

In the **Chapter-5**, binding affinity of the agonist (LA-1) was directly determined with different constructs of CD11bA domain. Energetics of binding of LA-1 to CD11bA domain, in the absence or presence of various metal ions (Mn^{2+} , Mg^{2+} , and Ca^{2+}) has also been reported. It concludes that binding affinity of leukadhrin-1 (LA-1) to CD11bA can be

regulated by certain factors such as temperature, pH of the buffer, conformation of integrin (I316G), various metal ions (Mn^{2+} , Mg^{2+} , and Ca^{2+}), and the length of activation sensitive helix. In short, our results suggest that LA-1 and metal ions mutually regulate their binding affinity to CD11bA domain.

In the **Chapter-6**, three different polyphenols (curcumin, resveratrol and EGCG) have been tested as the binding partner of the CD11bA domain. Energetics of the binding of these polyphenols to CD11bA domain has been reported with the order of binding affinity: curcumin > resveratrol > EGCG. It was also observed that curcumin and resveratrol significantly increases the binding affinity of Mn^{2+} , Mg^{2+} , and Ca^{2+} ion by increasing the favourable enthalpy during the binding reaction. In another set of experiments, Mn^{2+} and Mg^{2+} ion also increases the binding affinity of these polyphenols to CD11bA constructs by increasing the favourable enthalpy change during the binding process. This result indicates that these metal ions provides favourable binding environment to these polyphenols in contrast to CD11bA alone. Therefore, this chapter concludes that metal ions and these polyphenols mutually regulate their binding affinity to CD11bA protein.

At the last “**Chapter-7**” of this thesis is entitled as “**Summary and Future perspectives**” has outlined with the key observations and salient future of the whole thesis. Future perspectives of the whole study have also been reported.

सार

हमारे शरीर में प्रतिरक्षा प्रणाली की जटिलता और विनियमन को समझना एक चुनौतीपूर्ण कार्य है। इन प्रतिरक्षा प्रतिक्रियाओं के नियंत्रण को कई रोगों में बताया गया है जैसे कि कैंसर, पुरानी सूजन, पार्किंसंस जैसे न्यूरोलॉजिकल डिसऑर्डर, और अल्जाइमर के रूप में भिन्न। इन प्रतिरक्षा प्रतिक्रियाओं का विनियमन जटिल है और कई कारक शामिल हैं इंटीग्रस कोशिका झिल्ली में द्विदिश संकेतन में शामिल ट्रांस-झिल्ली रिसेप्टर प्रोटीन होते हैं। ल्यूकोसाइटिक "सीडी 11 बी / सीडी 18" इंटीग्रिन प्रोटीन कोशिका कोशिका या सेल-ईसीएम आसंजन में शामिल है, और इस तरह कोशिकाओं के विकास और कार्यों के लिए आवश्यक है। अंग विकास में उनकी भूमिका के अलावा, वे संक्रमण और स्वतः प्रतिरक्षी रोगों के प्रति प्रतिरक्षा प्रतिक्रियाओं में भाग लेते हैं। इसलिए, वे कई प्रकार के स्केलेरोसिस और सोरायसिस जैसे ऑटोइम्यून रोगों के उपचार में दवा की खोज के लिए संभावित चिकित्सीय लक्ष्य हैं। इस थीसिस को विभिन्न धातु आयनों और छोटे अणुओं के साथ $\beta 2$ (CD11b / CD18) इंजिनिन के सीडी 11 बीए डोमेन पर अध्ययन के रूप में हकदार "सात अध्यायों के होते हैं

अध्याय-1 "परिचय" ने समस्याओं के संदर्भ में प्रस्तावित कार्य की रूपरेखा के साथ-साथ एकीकृत प्रोटीन की कुछ महत्वपूर्ण विशेषताओं पर विचार-विमर्श किया है।

इस थीसिस "सामग्री और पद्धतियों" के अध्याय -2 अध्ययन के लिए इस्तेमाल की जाने वाली विभिन्न बायोफिजिकल तकनीकों के साथ, अभिव्यक्ति और शुद्धि प्रोटोकॉल का उपयोग अभिकलन के विभिन्न रूपों के लिए किया गया है।

इस थीसिस के अध्याय -3 अनुपस्थिति में सीडी 11 बीए प्रोटीन के लक्षण वर्णन के साथ संबंधित है या विभिन्न धातु आयनों और एगोनिस्ट (एलए -1) की उपस्थिति प्रतिदीप्ति और सीडी स्पेक्ट्रोस्कोपी का उपयोग करके किया गया था। इस अध्याय के नतीजे बताते हैं कि सीडी 11 बीए प्रोटीन की स्थिरता को इंटीग्रिन (आइसोलीयुसीन को हटाने), धातु आयनों और कुछ लिगैंड (एलए -1) के सक्रियण द्वारा नियंत्रित किया जा सकता है।

अध्याय -4 अनुपस्थिति या एगोनिस्ट (एलए -1) की उपस्थिति में धातु आयनों के साथ बाध्यकारी CD11bA डोमेन के ऊर्जागृहों से संबंधित है। इस अध्याय से पता चलता है कि धातु आयनों की सीडी 11 बीए डोमेन को बाध्य करने के संबंध में एग्निमिन और एलए -1 की सक्रियण अवस्था द्वारा विनियमित किया जा सकता है, एक एगोनिस्ट।

अध्याय -5 में, एगोनिस्ट (ला-1) के बाध्यकारी संबंध सीडी 11 बीए डोमेन के विभिन्न निर्माणों के साथ सीधे निर्धारित किए गए थे। अनुपस्थिति में या विभिन्न धातु आयनों (एमएन 2 +, एमजी 2 + और सीए 2 +) की उपस्थिति में एलए -1 से सीडी 11 बीए डोमेन के बंधन की ऊर्जा भी सूचित की गई है। यह निष्कर्ष निकाला है कि लेक्ट्रिन -1 (एलए -1) से सीडी 11 बीए को बाध्य करने के संबंध में कुछ कारक जैसे कि तापमान, बफर के पीएच, इंटीग्रिन की संरचना (आई 316 जी), विभिन्न धातु आयनों (एमएन 2 +, एमजी 2 + और सीए 2 +) से विनियमित किया जा सकता है, और सक्रियण संवेदनशील हेलिक्स की लंबाई संक्षेप में, हमारे परिणाम बताते हैं कि एलए -1 और मेटल आयनों ने सीडी 11 बीए डोमेन में पारस्परिक रूप से अपने बाध्यकारी संबंध को विनियमित किया है।

अध्याय -6 में, तीन अलग-अलग पॉलीफेनोल (कैर्क्यूमिन, रिबेरेट्रोल और ईजीसीजी) को सीडी 11 बीए डोमेन के बंधन भागीदार के रूप में परीक्षण किया गया है। बाध्यकारी आत्मीयता के आदेश के साथ इन पॉलीफेनॉल्स की बाध्यता के लिए सीडी 11 बीए डोमेन के एनरगेटिक्स की सूचना दी गई है: कैर्क्यूमिन > रिबेरेट्रॉल > ईजीसीजी यह भी देखा गया है कि कैर्क्यूमिन और रिबेस्ट्रेटोल एमएन 2 +, एमजी 2 + और सीए 2 + आयन की बाध्यकारी संबंध को काफी बाध्यकारी प्रतिक्रिया के दौरान अनुकूल उत्साह को बढ़ाकर बढ़ाता है। प्रयोगों के एक अन्य सेट में, एमएन 2 + और एमजी 2 + आयन बाध्यकारी प्रक्रिया के दौरान अनुकूल उत्साही बदलाव को बढ़ाकर सीडीओ पॉबोफेनोल की बाध्यता के संबंध को सीडी 11 बीए के निर्माण में बढ़ा देता है। यह परिणाम इंगित करता है कि ये धातु आयन अकेले सीडी 11 बीए के विपरीत इन पॉलीफेनॉल के लिए अनुकूल बाध्यकारी पर्यावरण प्रदान करते हैं। इसलिए, यह अध्याय यह निष्कर्ष निकाला है कि धातु आयनों और ये पॉलीफेनॉल पारस्परिक रूप से अपने बाध्यकारी संबंध सीडी 11 बीए प्रोटीन को विनियमित करते हैं।

इस थीसिस के आखिरी "अध्याय -7" में "सारांश और भविष्य के परिप्रेक्ष्य" के रूप में हकदार है, पूरे टिप्पणियों की प्रमुख टिप्पणियों और मुख्य भविष्य के साथ रेखांकित किया गया है। पूरे अध्ययन के भविष्य के परिप्रेक्ष्य भी सूचित किए गए हैं।

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LIST OF ABBREVIATIONS

1.	IPTG	β-D-1-thiogalactopyranoside
2.	ITC	Isothermal Titration Calorimetry
3.	LB Media	Luria Broth Media
4.	NMR	Nuclear Magnetic Resonance
5.	PBS	Phosphate-Buffered Saline
6.	PCR	Polymerase Chain Reaction
7.	SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
8.	TEV protease	Tobacco Etch Virus Protease
9.	EC	Endothelial cells
10.	TM	TM
11.	Tris	Tris (hydroxymethyl) Aminomethane
12.	ΔH	Change in Enthalpy
13.	ΔS	Change in Entropy
14.	ΔG	Change in Free Energy
15.	ΔT	Change in Temperature
16.	T_m	Melting Temperature
17.	ATP	Adenosine Triphosphate
18.	CD	Circular Dichroism
19.	DSC	Differential Scanning Calorimetry
20.	DTT	Dithiothreitol
21.	EDTA	Ethylene Diamine Tetraacetic Acid
22.	ECM	Extracellular Matrix
23.	GPCR	G Protein-Coupled Receptor
24.	GST	Glutathione S-Transferase
25.	FPLC	Fast protein Liquid Chromatography
26.	SEC	Size Exclusion Chromatography
27.	ICAM	Intercellular Adhesion Molecule
28.	WT	Wild Type
29.	ITGAM	Integrin Alpha M
30.	CD11b	Cluster of Differentiation Molecule 11b
31.	Mac-1	Macrophage-1 Antigen
32.	CR3	Complement Receptor 3
33.	LA-1	Leukadherin-1(agonists)
34.	mAb	Monoclonal Antibody
35.	I316G	Isoleucine Mutated by Glycine at position 316 of CD11bA
36.	B-H Method	Benesi-Hildebrand Method
37.	CD11bA/ $\alpha A/\alpha I/\alpha_m$)	Binding Domain of CD11b/CD18 Integrin
38.	CD11bA (active)	Open Conformation of CD11bA (or high affinity state)
39.	CD11bA (inactive)	Closed Conformation of CD11bA (or low affinity state)
40.	CD11bA ¹¹¹⁻³²¹ (inactive)	CD11bA Binding Domain Comprises Amino Acids From 111-321
41.	CD11bA ^{111-321(I316G)} (active)	CD11bA Binding Domain Comprises Amino Acids From 111- 321, With Substitution of Isoleucine By Glycine at Position 316
42.	CD11bA ¹¹¹⁻³¹⁸	CD11bA Domain Comprises Amino Acids From 111-318 (with Glycine at 316 Position)
43.	CD11bA ¹¹¹⁻³¹⁵ (active)	CD11bA Domain Comprises Amino Acids From 111-318 (without glycine at 316 position)