

**STABILITY, AGGREGATION AND REFOLDING STUDIES OF
HUMAN CARBONIC ANHYDRASE II**

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**DEPARTMENT OF CHEMISTRY
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**STABILITY, AGGREGATION AND REFOLDING STUDIES OF
HUMAN CARBONIC ANHYDRASE II**

by

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Department of Chemistry

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CERTIFICATE

This is to certify that the thesis entitled, “*Stability, aggregation and refolding studies of Human Carbonic Anhydrase II*”, being submitted by Ms **Preeti Gupta** to the **Indian Institute of Technology Delhi** for the award of the degree of **Doctor of Philosophy** in Chemistry is a record of bona fide research work carried out by her. Ms **Preeti Gupta** 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 dissertation have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

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ABSTRACT

Protein misfolding and aggregation have implications in number of devastating diseases and in production of large amounts of proteins in biotechnological industries. Hence, it is vital to understand and control undesirable aggregation process to prevent amyloid-based disorders and production of therapeutic proteins. The physico-chemical environment of protein exerts a strong influence on various aspects of protein aggregation such as onset, aggregation rate, and the final morphology of the aggregated state. Once it is established that how the physico-chemical determinants for aggregation modulate the stability and aggregation propensity of protein, the information can be used almost straightforwardly for getting insights into aggregation mechanism and for devising strategies to control/modulate them according to the requirement. With this aim, we tried to investigate the effect of changing protein environment or solution conditions on the aggregation tendency of protein. The thesis entitled “*Stability, aggregation and refolding studies of Human Carbonic Anhydrase II*” is concerned with the understanding of stability and aggregation behaviour of model protein human carbonic anhydrase II under various stress conditions. In this thesis, we also discussed the effect of intrinsic properties of the polypeptide chain on the stability of HCAII by introducing single point mutations in the amino acid sequence.

The thesis is composed of seven chapters. **Chapter 1 (Introduction)** provides an overview of different models proposed to explain the mechanism of protein aggregation, thermodynamic and kinetic aspects of aggregation phenomenon, and various intrinsic and extrinsic determinants that lie behind protein self-assembly. This chapter also describes the structural and functional aspects of model protein used in the present study- human carbonic anhydrase II. **Chapter 2 (Materials and Methodologies)** give details of the chemicals acquisition, construction of mutants,

expression and purification of protein as well as analytical techniques used to study the stability and aggregation of human carbonic anhydrase II (HCAII) and its variants. **Chapter 3** (*Intermediate conformation between native β -sheet to non-native α -helix is a precursor of trifluoroethanol-induced aggregation of Human carbonic anhydrase II*) deals with the study of conformational transitions and aggregation propensity of human carbonic anhydrase II (HCAII) in the presence of 2,2,2-trifluoroethanol (TFE). This chapter also discuss about the conformational preference of intermediate state to form amyloid-like fibrils over disordered aggregates. **Chapter 4** (*Salt mediated unusual switching in the aggregation kinetic profile of Human carbonic anhydrase II*) deals with the study of heat induced aggregation of HCA II in the presence of salt ions. The chapter describes the effect of protein concentration, temperature and salt concentration on the unusual biphasic kinetic profile of HCAII in the presence of salt. The efficiency of different cations in accelerating the protein aggregation was evaluated and analysed in terms of various possible models given in literature. The gross structural and morphological features of HCA II aggregates were also explored in a time dependent manner. **Chapter 5** (*Effect of disease linked single point mutations on the stability and aggregation of Human carbonic anhydrase II*) describes the effect of H94Y and G145R mutations on the structure and stability of HCA II. A systematic investigation involving chemical and thermal equilibrium unfolding studies, conformational studies using CD and NMR, and enzymatic assay were performed and comparison was made with wild type HCAII to ascertain the role of amino acids that are substituted in HCAII stability and aggregation. The structural stability of HCAII variants was also studied in the presence of small molecule acetazolamide with the aim of stabilizing the native state of mutant proteins. **Chapter 6** (*Competition between folding and aggregation during osmolyte-aided refolding of Human carbonic anhydrase II: Possible role*

of preferential exclusion and binding) presents the investigation of various osmolytes, including glycerol, sorbitol, ethylene glycol, sucrose and trehalose, on the refolding yield of guanidine-hydrochloride denatured human carbonic anhydrase II. The occurrence of competing aggregation pathway was assessed during refolding of denatured protein. Based on the results, the mode of action of co-solvents belonging to two different classes of osmolytes viz., polyols and sugars was explained. **Chapter 7 (Summery and Future Perspectives)** highlights the salient features of this work. The findings of this work have projected light on the various intrinsic and extrinsic factors that modulates the stability and aggregation of HCAII. These studies may help in better understanding of self-assembly of proteins under various stress conditions often encountered by the protein inside the cellular environment.

सार

प्रोटीन मिसफोल्डिंग और एकत्रीकरण ने विनाशकारी बीमारियों के प्रभाव में और जैव प्रौद्योगिकी उद्योगों में बड़ी मात्रा में प्रोटीन के उत्पादन पर प्रभाव डाला है। इसलिए, अमाइलॉइड-आधारित विकारों को रोकने और चिकित्सकीय प्रोटीन के उत्पादन को रोकने के लिए अवांछनीय समुच्चय प्रक्रिया को समझना और नियंत्रित करना महत्वपूर्ण है। प्रोटीन के भौतिक-रासायनिक वातावरण प्रोटीन एकत्रीकरण के विभिन्न पहलुओं जैसे कि शुरुआत, एकत्रीकरण दर और समेकित राज्य के अंतिम आकारिकी पर एक मजबूत प्रभाव डालता है। एक बार यह स्थापित हो जाता है कि एकत्रीकरण के लिए भौतिक-रासायनिक निर्धारक प्रोटीन की स्थिरता और एकत्रीकरण की प्रवृत्ति को कैसे व्यवस्थित करते हैं, तो जानकारी को एकत्रीकरण तंत्र में अंतर्दृष्टि प्राप्त करने और आवश्यकता के अनुसार उन्हें नियंत्रित करने / व्यवस्थित करने के लिए रणनीति तैयार करने के लिए लगभग सीधे उपयोग किया जा सकता है। इस उद्देश्य के साथ, हमने प्रोटीन के वातावरण या समाधान की स्थितियों को प्रोटीन की एकत्रीकरण प्रवृत्ति पर बदलने के प्रभाव की जांच करने का प्रयास किया। "स्थिरता, एकत्रीकरण और मानव कार्बोनिक एनहाइड्रेज II के रीफोल्डिंग अध्ययन" शीर्षक से प्राप्त सिद्धांत विभिन्न तनाव परिस्थितियों में मॉडल प्रोटीन मानव कार्बोनिक एनहाइड्रेज II के स्थिरता और एकत्रीकरण व्यवहार की समझ से संबंधित है। इस थीसिस में, हमने एमीनो एसिड अनुक्रम में सिंगल पॉइंट म्यूटेशन शुरू करने के द्वारा एचसीएआईआई की स्थिरता पर पॉलीपेप्टाइड श्रृंखला के आंतरिक गुणों के प्रभाव पर भी चर्चा की।

थीसिस सात अध्यायों से बना है अध्याय 1 (परिचय) प्रोटीन एकत्रीकरण, एकत्रीकरण घटना के गतिशील और गतिशील पहलुओं के तंत्र और प्रोटीन स्वयं-विधानसभा के पीछे झुकने वाले विभिन्न आंतरिक और बाहरी निर्धारकों की व्याख्या करने के लिए प्रस्तावित विभिन्न मॉडलों का अवलोकन प्रदान करता है। इस अध्याय में वर्तमान अध्ययन में उपयोग किए जाने वाले मॉडल प्रोटीन के संरचनात्मक और कार्यात्मक पहलुओं का भी वर्णन है- मानव कार्बोनिक एनहाइड्रेज III। अध्याय 2 (सामग्री और मेथोडोलॉजी) मानव कार्बोनिक एनहाइड्रेज II (एचसीएआईआई) और उसके प्रकारों की स्थिरता और एकत्रीकरण के अध्ययन के लिए रसायनों के अधिग्रहण,

म्यूटेंट का निर्माण, अभिव्यक्ति और प्रोटीन की शुद्धिकरण के साथ-साथ विश्लेषणात्मक तकनीकों का विवरण देता है। अध्याय 3 (गैर-मूल α -हेलिक्स के लिए देशी β -शीट के बीच की इंटरमीडिएट कन्फ्यूशंस मानव कार्बोनिक् अनहाइड्रेस ॥ के त्रिफ्लूरोएथेनॉल-प्रेरित एकत्रीकरण का अग्रदूत है) मानव कार्बोनिक् एनहाइड्रोजन ॥ (एचसीएआईआई) के गठनात्मक संक्रमण और एकत्रीकरण प्रवृत्ति के अध्ययन से संबंधित है 2,2,2-त्रिफ्लूरोएटेनॉल (टीएफई) की उपस्थिति इस अध्याय में मध्यवर्ती अवस्था के गठनात्मक वरीयता के बारे में भी चर्चा हुई है, जो अॉलेइड की तरह तंतुओं को बेतरतीब समुच्चय पर बना देती है। अध्याय 4 (मानव कार्बोनिक् एनाहाइडस ॥ का एकत्रीकरण गतिज प्रोफाइल में नमक मध्यस्थता असामान्य स्विचिंग) नमक आयनों की उपस्थिति में एचसीए ॥ के गर्मी प्रेरित समूह के अध्ययन के साथ संबंधित है। अध्याय नमक की उपस्थिति में एचसीएआईआई के असामान्य बिफेसिक कैनेटीक्स प्रोफाइल पर प्रोटीन एकाग्रता, तापमान और नमक एकाग्रता के प्रभाव का वर्णन करता है। साहित्य में दिए गए विभिन्न संभव मॉडल के संदर्भ में प्रोटीन एकत्रीकरण को गति देने में विभिन्न कारणों की दक्षता का मूल्यांकन और विश्लेषण किया गया था। एचसीए द्वितीय समुच्चय की सकल संरचनात्मक और रूपात्मक विशेषताओं का भी एक समय पर निर्भर तरीके से पता लगाया गया था। अध्याय 5 (मानव कार्बोनिक् एनाहाइडस ॥ की स्थिरता और एकत्रीकरण पर रोग से जुड़े एकल बिंदु म्यूटेशन का प्रभाव) एचसीईई ॥ की संरचना और स्थिरता पर एच 4 4 ई और जी -145 आर के उत्परिवर्तन के प्रभाव का वर्णन करता है। रासायनिक और थर्मल संतुलन के अध्ययन से जुड़ा एक व्यवस्थित जांच, सीडी और एनएमआर का उपयोग करने के लिए गठनात्मक अध्ययन, और एंजाइमेट परख प्रदर्शन किया गया और एचसीएआईआई स्थिरता और एकत्रीकरण में प्रतिस्थापित किए गए अमीनो एसिड की भूमिका का पता लगाने के लिए जंगली प्रकार एचसीएआईआई के साथ किया गया। उत्परिवर्ती प्रोटीन की देशी राज्य को स्थिर करने के उद्देश्य से छोटे अणु एसिटोजोलामाइड की उपस्थिति में एचसीएआईआई प्रकार की संरचनात्मक स्थिरता का भी अध्ययन किया गया। अध्याय 6 (मानव कार्बोनिक् एनहाइडस ॥ की ओस्मोलाईट-अनुदानित पुनः शमन के दौरान तह और एकत्रीकरण के बीच प्रतियोगिता: अधिमान्य बहिष्करण और बाध्यकारी की संभावित भूमिका) रिफाल्डिंग यील्ड पर ग्लिसरॉल, सोर्बिटोल, एथिलीन ग्लाइकोल, सोक्रोस और ट्रेहलोस सहित विभिन्न ओस्मोलाईट्स की जांच को प्रस्तुत करता है। गुआनडैनी-हाइड्रोक्लोराइड विकृत मानव कार्बोनिक् एनाहाइडस ॥ का विकृत प्रोटीन के रिफोल्डिंग के दौरान प्रतिस्पर्धात्मक एकत्रीकरण पथ की घटना का मूल्यांकन किया गया था। परिणाम के आधार पर, दो अलग-अलग वर्गों के संबंधित सह-सॉल्वेंट्स की कार्रवाई की विधि, जैसे पॉलीलीस और शक्कर

समझाया गया था। अध्याय 7 (समरी और भविष्य परिप्रेक्ष्य) इस काम की मुख्य विशेषताओं पर प्रकाश डाला गया है। इस काम के निष्कर्षों ने विभिन्न आंतरिक और बाह्य कारकों पर प्रकाश का अनुमान लगाया है जो एचसीएआईआई की स्थिरता और एकत्रीकरण को व्यवस्थित करता है। इन अध्ययनों से सेलुलर पर्यावरण के अंदर प्रोटीन से अक्सर कई तनाव की स्थिति में प्रोटीनों की स्वयं-विधानसभा की बेहतर समझ में मदद मिल सकती है।

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