PROTEIN DYNAMICS IN CROWDED SCENARIO: A SOLVATION APPROACH

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by

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Dedicated to

My

Parents

CERTIFICATE

This is to certify that the thesis titled "**Protein Dynamics in Crowded Scenario: A Solvation Approach**" being submitted by **Mr. Sanjib Kumar Mukherjee** 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 him.

Mr. Sanjib Kumar Mukherjee 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

This thesis entitled '**Protein Dynamics in Crowded Scenario: A Solvation Approach**' focuses on the study of the effect of macromolecular crowding on the dynamics of two serum albumin proteins, bovine serum albumin (BSA) and human serum albumin (HSA), both in their native and denatured states primarily through solvation studies.

Chapter 1 entitled '**Introduction**' describes dynamics of the biomolecules in detail with numerous examples. It also includes relevant details on the phenomenon of macromolecular crowding and its influence on protein structure and dynamics. This chapter ends with a brief overview of structural and functional aspects of HSA.

Chapter 2 entitled '**Materials and Methods'** describes chemical procurement, purification and storage along with techniques used during the investigation. Specifically, UV-VIS spectroscopy, Steady-state and Time-resolved fluorescence, Rotational Anisotropy, Circular Dichroism (CD), were used to carry out the requisite characterization.

Chapter 3 entitled **'Do macromolecular crowding agents exert only excluded volume effect? A protein solvation study'** describes the solvation of serum proteins in presence of increasing concentrations of synthetic and protein based crowding agents. We have observed that the synthetic crowders such as dextran and PEG of varying molecular weights at very low concentrations can have an appreciable effect on the extent of protein solvation thereby implying the presence of soft interactions that were initially very hard to detect. Since under such conditions the effect of excluded volume is appreciably low, this gives a direct evidence of soft interactions between the macromolecular crowding agents used and the serum proteins. Moreover, our data reveal, that since at these low crowder concentrations major perturbations to the protein structure are unlikely to take place while minor perturbations might not be readily visible, protein solvation provides a unique spectral signature of capturing such local dynamics, thereby allowing one to decouple hard sphere interactions from soft sphere ones. In other words our data reveal negligible changes at the equilibrium level but dramatic modulations in dynamics, implying therefore that the crowders show substantial transient/weak non-specific interactions with the proteins.

Chapter 4 entitled **'pH Dependent Domain Dynamics of HSA Controlled by Protein Based Crowding Agents'** describes the dynamics of domain I of human serum albumin

(HSA) in presence of bovine serum albumin and lysozyme as crowders. 6-bromoacetyl-2-dimethylaminonaphthalene (BADAN) covalently attached to cysteine-34 of HSA was used as the solvation probe and changes in its solvation pattern in presence of the protein-based crowders were monitored as a function of pH. Lysozyme induced increased retardation of solvation while BSA brought about faster dynamics. Our observations re-emphasize the importance of soft interactions even under conditions where repulsive charge-charge interactions dominate, thus reminding us of the enhanced level of complexity that the crowded milieu can possess.

Chapter 5 entitled **'Investigating the Solvent Characteristics of Macromolecular Crowders using Fluorescent Probes'** describes the solvation characteristics of the medium in presence of various crowding agents. Microenvironment studies by using suitable fluorophores revealed that the microenvironment undergoes some drastic changes in presence of crowding agents. Also self aggregation processes like the formation of micelles, excimers and J-aggregates are significantly affected in a crowder dependent manner. Moreover, PEG8000 behaves quite differently from all the crowding agents, implying that the microstructure of these crowding agents is different in the solution medium. Diffusion studies enable us to distinguish different concentration regimes of the polymer mesh which provide insights into the nature of the entanglement of the crowding agents. Therefore the molecular level picture of these crowding agents thus help us obtained understand in some detail, the characteristics of the medium, with these playing a significant role in various biological phenomenon.

Chapter 6 entitled **'Influence of Crowding Agents on the Dynamics of a Multidomain Protein in its Denatured State: A Solvation Approach'** describes the dynamics of BADAN attached to domain I of HSA in presence of different concentrations of various crowding agents at different urea concentrations. In absence of the macromolecular crowders, solvation time decreases as a function of urea concentration due to progressive unfolding of the protein. However, in presence of crowding agents, at low urea concentrations an initial drop followed by retardation in the solvation time was observed which we propose, implies the crossover between soft to hard sphere interactions between the protein and crowder molecules.

सार

'थियेटेड परिदृश्य में प्रोटीन डाइनेमिक्स: ए सोलवेशन दृष्टिकोण' नामक इस थीसिस में दो सीरम एल्बिनिन प्रोटीन, बोवाइन सीरम एल्बिनिन (बीएसए) और मानव सीरम एल्बमिन (एचएसए) की गतिशीलता पर मैक्रोमोल्यूलर भीड़ के प्रभाव के अध्ययन पर केंद्रित है, दोनों मुख्य रूप से उत्थान अध्ययन के माध्यम से देशी और विकृत राज्यों।

'परिचय' शीर्षक वाला अध्याय एक कई उदाहरणों के साथ जैव-अणुओं की गतिशीलता का वर्णन करता है। इसमें मैक्रोमोल्यूलर भीड़ की घटना और प्रोटीन संरचना और गतिशीलता पर इसके प्रभाव पर प्रासंगिक विवरण शामिल हैं। यह अध्याय एचएसए के संरचनात्मक और कार्यात्मक पहलुओं के संक्षिप्त विवरण के साथ समाप्त होता है।

'सामग्री और तरीके' नामक अध्याय दो जांच के दौरान उपयोग की जाने वाली तकनीकों के साथ रासायनिक खरीद, शुद्धिकरण और भंडारण का वर्णन करता है। विशेष रूप से, यूवी-वीआईएस स्पेक्ट्रोस्कोपी, स्थिर-राज्य और समय-संकल्प प्रतिदीप्ति, घूर्णन एनीसोट्रॉपी, सर्कुलर डिक्रोइज्म (सीडी) का उपयोग आवश्यक विशेषता को पूरा करने के लिए किया जाता था।

अध्याय तीन का शीर्षक है 'क्या मैक्रोमोल्यूलर भीड़ एजेंट केवल वॉल्यूम प्रभाव को छोड़ देते हैं? प्रोटीन सॉल्वेशन स्टडी 'सिंथेटिक और प्रोटीन आधारित भीड़ एजेंटों की बढ़ती सांद्रता की उपस्थिति में सीरम प्रोटीन के उद्घोषणा का वर्णन करती है। हमने देखा है कि बहुत कम सांद्रता पर भिन्न आणविक भारों के डेक्स्ट्रान और पीईजी जैसे कृत्रिम भीड़ प्रोटीन संवहन की सीमा पर एक सराहनीय प्रभाव डाल सकते हैं जिससे मुलायम बातचीत की उपस्थिति का अर्थ हो रहा है जो शुरू में बहुत मुश्किल था। चूंकि ऐसी परिस्थितियों में बहिष्कृत मात्रा का प्रभाव काफी कम है, यह मैक्रोमोल्यूलर भीड़ एजेंटों और सीरम प्रोटीन के बीच मुलायम बातचीत का प्रत्यक्ष सबूत देता है। इसके अलावा, हमारे आंकड़ों से पता चलता है कि चूंकि इन कम क्रॉडर सांद्रता प्रोटीन संरचना में प्रमुख परेशानी होने की संभावना नहीं है, जबकि मामूली परेशानी आसानी से दिखाई नहीं दे सकती है, प्रोटीन एकजुटता ऐसी स्थानीय गतिशीलता को पकड़ने का एक अद्वितीय वर्णक्रमीय हस्ताक्षर प्रदान करती है, जिससे एक को नरम क्षेत्र से हार्ड क्षेत्र इंटरैक्शन को कम करें। दूसरे शब्दों में, हमारे डेटा संतुलन स्तर पर नगण्य परिवर्तनों को प्रकट करते हैं लेकिन गतिशीलता में नाटकीय मॉड्यूलेशन, इसलिए यह दर्शाता है कि भीड़ प्रोटीन के साथ पर्याप्त क्षणिक / कमजोर गैर-विशिष्ट इंटरैक्शन दिखाते हैं।

अध्याय चार जिसका अर्थ है प्रोटीन आधारित भीड़ एजेंटों द्वारा नियंत्रित एचएसए की पीएच निर्भर डोमेन गतिशीलता 'मानव सीरम एल्बमिन (एचएसए) के डोमेन। की गतिशीलता का वर्णन करता है जो बोवाइन सीरम

एल्बिनिन और लाइसोइज्म की भीड़ में मौजूद है। 6-ब्रोमोसिटाइल-2-डायमेथिलामिनोफेथेलिन (बादान) को एचएसए के सिस्टीन -34 से सहसंयोजक रूप से जुड़ा हुआ था, जिसे प्रोटीन-आधारित भीड़ की उपस्थिति में इसके उत्थान पैटर्न में परिवर्तन की पीएच के एक समारोह के रूप में निगरानी की गई थी। Lysozyme प्रेरित उत्तेजना में वृद्धि में वृद्धि हुई जबकि बीएसए तेजी से गतिशीलता लाया। हमारे अवलोकन उन स्थितियों के तहत भी नरम अंतःक्रियाओं के महत्व पर दोबारा जोर देते हैं जहां प्रतिकूल चार्ज-चार्ज इंटरैक्शन पर हावी है, इस प्रकार हमें भीड़ की बढ़ी हुई स्तर की याद दिलाती है जो भीड़ वाले मिलियू के पास हो सकती है।

अध्याय पंज शीर्षक 'फ्लोरोसेंट जांच का उपयोग कर मैक्रोमोल्यूलर क्रॉडर के सॉल्वेंट लक्षणों की जांच करना' विभिन्न भीड़ एजेंटों की उपस्थिति में माध्यम की स्वीकृति विशेषताओं का वर्णन करता है। उपयुक्त फ़्लोरोफोर्स का उपयोग करके सूक्ष्म पर्यावरण अध्ययन से पता चला कि सूक्ष्म पर्यावरण को भीड़ एजेंटों की उपस्थिति में कुछ कठोर परिवर्तन हुए हैं। इसके अलावा स्वयं एकत्रीकरण प्रक्रिया जैसे कि माइक्रेल, एक्सीमर और जे-एग्रीगेट्स के गठन की तरह क्रॉउडर आश्रित तरीके से काफी प्रभावित होते हैं। इसके अलावा, पीईजी 8000 सभी भीड़ एजेंटों से काफी अलग व्यवहार करता है, जिसका अर्थ यह है कि इन भीड़ एजेंटों का सूक्ष्म संरचना समाधान माध्यम में अलग है। डिफ्यूजन अध्ययन हमें बहुलक जाल के विभिन्न एकाग्रता शासनों को अलग करने में सक्षम बनाता है जो भीड़ एजेंटों के विघटन की प्रकृति में अंतर्दृष्टि प्रदान करता है। इसलिए इन भीड़ वाले एजेंटों की आणविक स्तर की तस्वीर इस प्रकार हमें कुछ विवरण, माध्यम की विशेषताओं में समझने में मदद करती है, इन विभिन्न जैविक घटनाओं में महत्वपूर्ण भूमिका निभाते हैं।

अध्याय छह का शीर्षक 'बहु संख्यक प्रोटीन की गतिशीलता पर भीड़ एजेंटों का प्रभाव, इसके डेनेचरड राज्य: ए सोलवेशन दृष्टिकोण' विभिन्न यूरिया सांद्रता पर विभिन्न भीड़ एजेंटों की विभिन्न सांद्रता की उपस्थिति में एचएसए के डोमेन। से जुड़े बादान की गतिशीलता का वर्णन करता है। मैक्रोमोल्यूलर भीड़ की अनुपस्थिति में, प्रोटीन के प्रगतिशील प्रकट होने के कारण, यूरिया एकाग्रता के एक समारोह के रूप में सॉल्वेशन समय कम हो जाता है। हालांकि, कम यूरिया सांद्रता पर भीड़ वाले एजेंटों की उपस्थिति में, प्रारंभिक बूंद के बाद हलचल समय में मंदता के बाद देखा गया था, जिसे हम प्रस्तावित करते हैं, प्रोटीन और क्रॉडर अणुओं के बीच नरम से कठिन क्षेत्र के बीच पारस्परिक क्रिया के बीच क्रॉसओवर का तात्पर्य है।

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