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PII: S0039-9140(16)30889-X
DOI: http://dx.doi.org/10.1016/j.talanta.2016.11.018
Reference: TAL17046

To appear in: *Talanta*

Received date: 3 August 2016
Revised date: 7 November 2016
Accepted date: 9 November 2016

Cite this article as: Xiaoyun Gong, Xingchuang Xiong, Lin Qi and Xiang Fang Investigating the Structural Transitions of Proteins during Dissolution by Mas Spectrometry, *Talanta,* http://dx.doi.org/10.1016/j.talanta.2016.11.018

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Investigating the Structural Transitions of Proteins during Dissolution by Mass Spectrometry

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ABSTRACT: An appropriate solvent environment is essential for the implementation of biological functions of proteins. Interactions between protein residues and solvent molecules are of great importance for proteins to maintain their active structure and catalyze biochemical reactions. In this study, we investigated such interactions and studied the structural transitions of proteins during their dissolution process. Our previously developed technique, namely solvent assisted electric field induced desorption/ionization, was used for the dissolution and immediate ionization of proteins. Different solvents and proteins were involved in the investigation. According to the results, cytochrome c underwent significant unfolding during dissolution in the most commonly used NH_4Ac buffer. The unfolding got more serious when the concentration of NH_4Ac was further increased. Extending the dissolution time resulted in the re-folding of cytochrome c. In comparison, no unfolding was observed if cytochrome c was pre-dissolved in NH_4Ac buffer and detected by nano-ESI. Furthermore, no unfolding was observed during the dissolution process of cytochrome c in water. Interactions between the residues of cytochrome c and the solute of NH_4Ac might be the reason for the unfolding phenomenon. Similar unfolding phenomenon was observed on holo-myoglobin. However, the observed dissolution feature of insulin was different. No unfolding was observed on insulin during dissolution in NH_4Ac buffers. Insulin underwent observable unfolding when water was used for dissolution. This might be due to the structural difference between different proteins. The obtained results in the present study furthered our insights into the interactions between proteins and the solvents during the phase transition of dissolution.

Key words: Structural transition; Protein; Dissolution; Mass spectrometry

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1. Introduction

The biological functions of proteins are remarkably related to their structures at molecular level. Different proteins perform varied functions of their own. The biological mechanism of a certain protein is determined by its native three-dimensional (3D) structure, which in turn is encoded in the 1D string of the amino acid backbone of the protein [1]. Efforts have been made to investigate the structural transitions of proteins under various conditions, namely the folding and unfolding events [2-4]. Computational modelling methods are developed to simulate the folding process of proteins, generally from a random configuration into their native structure [5]. The simulation matched well with experimental results for a limited set of small proteins [6]. Nuclear magnetic resonance (NMR) based methods offer a more practical way [7]. Assignment of folded and unfolded proteins in solutions is possible [8]. But such methods require large amounts of targeted proteins, and the high concentration of proteins needed can sometimes lead to aggregation. Besides, the investigation of large proteins using NMR is still challenging [9].

Recently, electrospray ionization (ESI) mass spectrometry (MS) based methods are gaining increasing attention in the investigation of protein structures [10-13]. They are fast and sensitive, and can

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