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RESEARCH PAPER

Charged Bubble Extractive Ionization Mass Spectrometry for Protein Analysis

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Abstract: Rapid mass spectrometry analysis of protein in liquid sample was carried out by using a charged bubble extractive ionization device. In this work, experimental parameters including gases (N_2 and CO_2), bubble path length, voltage, and gas pressure on the charged bubble extractive ionization of lysozyme were investigated. Under the optimum experimental parameters including CO_2 as extraction gas, bubble path length of 32 cm, solution voltage of 2 kV, and gas pressure of 0.05 MPa, lysozyme in liquid sample were successfully detected with the limits of detection of 1×10^{-8} M in aqueous solution and 1×10^{-7} M in diluted urine (200 times with ultrapure water), respectively. In addition, the limit of detection of 1×10^{-5} M was obtained for undiluted urine sample. By comparing the desalting effects of charged bubble extractive ionization mass spectrometry with ESI-MS, it was found that the charged bubble extractive ionization method could obtain a wider and lower charge state distribution of protein ions, and showed higher tolerance facing non-volatile inorganic salts. Off-line study of the collected catalase after charged bubble extractive ionization showed that 53.9% enzyme activity was remained, which indicated that the proposed method was a soft ionization method. The method had merits including no sample pretreatment, no chemical reagent contamination and high speed, showing potential application to mass spectrometry analysis of protein components in solution samples.

Key Words: Protein sample; Bubble; Charged bubble extractive ionization; Mass spectrometry

1 Introduction

Separation and analysis of protein components in complex biological samples are of great importance in life science research. The commonly used protein separation techniques include chromatographic separation[1], electrophoresis separation[2], chromatographic separation[3,4], etc. Especially, gel electrophoresis[5] is one of the most widely used methods for protein separation from complex biological samples and the separated protein spots can be extracted after dying, which are subsequently subjected to chromatography-mass spectrometry. However complex procedures including hydrolysis, extraction, desalting, etc. are required, resulting in time-consuming, lower efficiency, and apt to protein-loss. In general, in order to obtain purified proteins, solute precipitation, dialysis, ultrafiltration, chromatographic separation, chemical extraction, lyophilization, recrystallization, and other multi-step fine processings are routinely needed, which make it cannot fit the demands of high-throughput analysis of complex biological samples. Therefore, it is of great significance to develop highly sensitive method for direct analysis of proteins in complex biological samples.

Atmospheric pressure ionization mass spectrometry technologies developed in recent years have been used to directly detect trace components in complex samples without sample pretreatment. Atmospheric pressure ionization technologies such as desorption electrospray ionization (DESI)[6], ambient corona discharge ionization (ACDI)[7] extractive electrospray ionization (EESI)[8,9], ultrasonic spray ionization (SSI)[10] and paper spray ionization (PS)[11] have been used for rapid analysis of proteins in complex samples. Significant progresses have been made in

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determination of protein structure[12], enzyme activity detection[13] and protein-protein interaction[14]. However, it is still of practical significance to explore new ionization methods for rapid mass spectrometry analysis of trace proteins in biological samples due to their complexity and specificity.

In this study, a homemade charged bubble extractive ionization (CBEI) source was developed for mass spectrometry determination of protein components in solution samples. Parameters including extraction gases (*eg.*, N_2, CO_2), bubble path length, solution voltage, and gas pressure on the ionization of lysozyme were investigated. The mass spectra obtained by charged bubble extractive ionization mass spectrometry (CBEI-MS) were compared with that in ESI-MS under the same experimental conditions, suggesting that CBEI-MS could obtain protein ions with a wider and lower charge state distribution, and even had better tolerance to non-volatile inorganic salts. The results revealed that the established CBEI-MS method could be used to rapidly detect protein components in the liquid samples without sample pretreatments and chemical contamination. CBEI-MS shows potential application for biological protein samples analysis.

2 Experimental

2.1 Instruments and reagents

A homemade charged bubble extractive ionization source was used in this study. All the experiments were performed using an Orbitrap Fusion™ Tribrid™ mass spectrometer (Thermo Scientific, San Jose, CA, USA) with Xcalibur® 3.0 data processing system. Lysozyme (purity 95%), cytochrome C (purity 95%) and myoglobin (purity 95%–100%) were purchased from Solarbio Technology Co., Ltd. (Beijing, China). Horseradish peroxidase (HRP, enzyme activity ≥ 250 u/mg) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Ammonium acetate (Chromatographic purity) was purchased from Fisher Scientific Ltd. (USA). Deionized water was obtained from a Millipore water purification system (Milli-Q, Millipore; Bedford, MA, USA).

2.2 Experimental devices and detection methods

Two kinds of CBEI devices are shown in Fig. 1. In the case of device 1, the distance (a) between aerosol exit and MS inlet was 7.5 mm. Accordingly, the inside diameters of aerosol exit (b) and container (c) were 25 mm and 110 mm, respectively. The volume of the container was about 600 mL and the volume of sample solution was about 250 mL. The pore size of the gas diffuser was 100 μm. A high voltage was applied to the solution through a metal wire and the outer wall of the glass container was carefully grounded. In the case of device 2, the distance (a) between the top of the glass tube and the MS inlet was 7.5 mm. The diameters of aerosol exit (b) and glass tube (c) were 5 mm and 25 mm, respectively. The volume of the glass tube container was about 520 mL and the added sample solution volume was 6–160 mL. The pore size of the gas diffuser was 100 μm. The voltage was added to the solution through a metal wire, and the outer wall of the glass tube was grounded using foil wrapped outside. Mass spectra were collected in mass range of *m/z* 100–4000 with positive ion detection mode, and the temperature of ion capillary was set at 320 °C. Other parameters were set to default instrument values. The working principle of CBEI was as follows: during the bubble rising process, the protein molecules in solution were enriched on the bubble surface, and the adhered protein molecules were released as the bubbles busting on the surface of the sample solution, forming charged aerosol (containing target protein molecules) for subsequent MS interrogation.

3 Results and discussion

3.1 Charged bubble extractive ionization mass spectrometry analysis of lysozyme solution

Firstly, device 1 was used to analyze lysozyme solution (1 μM) containing 2 mM ammonium acetate, with CO_2 gas pressure of 0.2 MPa and bubble path length of 3 cm. As shown in Fig.2A, the characteristic peaks of lysozyme at *m/z* 2862 (*z* = 5), *m/z* 2385 (*z* = 6), *m/z* 2044 (*z* = 7), *m/z* 1789 (*z* = 8) and *m/z* 1590

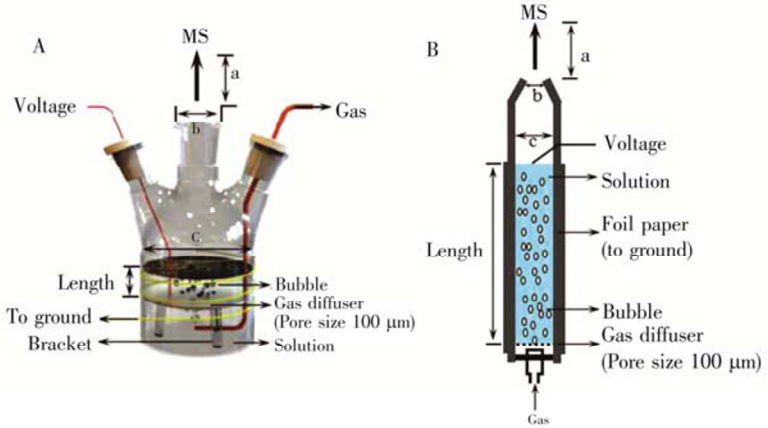

Fig.1 Schematic diagram of the charged bubble extractive ionization mass spectrometry platform (A) device 1; (B) device 2