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Ultra-sensitive quantification of lysozyme based on element chelate labeling and capillary electrophoresis–inductively coupled plasma mass spectrometry

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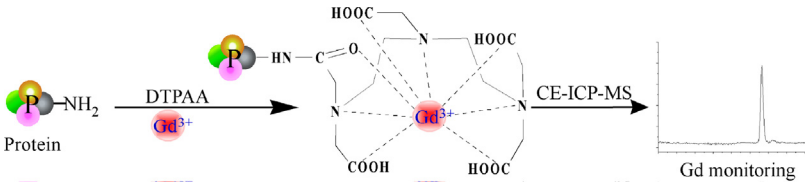
HIGHLIGHTS

- An ultra-sensitive method for detecting lysozyme based on CE–ICP–MS was described.
- The proposed method has an extremely low detection limit of 3.89 attomole.
- It can be used to detect trace lysozyme in saliva sample with a satisfied recovery.
- The method provides a new potential for sensitive detection of low-abundant proteins.

GRAPHICAL ABSTRACT

An ultra-sensitive method for the determination of lysozyme was developed based on the Gd^{3+} chelate labeling and CE–ICP–MS. The proposed method has an extremely low detection limit of 3.89 attomole and has been successfully used to detect lysozyme in saliva sample, showing excellent reliability. The success of the present method provides a new possibility for biological assays and clinical diagnoses.



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ABSTRACT

In this study, an ultra-sensitive method for the quantification of lysozyme based on the Gd^{3+} diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid labeling and capillary electrophoresis–inductively coupled plasma mass spectrometry (CE–ICP–MS) was described. The Gd^{3+}-tagged lysozyme was effectively separated by capillary electrophoresis (CE) and sensitively determined by inductively coupled plasma mass spectrometry (ICP–MS). Based on the gadolinium-tagging and CE–ICP–MS, the lysozyme was determined within 12 min with an extremely low detection limit of 3.89 attomole (3.89×10^{-11} mol L^{-1} for 100 nL of sample injection) and a RSD < 6% ($n = 5$). The proposed method has been successfully used to detect lysozyme in saliva samples with a recovery of 91–106%, suggesting that our method is sensitive and reliable. The success of the present method provides a new potential for the biological assays and sensitive detection of low-abundant proteins.

1. Introduction

Increasing efforts are endeavored in the ultra-sensitive quantification of proteins, because many important proteins are present at ultra-low level and only the quantity of them or the change in their abundance really reflects the status and changes of a biological system. Lysozyme is a ubiquitous protein existing in various

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organisms. It contains 129 amino acids and is well known for the protection of food from bacterial deterioration [1]. Lysozyme is an important antibacterial protein, which is active toward many bacteria such as neisseria, micrococcus, sarcina, klebsiella, streptococcus, staphylococcus and mycobacteriuiii, because it can catalyze the breaking of bacterial cell wall though enzymes hydrolysis of cytoskeleton [2]. Unlike antibiotic, lysozyme is totally nature, so it has been widely used in food industry for preserving food from deteriorating and in clinic for diminishing inflammation etc. Previous study showed that the concentration of lysozyme in the serum and saliva is also correlated with breast cancer [3,4]. For above reasons, the development of the sensitive and reliable method for the quantification of lysozyme in biological sample and food is of great significance.

So far, many methods for the quantification of lysozyme have been reported. For example, aptamer-based electrochemical or optical biosensors [5–11], electrospray ionization mass spectrometry (ESI–MS)-based method or label-free liquid chromatography mass spectrometry (LC–MS) and capillary electrophoresis mass spectrometry (CE–MS) methods [12–16], and enzyme-linked immunosorbent assay (ELISA) [17]. Aptamer-based biosensors have high sensitivity. However, the practical application of these biosensors was hampered by one or more of the following drawbacks such as poorer selectivity and stability, complex experiment procedure, poor resistance to complex matrix of real sample and so on. ESI–MS-based or label-free LC–MS and CE–MS methods showed the versatility of mass spectrometry in analytical field and provided the detail molecular information of lysozyme. However, these methods have obvious deficiencies such as complex isotopic labeling, higher cost due to the use of isotopic labeling and isotopic-labeled internal standard, and/or long analysis time, insufficient sensitivity and so on. ELISA is easy to use and has a better specificity, whereas, it has relatively high cost, lower sensitivity and poor stability due to the utilization of antibody.

Inductively coupled plasma mass spectrometry (ICP–MS) is a sensitive technique for the detection and quantification of elements. It has various characteristics including a broad dynamic range, multi-element detection capabilities and excellent mass resolution for metallic ions. All these features make it a promising detector for the quantification of proteins via the definite element stoichiometry in protein. However, proteins, as well as most bio-molecules, cannot be efficiently detected by ICP–MS since ICP–MS has extreme low sensitivity for the hydrogen, oxygen, carbon and nitrogen, which are the main components of proteins [18]. Therefore, it is necessary to label metallic ion to proteins in order to sensitively determine them with ICP–MS-based techniques. The combination of metallic labeling and ICP–MS-based techniques promises a powerful technique for the quantification of proteins, and has attracted an increasing attention [19]. So far, some strategies, which are based on isotopic labeling [20–22] or elemental-labeled antibodies [23–25] or element chelate tag [25–29] or gold nanoparticles (AuNPs) labeling [18,30] and liquid chromatography–ICP–MS (LC–ICP–MS) or CE–ICP–MS or direct ICP–MS detection, have been developed for the sensitive quantification of proteins. However, the methods previously reported have one or more of following shortages, such as expensive and complex isotopic labeling, relatively low sensitivity, and the utilization of antibody etc. In addition, the use of CE–ICP–MS for the sensitive detection of lysozyme has been rarely reported due to the difficulty of the separation of proteins. In this work, we report a novel method for the sensitive quantification of lysozyme based on gadolinium (Gd) chelate tagging and CE–ICP–MS, in hope of providing a realistic approach for the study of physiological effect and clinical pathology of lysozyme in human body.

2. Experimental

2.1. Reagents

The lysozyme was purchased from Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China), its activity > 70000 U mg^{-1}. The diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic dianhydride (DTPAA) was purchased from Tokyo Chemical Industry (Tokyo, Japan). The 100 μg mL^{-1} Gd^{3+} stock standard solution was prepared by dissolving pure Gd_2O_3 (Wako Pure Chemical Industries Co. Ltd, Japan) in 1% HNO_3 solution, and the 10^{-3} mol L^{-1} Gd^{3+} used in the experiment was prepared by diluting the stock solution with Milli-*Q* water. The running buffer solution of 15 mmol L^{-1} NaH_2PO_4–3.75 mmol L^{-1} $Na_2B_4O_7$ was prepared by dissolving sodium dihydrogenphosphate ($NaH_2PO_4{\cdot}2H_2O$) and sodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$), which were purchased from Shanghai Reagents Co., Ltd. (Shanghai, China), in Milli-*Q* water. Dimethyl sulfoxide (DMSO), hydrochloric acid and nitric acid were purchased from Shanghai Reagents Co., Ltd. (Shanghai, China). ELISA kit for human lysozyme was purchased from Beijing Qisong Biotech. ELISA experiment was measured with microplate reader (Multiskan MK3, Thermo Scientific, China). All reagents are analytical grade or ultra-pure grade, and the water used in the experiment is Milli-*Q* water (18.2 MΩ cm^{-1}).

2.2. CE–ICP–MS system

The CE–ICP-MS system consists of a CEi–SP20 CE-interface system (Reeko Instrument Co. Ltd., Xiamen, China) and an Agilent 7500ce ICP-MS (Agilent Technologies, USA). The CE-interface was made according to the principle reported in our previous paper [31]. Sample solution was injected into CE–ICP–MS for determination with electro-migration injection. The CE capillary was conditioned daily by purging it with Milli-*Q* water for 10 min, 0.1 mol L^{-1} NaOH solution for 10 min, Milli-*Q* water for 10 min and running buffer solution for 10 min, respectively. Between each run, the CE capillary was flushed with Milli-*Q* water and running buffer solution for 2 min, respectively. All CE–ICP–MS experiment was performed at room temperature in which the temperature was regulated in 23–25 °C by an air conditioner.

2.3. Measuring procedure

About 10 μL of 8×10^{-5} M lysozyme solution (in DMSO) was putted into a 1.5 mL centrifuge tubes, and 50 μL of 8×10^{-3} M DTPAA solution (in DMSO) was added into it. Then, the mixture was incubated at 37 °C for 2 h under full agitation to couple lysozyme to DTPA (diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid). Subsequently, 4×10^{-7} mol of Gd^{3+} (in 10^{-3} mol L^{-1} solution) and proper volume of 15 mmol L^{-1} NaH_2PO_4–3.75 mmol L^{-1} $Na_2B_4O_7$ buffer solution (pH 6.0) were added (to a total volume of 1.0 mL). The mixture was continuously incubated at 37 °C for 1 h under full agitation again to finish the Gd^{3+} labeling. The final solution was then diluted to desired concentration with 15 mmol L^{-1} NaH_2PO_4–3.75 mmol L^{-1} $Na_2B_4O_7$ buffer solution (pH 6.25), and was analyzed by CE–ICP–MS with electro-migration injection under the conditions shown in Table 1.

3. Results and discussion

3.1. Principle for the determination of lysozyme with CE–ICP–MS

As we mentioned above, proteins, as well as most bio-molecules cannot be sensitively detected by ICP–MS-based technique since ICP–MS has extreme low sensitivity for the hydrogen, oxygen,