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Full Length Article

Investigation of the interaction between superoxide dismutase and caffeoylquinic acids by alkali metal assisted cationization-ion mobility mass spectrometry

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ABSTRACT

Misfolding and dissociation of Cu, Zn-superoxide dismutase (SOD1) is closely related to the pathogenesis of Amyotrophic Lateral Sclerosis (ALS), so that, it is important to discover the compounds which can stabilize the structure of SOD1 for the prevention and treatment of ALS disease. Caffeoylquinic acids (CQAs), as potential antioxidants, are important bioactive components in multiple vegetables and fruits. In this research, we studied the interactions between the CQAs and SOD1, and explored their effects on stabilizing the SOD1 structure and inhibiting the misfolding of SOD1. Because metal-free SOD1 (apo-SOD1) may be one of the precursor compounds of SOD1 dissociation and aggregation, we investigated the non-covalent complex between CQAs and apo-SOD1 by the electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (MS/MS). Among the CQAs observed in this research, dicaffeoylquinic acids (di-CQAs) have the stronger binding affinity to apo-SOD1 than monocaffeoylquinic acids (mo-CQAs). In order to study the interactions of di-CQA isomers with apo-SOD1, a novel method based on alkali metal assisted cationization-ion mobility mass spectrometry was established and used to investigate the binding affinity between the di-CQA isomers and apo-SOD1 by distinguishing and quantifying the unbound di-CQA. Collision induced unfolding (CIU), as a new methodology, was further used to assess the stabilities of di-CQAs and apo-SOD1 complexes. The results show that there were no distinct differences in the binding affinity between different di-CQA isomers and apo-SOD1. Furthermore, CIU data obtained in this work reveal that di-CQAs can stabilize apo-SOD1 dimer and retard the unfolding of apo-SOD1 dimer. The strategy developed by us can be also used to study the interactions between other isomeric compounds and target protein in order to discover more potential drug-like small molecules.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disease which has been a grave health threat by progressive injury and death of motor neurons [1,2]. Through in-depth study of the causes of ALS, it is found that about 10% of all cases are familial ALS (FALS), and about 20% of FALS is caused by abnormal accumulation of SOD1 [3,4]. A major pathological hallmark of this disease is abnormal accumulation of SOD1 protein in the spinal motor neurons of FALS patients [5,6]. The pathogenic mutation trigger is the misfolding of SOD1 leading to the formation of insoluble SOD1 oligomers/aggregates, so the misfolding and aggregation of SOD1 is the prime cause for the SOD1-mediated ALS [7,8]. Stabilizing the native conformation of SOD1 can suppress the misfolding of SOD1 into the toxic conformation, which is well expected to develop a leading drug to protect the misfolding and oligomerization of the native structure of SOD1 [9–12]. Studies have reported that the interactions of aromatic compounds with protein played an important role in amyloid formation and amyloid structure stabilization [13–15]. Epi-gallocatechine-3-gallate (EGCG), resveratrol and flavones containing aromatic structure can inhibit and remodel the formation of amyloid fibrils [16–20]. CQAs, the most representative phenolic compounds, are a kind of important bioactive components with versatility and universality distribution in the plants [21]. Structure-activity relationship studies showed that these compounds have antioxidant, antimicrobial, antibacterial, antiviral, and anti-inflammatory properties [22–25]. Studying the interaction of CQAs with SOD1 should be helpful for confirming that

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whether CQAs are an attractive therapeutic candidate that inhibits the misfolding and aggregation of SOD1.

The application of native mass spectrometry (Native MS) in biology have continuously evolved over the past decade, the characterization of non-covalent complexes of protein/ligand by electrospray ionization mass spectrometry (ESI-MS) has been successfully integrated in pharmaceutical drug-discovery and structural biology programs [26–28]. Native MS is complementary to the classical biophysical methods of spectrum, calorimetry, crystallography, and NMR or surface plasmon resonance. Comparing with these conventional biophysical methods, the advantages of ESI-MS for studying the non-covalent complexes possess high sensitivity, high resolution, fast acquisition speed and extended mass range [29–31].

Due to the presence of multiple isomers of the dicaffeoylquinic acids (di-CQAs), it is difficult to study the binding affinity between the di-CQAs and protein in their mixed systems. In recent years, ion mobility separation -mass spectrometry (IMS-MS) has been successfully applied to distinguish and quantitate the enantiomers [34]. IMS-MS is analogous to electrophoresis technique that allows gas-phase ions to be distinguished on the basis of their size and shape [35–37]. Ions are guided through the cell by a weak electric field, collisions with the buffer gas and traverse to the mass analyzer followed by a detector, then the drift time of each ion package is recorded [28,38]. In our report, a novel method based on alkali metal assisted cationization-ion mobility mass spectrometry was used to maximize structural differences of four di-CQA isomers and guide us to select suitable charge carrier to distinct the isomers. Furthermore, the differentiation and quantification of the isomers can be performed easily and quickly using travelling ion mobility mass spectrometry (TWIMS) coupled with ESI-MS, then the relative binding affinities between the isomers and apo-SOD1 can be determined.

In this study, because metal-free SOD1 (apo-SOD1) may be one of the precursor compounds of SOD1 dissociation and aggregation, we selected apo-SOD1 as target protein which interact with CQAs. The non-covalent interaction between the CQAs and apo-SOD1 were investigated based on the ESI-MS and electrospray ionization-tandem mass spectrometry (ESI-MS/MS). The results suggested that the binding strength of di-CQAs is higher than that of mo-CQAs, which is expected that the numbers of caffeoyl group in the molecular structure may contribute effects to the interaction of ligand/protein. Since di-CQAs present different position isomers, the structural diversity of these isomers hampers accurate affinity determination among the interaction of CQAs regioisomers with apo-SOD1. In our study, we distinguished and assessed the relative binding affinities of four di-CQA isomers with apo-SOD1 by alkali metal assisted cationization-ion mobility mass spectrometry method that increased ion mobility separation by introducing cations (alkali metal ions) in positive ion mode. And the stability of di-CQAs and apo-SOD1 complex was further assessed by collision induced unfolding (CIU), which showed that the extended unfolding of apo-SOD1 under the actions of four di-CQAs was slowed down. The results showed that the four di-CQA isomers have little differences on the binding affinities and unfolding of apo-SOD1. The analytical strategy should be helpful to detect the interaction of SOD1 and small molecules so as to find the potential drugs.

2. Materials and methods

2.1. Materials

Bovine SOD1 was purchased from Beyotime Biotechnology (Shanghai, China), isochlorogenic acid B (3,4-di-CQA, purity≥98%), isochlorogenic acid C (4,5-di-CQA, purity≥98%), 1,5-dicaffeoylquinic acid (1,5-di-CQA, purity≥98%), isochlorogenic acid A (3,5-di-CQA,purity≥98%), quinic acid (QA, purity≥98%), caffeic acid (CA, purity≥98%), cryptochlorogenic acid (4-CQA, purity≥99%), neochlorogenic acid (5-CQA, purity≥98%) and chlorogenic acid (3-CQA, purity≥98%) were obtained from Chengdu mansite bio-technology co., LTD. Lithium chloride (LiCl), sodium chloride (NaCl), potassium Chloride (KCl), rubidium chloride (RbCl), and cesium chloride (CsCl) were purchased from Aladdin (Shanghai, China). Ammonium acetate (NH_4OAC) was obtained from Sigma-Aldrich (St.louris.MO). HPLC grade formic acid and acetonitrile (ACN) were supplied by Fisher Scientific (Lough borough, UK). Glacial acetic acid (HAc) was purchased from TEDIA COMPANY, INC. Ethylenediaminetetraacetic acid (EDTA) was purchased from J&K Scientific Ltd. (Beijing, China). The Micro Float-A-Lyzer (MW-cut: 10 kDa) used in the dialysis experiment was purchased from Spectrum Laboratories Inc. (Shanghai, China). The ultrapure water was prepared by Milli-Q (Millipore, Bedford, MA).

2.2. Sample preparation

2.2.1. Preparation of apo-SOD1

According to previous report [32], the method of apo-SOD1 preparation was as follows: SOD1 stock solution was dialyzed in a Float-A-Lyzer G2 (10-kDa MW-cut) against a 20 mM aqueous NH_4OAc buffer (pH 3.2) containing 5 mM EDTA. After 24 h, the dialysis buffer was changed with a 20 mM NH_4OAc buffer (pH 3.2) to remove EDTA. In the end, the buffer was replaced with 20 mM NH_4OAC (pH 6.8) to yield the apo-SOD1 dimer.

2.3. ESI-MS experiment

The apo-SOD1 sample was dissolved into 10 mM NH_4OAC to a stock concentration of 200 μM. The ligand samples were dissolved into 1.2 mM stock solution. Before experiment, the protein stock solution was stored at −20 °C, and the ligand stock solution was kept at 4 °C. Binding experiments of apo-SOD1 with QA, CA and CQA derivatives were performed in 1:6 ratio of protein: ligand after the protein-ligand mixture was incubated at 37 °C for 30 min, respectively.

2.4. Conditions for ESI- MS and ESI-MS/MS

Binding and MS/MS experiments were carried out on a quadrupole-ion mobility-time-of-flight mass spectrometer with ESI source (Synapt G2-Si HDMS, Waters Corp., Manchester, UK). Data were acquired in positive ion mode. The MS tune parameters were carefully adjusted to maintain the non-covalent interaction of the protein dimer and the protein – ligand complexes. Optimized parameters were set as follows: 20 V cone voltage; 2.25 kV the capillary voltage; 60 °C source temperature; 5 $L\,h^{-1}$ cone gas flow; 100 $L\,h^{-1}$ desolvation gas flow rate; 150 °C desolvation temperature; for CIU experiment, the selected wave velocity and wave height are 650 $m.s^{-1}$ and 40 V, 8 $\mu l.min^{-1}$ sample injection flow rate. The data were acquired and processed by the Masslynx 4.0 (Waters Corp., Manchester, UK).

2.5. IMS-MS experiments

2.5.1. Calibration of TWIM drift times and measurements

For conventional ion mobility separation (IMS) measurements, the obtained drift time values are linearly related to Collisional Cross Section (Ω). However, in the T-wave IMS system, because of the limit of the nonuniformity of the electric field, it was found that Ω was proportional to t_D^X, where t_D is the measured TWIM drift time, and X is the proportion constant that can be obtained