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A new strategy to determine the protein mutation site using matrix-assisted laser desorption ionization in-source decay: Derivatization by ionic liquid

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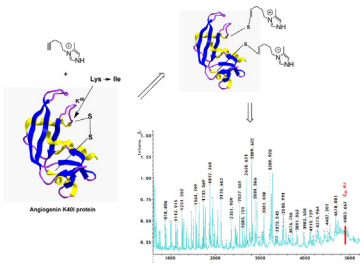
HIGHLIGHTS

- We have designed a new MALDI-ISD strategy to identify the protein mutation site.
- 4-Methyl-3-(pent-4-yn-1-yl)-imidazol-3-ium was synthesized to derive the protein.
- The protein after derivatization directly occurred ISD with the normal matrix CHCA.
- The protein after derivatization produced much larger range of fragments ions.

GRAPHICAL ABSTRACT



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ABSTRACT

Matrix-assisted laser desorption ionization (MALDI) time-of-flight mass spectrometry (TOF-MS) can be considered as state of the art in the field of proteins and peptides analysis. In this work, we have designed an ionic liquid derivative strategy to obtain abundant fragment ions in MALDI in-source decay (ISD) and used the analysis of angiogenin with mutation in the fortieth (K40I) as an instance. Firstly, we have synthesized two types of ionic liquids, 3-allyl-4-methyl-1*H*-imidazol-3-ium and 4-methyl-3-(pent-4-yn-1-yl)-1*H*-imidazol-3-ium. Then in the light-catalyzed reaction, the alkenyl ionic liquid can open the disulfide bond of K40I protein and add to the thiol. And the derived protein can process in-source decay under the effect of ionic liquid group to produce c–z type ions. Additionally this fragmentation is potentiated to support widely range of fragment ions which can cover the location of mutation. Our results have supplied a new top-down method about how to analyze the mutation or even post-translational modification of proteins in MALDI mass spectrometry.

1. Introduction

Mass spectrometry has been developed successfully to analyze high molecular weight compounds with soft ionization

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techniques [1]. In particular, matrix-assisted laser desorption/ionization (MALDI) has increased the applicability to large biological molecule because it can easily ionize large nonvolatile proteins and nucleotides [2,3]. During the past decade, MALDI-TOF mass spectrometry has become a powerful technique giving access to the distribution of a large range of different compounds such as proteins, peptides, lipids, drugs and their metabolites [4–6]. In protein identification, there are two main approaches for the determination of protein/peptide primary structure (amino acid sequence) by MS: "bottom-up" and "top-down" [7]. Both approaches can produce series types of fragment ions in mass spectrum, that is a-, b-, c- and x-, y-, z- type ions [8,9]. In addition, top-down sequencing depends on the fragmentation of the proteins which usually produced in mass analyzer because there is not any enzymatic digestion prior to analysis [10]. And the MALDI in-source decay (ISD) technique occurred in MALDI ion source with fragmentation induced by laser during the ionization processes can help the top-down methodology of intact proteins [11]. ISD technique based on hydrogen radicals produced after matrix such as 1,5-diaminonaphthalene (1,5 DAN) ionization and leads to c-, z-, w- and d- types ions which correspond to the dissociation of the N—Cα bond on the peptide backbone [12–15]. The fragments formed by ISD can be detected in full scan MS mode of MALDI-TOF and then undergo further fragmentation to produce more sequencing ions by MS/MS [10,16,17].

Beside MALDI-TOF instrument development in biomolecular analysis, the scientists also tried to employ some chemical methods such as derivatization for enhancing the ionization and fragmentation of the compounds. The derivatization usually locates in the N or C terminal of proteins [18–20]. Taking the peptides with lysine at the C-terminus for instance, Peters et al. [19] derived this type lysine with 2-methoxy-4,5-dihydro-1*H*-imidazole to achieve better ionizability and a series of y-ions which simplified the analysis in MS/MS spectra. Hanash and co-workers [21,22] have provided a robust method for quantitative analysis of complex proteomes by using acrylamide for the labeling in LC-MS/MS. Additionally the protein almost has disulfide bond as the basic structure. Besides in bottom-up method the disulfide bond must be open by dithiothreitol (DTT) and protected by iodoacetamide before enzymatic hydrolysis [23,24]. This process can also be considered as derivatization [25]. In organic chemistry the reactions of thiols with reactive carbon–carbon double bonds, or simply "enes", were recorded in the article in 1905 [26]. Then today this type of reaction is described with a wide variety of unsaturated functional groups by a mixed mechanism, including the classical radical addition but also Michael-type nucleophilic addition [27,28]. Furthermore, Conte et al. [29] have achieved the free-radical hydrothiolation of alkynes (thiol-yne coupling) by exclusive 1,2-addition of uniting two thiol fragments across the carbon–carbon triple bond. This reaction can be used to derive the disulfide bond directly and attaching two modifications at the single site in protein, which is regarded as the powerful strategy of the protein pretreatment before MS analysis.

In this work, we have proposed to develop a new method which can bring more abundant fragments of proteins in MALDI-ISD analysis. And we also applied it into the analysis of angiogenin which has mutation from Lys to Ile in the fortieth amino sites (K40I protein) as an example. Angiogenin has important biological functions in inducing a series of biological processes such as cell migration, invasion and proliferation by activating vessel endothelial and smooth muscle cells [30]. It is a single-chain protein with 123 amino acids (about MW 14400) and containing three disulfide bonds. The role of Lys-40 in angiogenin has been demonstrated having closely related with the function of anagiogenin as rebonucleolytic. Replacement of this residue by Ile decreases the function of anagiogenin seriously [31–33]. So a convenient strategy which can identify the mutation faster by mass spectrometry is badly in need. Then the MALDI in-source decay technique was chosen. However, the fragmentation of K40I protein in MALDI-ISD with common matrix such as 1,5-diaminonaphthalene or 2,5-dihydroxybenzoic acid hardly produces the fragments over MW 3000, but the mutation site of the K40I protein is about MW 5000. Therefore, the derivatization of disulfide bond of K40I protein can be participated before ISD analysis because these three disulfide bonds are relatively uniform distribution in amino acid sequencing of protein and may magnify the range of fragment ions in ISD by adding some groups which can enhance the fragmentation. In this derivatization experiment ionic liquid has been employed as reagent.

Ionic liquids are a heterogeneous type of compounds typically formed by heterocyclic cation based on substituted imidazole or pyridine systems and inorganic counter anion [34,35]. It has good stability, wide liquid temperature range and other characteristics. Especially it exists as ionic form thereby has intense abundance without protonation in the ion source of mass spectrum (like the ionization of metal ions). However, its high residual will also contaminate the electrospray ion source. Only the MALDI ion source can analyze the ionic liquid with a high tolerance. Recently the ionic liquids have been employed as solvent for liquid–liquid extraction, as chromatographic stationary phase in analysis of biological molecule. In particular, a number of literatures have been reported about the use of ionic liquids as the matrices for MALDI-MS analysis of proteins and DNA oligomers according to its outstanding properties such as good stability, negligible vapor pressure and electrical conductivity [36–40]. On this base, Horvatić et al. [41] have obtained collision-induced dissociation like in-source decomposition of peptide by addition of ammonium persulfate in the matrix solution in MALDI-MS.

From the above, in this work we have designed two ionic liquids, 3-allyl-4-methyl-1*H*-imidazol-3-ium and 4-methyl-3-(pent-4-yn-1-yl)-1*H*-imidazol-3-ium as the derivatization reagent and used these two ILs reacting with peptide and protein, respectively. Our objectives are obtaining richer fragment ions in MALDI-ISD by the inducement of derivatization ionic liquid to rapidly authenticate K40I protein. Additionally, the fragmentation mechanisms are also investigated.

2. Experimental

2.1. Materials

The ionic liquid synthesis reagents which contain 5-chioropent-1-yne, bromopropene, methylimidazole and photocatalyst, benzoin dimethyl ether (DMPA) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Pre-experiment peptide which includes one disulfide bond was purchased from Top Biotechnology Co., Ltd. (Shanghai, China) and its sequence was RPCLPHCNSTSD. K40I protein, the fortieth amino acid mutation of angiogenin, was provided by Pro. Xu Zhengping, School of Medicine, Zhejiang University. α-Cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxy-benzoic acid (DHB) were obtained from Bruker Franzen Analytik GmbH (Germany). The model of filter membrane was Omega from Pall Corporation Company (USA).

2.2. Ionic liquid synthesis

The bromopropene and methylimodazole were mixed as equivalent weight 1:1.1 m/m in reaction flask and heating reflux for 12 h at the temperature 80 °C. Then the reaction solution was dissolved in ethyl acetate and extracted by water for three times. After that the extraction liquid was concentrated through reduced pressure distillation and analyzed by mass spectrometer.