Utility of mass spectrometry in protein science

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Facing the post-genome era, the importance of mass spectrometry (MS) in biological science is increasing. In addition to the identification of proteins, we have been exploring new applications of MS to solve biological problems including post- translational modification, protein-protein and protein-ligand interactions, higher order structure of proteins and functional aspects of probable proteins.

Introduction

With the end of the human genome project in sight, mass spectrometry (MS) has become an essential tool for biochemical analyses. Genome projects have made enormous amounts of genetic information available for protein characterization, although large portions of the genes unveiled remain to be worked out for functions. Important information is lacking regarding the processing of nascent polypeptide chains to mature functioning forms. With recent advancements, today's MS is a powerful tool for the analysis of biological macromolecules, especially proteins. Having high sensitivity,[*2] high resolution and rapid processing, MS is considered to be the method of choice for the proteomic era. It can be applied not only to identify proteins and their post-translational processings, but also to study protein-protein and protein-ligand interactions and higher order structures of proteins. Since the early 1990s, we have been exploring new applications of MS for protein characterization in many aspects. Here, we describe some of our applications.

Post-translational modification

Since MS is based on the behaviour of charged particles in an electrical and/or magnetic field in vacuum, it provides straightforward numerical information (mass per unit charge) on the post-translational processing of nascent polypeptides translated from mRNA.

Brains of Alzheimer's disease patients are characterized by the presence of two structures: neurofibrillary tangles (NFTs) and senile plaques. Paired helical filaments (PHFs) and amyloid fibrils, respectively, are deposited to form those structures. The major components of PHFs and amyloid fibrils have been identified as tau, a microtubule-associated protein, and amyloid β-protein (Aβ) derived from β- amyloid precursor protein (APP), respectively. Extensive protein chemical and mass spectral analyses revealed that those hardly soluble deposits are complex mixtures of aberrant processing products of tau and APP. Tau had been extensively phosphorylated,[1)] ubiquitinated,[2)] and isomerized,[3)] and Aβ had been generated by aberrant proteolytic processing of APP.[4)]

A novel essential structure of the active center was revealed in photo-reactive nitrile hydratase by using MS together with X-ray crystallography. Non-heme iron was attached to a short cysteine clustered stretch of the polypeptide chain, in which all three cysteine residues exist in different oxidation states, namely, cysteine, cysteine sulfenic acid and cysteine sulfinic acid.[5,6)] Three sulfur atoms of Cys109, CysSO_2H112 and CysSOH114 and two amide nitrogen atoms of Ser113 and CysSOH114 are coordinated to the ferric iron. Three oxygen atoms of CysSO_2H112, CysSOH114 and Ser113 are in the position to surround the nitrogen atom of the substrate or the photosensitive inhibitor, NO, in a claw setting fashion.[5)]

Protein-ligand, protein-protein interactions, and higher order structures

It is now clear that by selecting ionization conditions, non-covalent complexes can be analyzed directly. Using sinapinic acid as a matrix, non-covalent complexes of Ras-GDP and Ras-GppNp were observed by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) MS.[7)] Electrospray ionization (ESI) of a tryptic digest in weakly alkaline solution conserved all the iron-peptide coordination of the active center in photo-reactive nitrile hydratase. Upon addition of acetic acid, the complex was destroyed with the release of ferric iron and the spontaneous formation of a disulfide bond.[5)]

The binding affinity difference of truncated forms of a cysteine protease inhibitor, cystatin (Fig. 1), to papain was clearly demonstrated (Fig. 2) using ESI along with a Fourier-transform ion cyclotron resonance (FTICR) mass spectrometer.[8)]

It has been a common practice to use chemical modifications to study surface localization, protein-ligand and protein-protein interaction sites.[9)] However, its applicability is severely limited by the limited sidechain reactivity, the solubility of the reagent, and the reaction conditions,

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[*2] The amount of sample required depends on the purpose of the analysis. Molecular mass of a protein or identification of the gene encoding a protein may need only sub-picomole quantities since only one spectrum or partial structural information, respectively, is needed. Analysis of protein-protein or protein-ligand complex or a certain specific region of a protein may require even nanomolar quantities because of the necessity of sub-optimal conditions or the intrinsic character of the region, respectively. The actual amount also depends on the conditions of the sample.

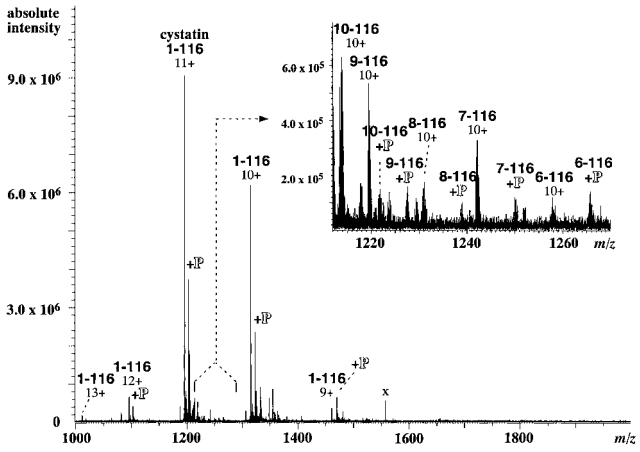


Fig. 1. ESI-FTICR mass spectrum of cystatin (Sigma, Lot #78H40891). Multiply protonated molecules of the full-length cystatin are indicated by "1-116" together with the number of charges. Those of N-terminally truncated forms are indicated by "10-116", "9-116", "8-116", "7-116", or "6-116" accompanied by the number of charges. Molecular ions of phosphorylated species are marked with "+ℙ". A noise peak is indicated by x.

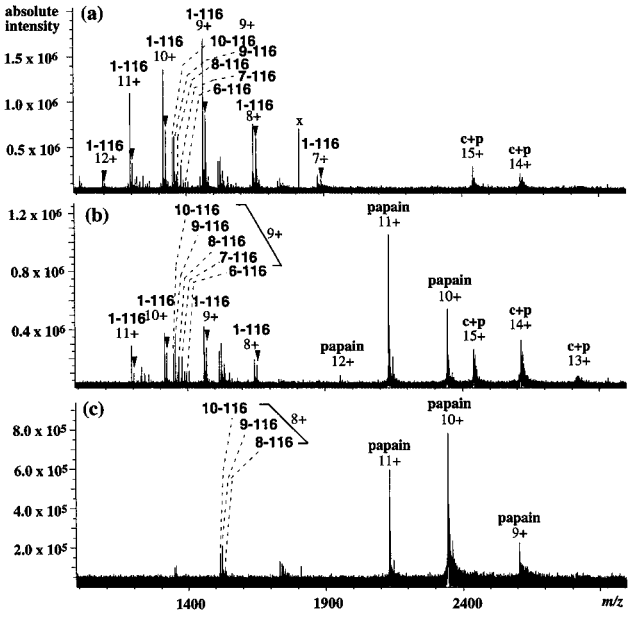
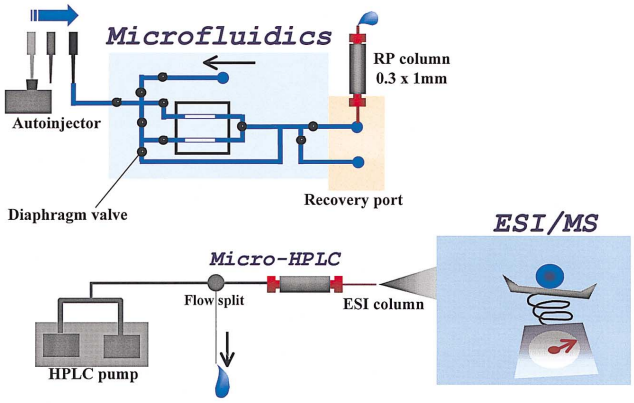


Fig. 2. ESI-FTICR mass spectra of (a) 2.8:1, (b) 1:1.3, and (c) 1:3.2 mixtures of cystatin and papain. Multiply charged molecular ions of cystatin-papain complex are indicated by "c+p" accompanied by the number of retaining charges. Those of papain are denoted "papain" with the number of charges. Multiply protonated molecules of truncated cystatin are indicated as in Fig. 1. Arrowheads indicate phosphorylated forms of the intact cystatin.

as well as the secondary effects of chemical modification. With the use of MS, hydrogen/deuterium (H/D) exchange of amide hydrogens has become a powerful tool for such studies. Although detailed information can be obtained by NMR or X-ray crystallography, MS is quick and requires much less materials. We applied H/D exchange along with MS to study enzyme-inhibitor complex interfaces in papain-cystatin[10] and thrombin-thrombomodulin[11] complexes.



Schematic diagram of semi-on-line microfluidic-LC-MS/MS system

Fig. 3. Micro-quantity target proteins (fmol amount) present in a biological mixture are isolated on the sensor chip surface (indicated as microfluidics), and the captured proteins are digested on the sensor chip. Generated peptides are recovered in an on-line RP micro-precolumn. The precolumn is transferred to HPLC-MS/MS. The resulting peptides are further separated on the micro-ESI column and analyzed by MS/MS.

Conformational changes can also be studied by H/D exchange along with MS. pH- and organic solvent-dependent conformational changes of ubiquitin, cytochrome c and myoglobin were probed and the results were in accord with those obtained by circular dichroism.[12] Higher order structure information on ubiquitin was obtained by H/D exchange in conjunction with collision-induced dissociation (CID) in an rf-only hexapole ion guide with ESI-FTICR MS.[13]

Functional analysis through biomolecular interaction

Genome projects have revealed the presence of many probable proteins with totally unknown functions. Information on the functional aspects of those probable proteins can be collected from proteins that interact with the protein expressed artificially using the probable protein gene.

Using microfluidics together with surface plasmon resonance, biomolecular interaction analysis (BIA) is a highly sensitive method to analyze biomolecular interactions quantitatively. Combining BIA with micro-LC/ESI MS, we have shown that it is possible to identify bound protein at low femtomole levels.[14] Cell lysate was passed through a ligand-immobilized sensor chip. The flow path was washed and bound protein was quantified. A digestion buffer containing a proteolytic enzyme was introduced to digest the bound protein. The peptide mixture thus generated was recovered on a reversed-phase micro column and analyzed by ESI MS/MS (Fig. 3). This BIA-MS system is a powerful tool to reveal the functional significance of those probable proteins.

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