Current Topics

Challenge of Mass Spectrometry toward the Elucidation of Life Phenomena

Quantitative Approach for Small Molecules Using Laser Desorption/Ionization Mass Spectrometry

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Recent advances in quantitative approaches to laser desorption/ionization mass spectrometry (LDI-MS) are selectively reviewed. Numerous efforts have been made to use matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and related techniques for rapid screening and/or monitoring of biological events. Among the various mass ionization spectrometric analysis techniques, MALDI-MS has exceptional potential for high-throughput analysis. Although quantitative analysis with MALDI-MS is challenging, the expansion of its utility is inevitable. It is notable that practical improvement of MALDI-MS can be achieved by preparation of an uniform matrix using binary matrix systems. Broad applications have been established with a self-assembled monolayer for MALDI-MS, referred to as SAMDI-MS. The method could be an essential tool not only for rapid screening but also as a sensitive probe for surface sciences. Complementary to these approaches, labeling of molecules for LDI-MS is introduced as potential tool for the selective detection of specific target molecules.

Key words laser desorption ionization mass spectrometry; quantitative analysis; labeling; photocleavable molecule; matrix-assisted laser desorption/ionization mass spectrometry; small molecule analysis

1. INTRODUCTION

Mass spectrometric detection of small target molecules is attractive method to analyze biological reactions.[1,2] Among many detection methods utilizing optical phenomena such as UV, visible absorption, fluorescence, luminescence, and radioactive isotopes, mass spectrometric detection of target molecules is attractive for the detection of target molecules because of its ability to obtain qualitative information related to their molecular weight. Any minor modification of target molecules, which is accompanied by a change in molecular mass, can be assessed unambiguously as the change in the mass-to-charge ratio (m/z). This feature allows the observation of multiple target molecules simultaneously even in a complicated experimental system. Although the method is destructive, generally consumption of samples is negligibly small. Thus, the great potential of mass spectrometry (MS) is attracting the attention of many chemists and biochemists as it allows expansion of the m/z region of target molecules.

Matrix-assisted laser desorption ionization (MALDI)-MS is one attractive method for analyses of complex biological systems because of its benefits, such as simple operation and sample preparation, high-throughput analyses, applicability to high m/z regions, and sample preservation on target plates. On the other hand, MALDI-MS analysis has the disadvantages of rather poor reproducibility of signals and chemical noise in low-mass regions (<1000 Da), mainly originating from excess matrices. Numerous efforts have been made to overcome these drawbacks. In this paper, impressive recent advances related to laser desorption ionization (LDI)-MS and the potential for future endeavors in the field are described.

2. OPERATIONAL IMPROVEMENT OF MALDI-MS

Many attempts have been made aimed at achieving high reproducibility and applicability to low-mass regions, including high-throughput and qualitative and quantitative analyses of complicated biological systems. Various improved sample preparation methods have been reported for the quantitative use of MALDI-MS targeting peptides, proteins, oligosaccharides, bile acids, lipids, *etc.*[3] Impressive binary matrices, such as the combination of 2,5-dihydroxybenzoic acid/*N*,*N*-dimethylaniline, were reported by Snovida *et al.* for the quantitative analysis of oligosaccharides.[4,5] The observed highly reproducible MALDI spectra were explained as a result of a uniform crystal layer.[6] Since the operation is simple and readily feasible, the approach would be of further utility and applicability for a variety of target molecules. In addition, the results may encourage researchers to seek the best measurement conditions using MALDI-MS. Such activity should lead to a better understanding of the MALDI process.

3. SELF-ASSEMBLING MONOLAYER FOR MALDI-MS

One of the most successful applications was established with a self-assembling monolayer for MALDI-MS, which is referred to as SAMDI-MS. Aligned spots of a thiol monolayer on a gold target plate are used for chemical screening.[7] Since the substrate is fixed on the surface of the gold target plate, it is easy to remove applied reagents before measurement. Surfaces are stabilized on the surface layer, and a minimal amount of matrix is required for measurement. The use of a

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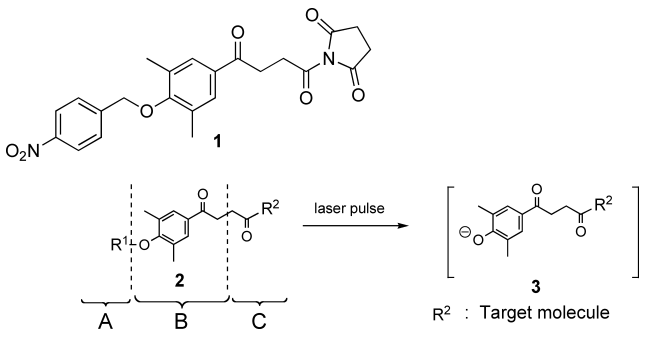


Fig. 1. Photocleavable Molecule for Laser Desorption Ionization Mass Spectrometry

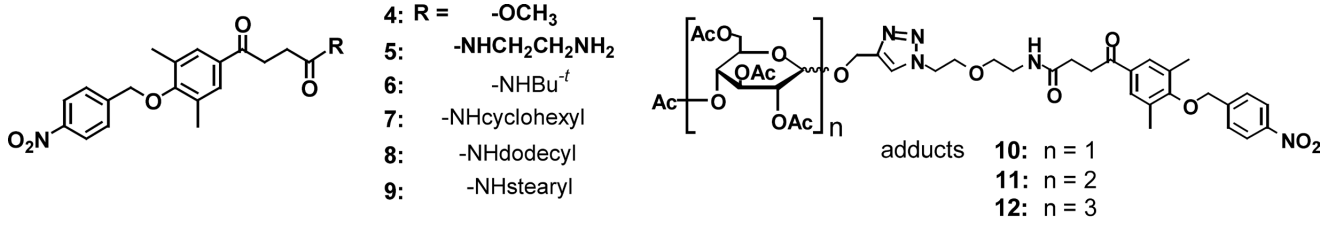
(A) A moiety to afford cationic species after bond breaking by laser-pulse irradiation. Electron-deficient groups are preferable for achieving fast bond breaking, which affords high sensitivity.[15] (B) Phenacyl substructure to generate phenolate ion **3** by laser-pulse irradiation. (C) Tether to capture a target molecule. A variety of functional groups can be introduced depending on the nature of target molecules.

polyethylene glycol chain shifts the observed *m/z* value from the low-mass region to the high (detectable)-mass region in which signals originating from the matrix do not hamper the spectra. Significantly stabilized surface conditions with the self-assembling monolayer make it possible to treat mass spectra in a quantitative or semiquantitative manner. A wide range of applications was reported by groups working with Mrksich.[7–13] Measurement of each single spot is completed in several seconds, thus enabling high-throughput screening for kinases,[8] β-1,3-*N*-acetylglucosaminyltransferase,[9] histone deacetylase,[10] lysine deacetylases,[11] nucleic acid ligases,[12] and inhibitors of the anthrax lethal factor.[13] The process may maximize the utility of LDI-MS, and its usefulness and future potential have been confirmed.

4. LABELING METHOD FOR LDI-MS

Labeling of molecules is an attractive technique that enables the monitoring of specific molecules in a complicated system.[14] Although a broad range of fluorescence-based molecular tags has been developed, molecular tags for mass spectrometric detection have been relatively unexploited. A photocleavable molecule emerged as a new functional molecule that selectively affords detectable ions under LDI-MS conditions.[15] A representative molecule **1** is composed of three parts: chromophores A and B; and tether C (Fig. 1). After labeling the target molecule with tether C, negative-mode LDI-MS can detect the *m/z* of **3**, which involves information on the molecular weight of the target.

Since chromophores A and B were designed to absorb energy directly from the laser pulse, the system does not require a matrix to assist ionization. An interesting structural motif of the photocleavable molecule was elucidated in which strengthening of the carbon-oxygen bond by an electron-withdrawing group on the cationic part of the molecular tag was observed to be favorable for high sensitivity. Thus, signals in the low-mass region can be selectively monitored after labeling. The reproducible response is suitable for quantitative and/or semiquantitative treatment of ratios of signal intensities. For



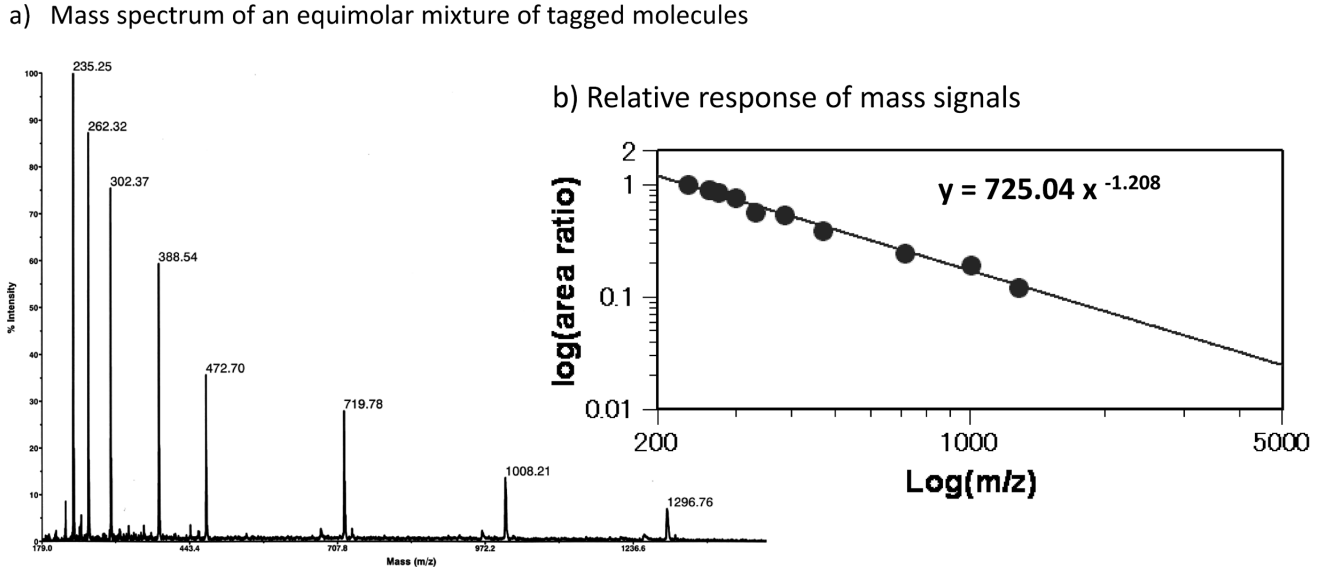


Fig. 2. LDI-MS Response for a Variety of Derivatives with Photocleavable Molecules

(a) The spectrum of LDI-MS acquired for a mixture of photocleavable molecule derivatives **4**–**12** that have various molecular weights and structures. (b) The graph shows a linear relationship between the logarithmic signal-area ratio and mass-to-charge ratio (*m/z*) of the mass spectrum. The sample was prepared as 10 μM of solution for each substrate and 0.2 μL of solution was applied on a MALDI target plate (2 pmol/well) without matrix.[15]

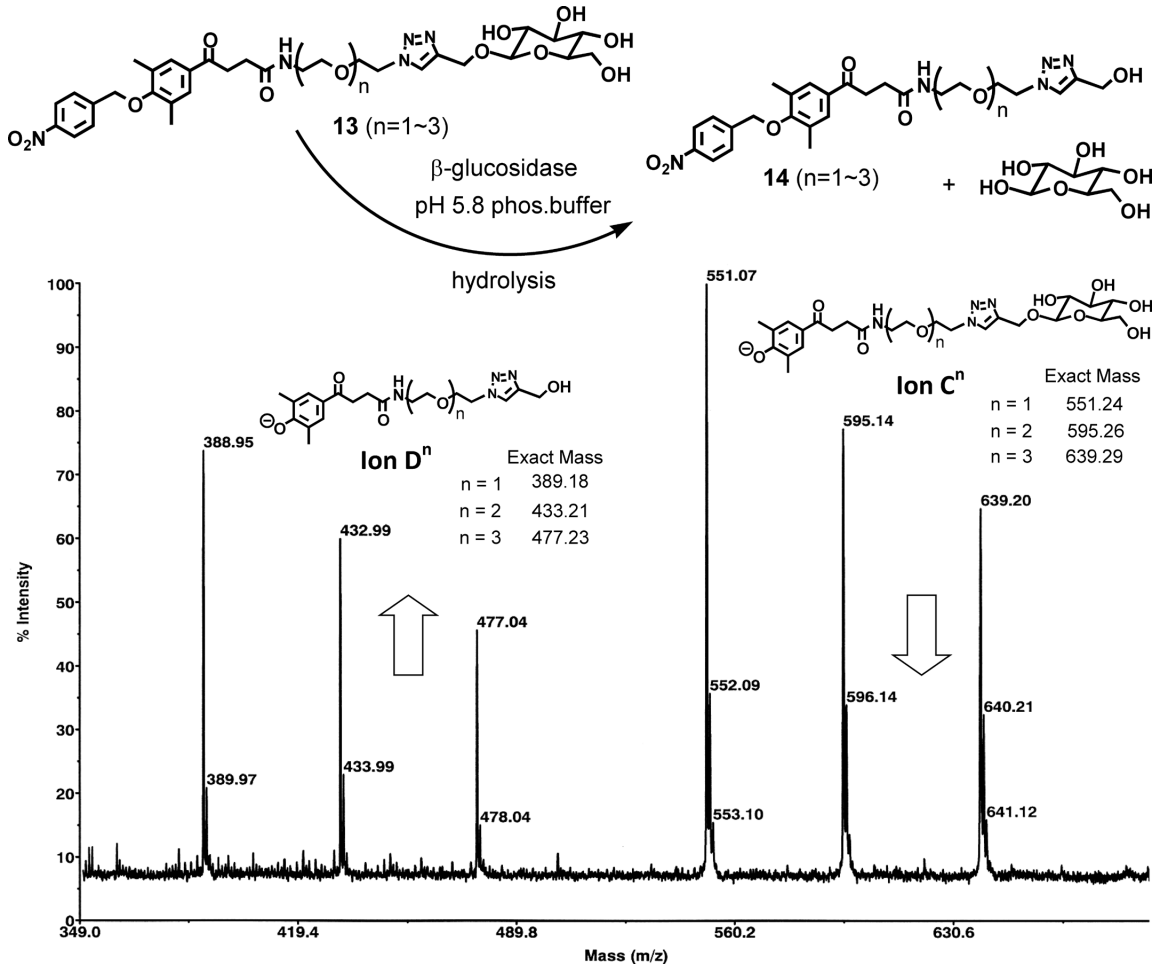


Fig. 3. Photocleavable Probe for β-Glucosidase

The mass spectrum for measuring the activity of β-glucosidase using a probe simply prepared by introducing a photocleavable molecule into β-glucose *via* ethereal chains (ethylene glycol unit numbers 1–3). The spectrum shows an increasing set of signals originating from products (388–477) with a decreasing set of signals from probe molecules (552–639). The reaction sample was mixed with the same volume of acetonitrile to remove excess buffer salts by precipitation, and the supernatant was applied on a MALDI target plate (10 pmol/well) without matrix.[17]

example, molecule **1** can react with the amino functionality of target molecules. Also, 2+3 cycloaddition between the carbon triple bond and azide (click chemistry)[16] is usable as a reliable chemical reaction. Since the photocleavable bond is stable under standard chemical reaction conditions (moderate acidity and basicity), a variety of reaction conditions are applicable for derivatization.

Based on the high reproducibility of the labeling method, even the molar ratio of unknown molecules can be estimated. A mass spectrum of an equimolar mixture of labeled molecules, which have a variety of molecular weights, exhibited steady responses that were correlated with *m/z* alone (Fig. 2). When the photocleavable molecule is introduced into a sugar, it can act as a probe for enzyme activity. Mass spectrometric detection can be readily differentiated based on the *m/z* of each different length of the polyethylene glycol tether. It was reported that the reaction profile was successfully monitored by readily derivatized compound **13**[17] (Fig. 3).

Five-step preparation of **1** without chromatographic purification has recently been achieved from commercial, abundantly available resorces.[18] Furthermore, compound **1** is now commercially available and used for the detection of amino acids and small peptides.[19] A molecular probe utilizing UV absorption or fluorescence must be well designed to change signals upon target enzymatic reactions, which largely limits the structure of molecular probes. In addition to this limitation, molecular probes based on absorption and fluorescence cannot detect unexpected reactions. Mass spectrometric detection makes flexible and quick design of molecular probes and assessment of unknown side reactions possible.

5. CONCLUSION

Quantitative analysis with LDI-MS is challenging when compared with other ionization methods in MS. However, because of its simplicity and undisputed high-throughput inherencies, MALDI-MS and LDI-MS are still attractive. Thus efforts to improve quantitative or semiquantitative analyses should continue and may open a new stage in biological elucidation.

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