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Effect of solvent on ionization efficiency in matrix-assisted laser desorption/ionization mass spectrometry of peptides

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ABSTRACT

Although various theoretical models of matrix-assisted laser desorption/ionization (MALDI) have been proposed, no model can explain all of the experimentally observed phenomena. It has been reported that water content in the solvent used in the sample preparation has influence on the ion yield of MALDI. However, the previously proposed models do not take account of the solvent. Therefore, we have investigated the influence of the solvent on MALDI mass spectrometry of peptides. Among the solvents of water, methanol, ethanol, tetrahydrofuran, and acetonitrile, the signal peak area of the peptide ion was the largest when the water solvent was used. It is suggested that the residual water solvent contained in the dried matrix crystal has influence on the ionization processes of MALDI. It is inferred that inclusion of the influence of the residual solvent into the theoretical model of MALDI could contribute to the improvement of the precision of the model.

1. Introduction

Mass spectrometry is an analytical technique by ionizing analyte to generate charged particles and by separating them according to their mass-to-charge ratio. So that, ionization is one of the most essential processes in mass spectrometry. Because the chemical and physical properties of the analyte vary with the application fields, various ionizing methods have been developed according to the object of analysis. Among them, matrix-assisted laser desorption/ionization (MALDI) has widely been used for mass spectrometric analysis of peptides, proteins, synthetic polymers, etc. [1]. However, ionization mechanism of MALDI has still not been elucidated. On the other hand, low reproducibility of the ion yield in MALDI makes quantitative measurement difficult. Ion suppression effects are also important problem to analyze complex mixtures. By understanding and controlling the ionization processes of MALDI, these problems will be overcome and much more applications will become possible.

In order to explain the phenomena experimentally observed in MALDI mass spectrometry, various ionization models have been proposed, e.g., the photochemical ionization (PI) model [2], the cluster ionization (CI) model (or the Lucky Survivor model) [3], the quantitative model [4–6], and the energy transfer induced disproportionation (ETID) model [7]. The PI model is considered to involve a two-step ionization process, i.e., photoionization of matrix molecules and subsequent ionization of analytes in the gas phase reactions with ionized matrix molecules. Energy pooling and multiphoton absorption are the major processes to the photoionization of matrix molecules. Analyte ions are produced by protonation or deprotonation via the collision process with ionized matrix molecules. The CI model assumes large protonated analyte polymers already exist in the acidic matrix environment, and these clusters are desorbed during the laser irradiation. Then analyte ions are produced in the gas phase by the desolvation of neutral matrix molecules. The quantitative model proposed by Knochenmuss in 2002 is a model solving continuum rate equations taking account of primary ionization processes which include both photochemical processes and desorption dynamics [4]. Then the quantitative model has been extended to include secondary ion-molecule reactions in 2003 [5] and to include bipolar ions in 2009 [6]. In the ETID model, two analyte molecules share an active proton through hydrogen bonding in the solid phase, and a nearby matrix molecule is closely coupled to the bonding proton that is shared by the two analyte molecules. With absorption of a laser photon by the nearby matrix molecule, the excited matrix molecule can transfer its energy through short range energy transfer mechanism to an analyte dimer to produce disproportionation which leads to one protonated and one deprotonated analyte ion simultaneously. However, the validity of this model has recently been denied by the same research group by measuring both positive and negative ions simultaneously using a home-made dual-polarity mass

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spectrometer [8]. Recently, Jaskolla and Karas have proposed the combined Lucky Survivor and gas phase protonation model [9].

Although various models have been proposed as described above, no model can explain all of the experimentally observed phenomena. It has been reported that water content in the solvent used in the sample preparation of MALDI has influence on the ion yield of MALDI [10]. In the report, the influence of the residual solvent on the ionization processes in MALDI has been pointed out. However, all models described above do not take account of the effect of the solvent. Therefore, we have investigated the influence of the solvent on the ionization efficiency in MALDI mass spectrometry of peptides.

2. Materials and methods

2.1. Materials

Bradykinin fragment 1–7 (B4181, Sigma–Aldrich, St. Louis, MO, USA) and human angiotensin II (A8846, Sigma–Aldrich) were used as the peptide analytes, and 2,5-dihydroxybenzoic acid (DHB, 149357, Sigma–Aldrich) was used as the matrix. All were used without further purification. Water, methanol (MeOH), and ethanol (EtOH) were used as protic solvents, and tetrahydrofuran (THF) and acetonitrile (ACN) were used as aprotic solvents.

Bradykinin fragment 1–7 or angiotensin II was dissolved at a concentration of 1 pmol/μL in each solvent, while DHB was dissolved at a concentration of 10 mg/mL in each solvent. Each peptide solution was mixed with each matrix solution with the same solvent at 1:1 (v/v) ratio. Then 1 μL of the mixture was deposited in a circle with a diameter of 2.5 mm on a gold coated target plate (V503476, AB SCIEX, Framingham, MA, USA) and dried in ambient air.

2.2. Methods

A MALDI time-of-flight mass spectrometer (Voyager-DE PRO, AB SCIEX) equipped with a nitrogen laser (VSL-337ND, Laser Science, Franklin, MA, USA) was used. The nitrogen laser was irradiated to the sample at a repetition rate of 20 Hz. A laser energy meter (PE10, Ophir Optronics, Israel) was used to measure the irradiated laser pulse energy. Positive ions were accelerated by a voltage of 20 kV with delayed extraction and detected through an ion reflector. Each mass spectrum was averaged over 100 laser shots, and 20 mass spectra obtained by irradiating the laser to randomly selected 20 points within the sample spot with a diameter of 2.5 mm were averaged to obtain a single mass spectrum from each sample spot.

3. Results and discussion

Fig. 1 shows typical mass spectrum for angiotensin II obtained with the water solvent. Signal peak areas of the monoisotopic ions of the matrix and peptide were calculated using Data Explorer 4.0 software (AB SCIEX). Because both the radical ion m^+ and protonated ion $[m + H]^+$ of the matrix were observed, the peak at m/z 155.03 contains the monoisotopic ion of $[m + H]^+$ and the second isotopic ion of m^+. Therefore, 7.9% of the peak area at m/z 154.03 was subtracted from the peak area at m/z 155.03 to obtain the peak area for the monoisotopic ion of $[m + H]^+$ taking account of the natural isotopic distribution of m^+. On the other hand, only the protonated ion $[A + H]^+$ was detected for the analyte angiotensin II.

Fig. 2 shows the relationships between the solvent and the ion signal peak areas of m^+, $[m + H]^+$, and $[A + H]^+$. For both bradykinin fragment 1–7 and angiotensin II, the peak area was the largest

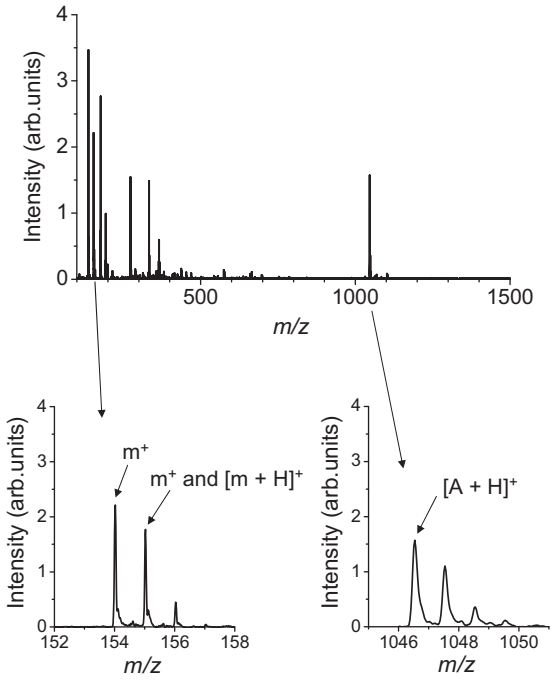

Fig. 1. Typical mass spectrum, where the analyte and solvent were angiotensin II and water, respectively.

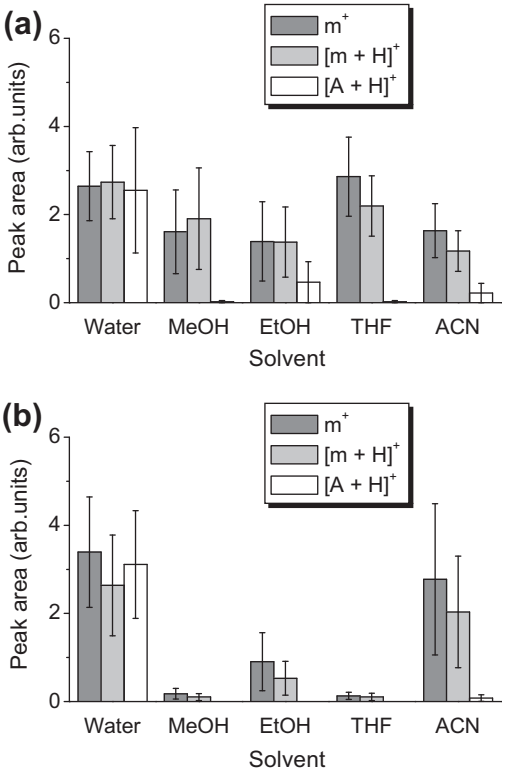

Fig. 2. Relationships between the solvent and the ion signal peak area for (a) bradykinin fragment 1–7 with laser fluence of 12 mJ/cm^2 and (b) angiotensin II with laser fluence of 18 mJ/cm^2. The averaged values and the standard deviations from eight sample spots are shown.

when the water solvent was used. If the matrix ions directly contribute to the ionization of the peptide, correlation between the peak areas of the matrix and peptide ions should be observed. However, evident correlation between the peak areas of the matrix