

Influence of Silver Salt Types on Formation of Silver Cluster Ions in MALDI with DHB as Matrix

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Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a soft ionization technique used for the analysis of large nonvolatile molecules such as biopolymers and synthetic polymers.¹⁻⁴ The goal of sample preparation in MALDI analysis is homogeneous cocrystallization of analyte and matrix molecules. The role of the matrix is very crucial in MALDI and many researchers have studied on what is a good matrix. Macha and Limbach⁵ discussed about the role of matrix in MALDI analysis. Matrix compounds are still discovered through trial and error because there has not been enough research to investigate what are the factors that affect the functioning of such compounds in MALDI process. The matrix dilutes and isolates polymers in the sample preparation step and plays a role in the analyte desorption and ionization process by absorbing the laser energy at the wavelength used.⁶ The ratio of the analyte to the matrix depends on the molecular weight and chemical properties of the analyte. The ratio increases when higher molecular weight sample is analyzed. In general, the molar ratio of the analyte to the matrix ranges from 1:10³ to 1:10⁵.

Dihydroxybenzoic acid has many isomers such as 2,3-, 2,4-, 2,5-, 2,6-, and 3,4-dihydroxybenzoic acids. Among them, 2,5-dihydroxybenzoic acid has the most absorption coefficient at a wavelength of 337 nm.⁷ 2,5-Dihydroxybenzoic acid (DHB) is commonly used for the analysis of synthetic polymers as well as biopolymers. Some polar matrices such as 4-hydroxy-3,5-dimethoxy-cinnamic acid, α -cyano-4-hydroxy-cinnamic acid, and methyl salicylic acid interact well with biopolymers while some polar matrices such as 2-(4-hydroxyphenylazo)-benzoic acid (HABA), *all-trans*-retinoic acid (RTA), and dithranol work for synthetic polymers. Nonpolar matrices such as anthracene, pyrene, acenaphthene, and terthiophene are suitable for nonpolar samples.

In a MALDI mass spectrum, the protonated molecule and cation adduct of an analyte give an important information about the molecular ion mass. Some researchers⁸⁻¹⁰ reported that silver clusters can be effectively produced under MALDI conditions from silver salts in the presence of matrices. During the analysis of organic molecules when Ag⁺ is used as the cationizing agent, it can be recognized that besides the desired adduct ions, [M + Ag]⁺, an additional silver cluster ions, Ag_n⁺. Study on metal cluster formation in gas-phase by

mass spectrometry provides valuable information on the stability and electronic properties of the metal clusters depending on the sizes and this information can be utilized when the clusters are immersed into a liquid or solid environment. One of useful methods to generate silver cluster ions is MALDI using silver salts and matrices.^{8,9} Kéki and coworkers⁹ studied the matrix effect on the formation of the silver cluster ions under MALDI conditions using reductive polar organic matrices and silver trifluoroacetate (AgTFA) and reported that the silver cluster ions were effectively produced and the matrix greatly influenced the resulting cluster ion abundances. Properties of metal salts are depending on the kind of the counter anions as well as the metal types. It can be expected that the formation of the silver cluster ions in MALDI will be affected by the counter anions of silver salts. However, this investigation has not been sufficiently carried out. In the present work, we investigated the influence of the silver salt types on the formation of silver clusters in MALDI conditions and experimental results were explained with the interactions and reactions between the silver salt and the matrix, DHB.

The MALDI mass spectrum of the AgBz shows the silver cluster ion intensity distribution with an odd-even alternation pattern as shown in Figure 1. The intensity of the odd-number cluster ions tends to be stronger than that of the neighboring even-number cluster ions. The mass spectrum also shows the clear magic numbers of n = 21 and 35 of Ag_n⁺. The magic number of n = 21 is in good agreement

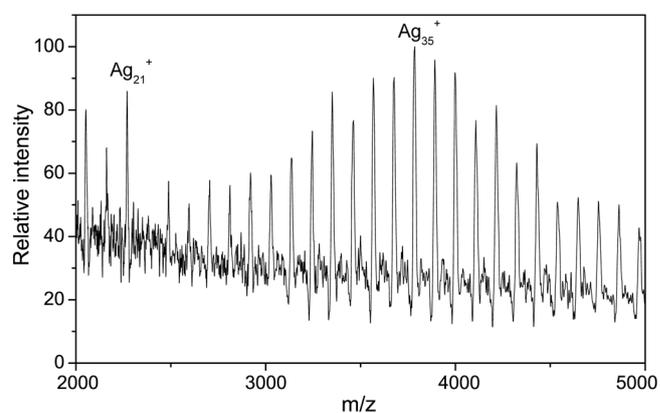
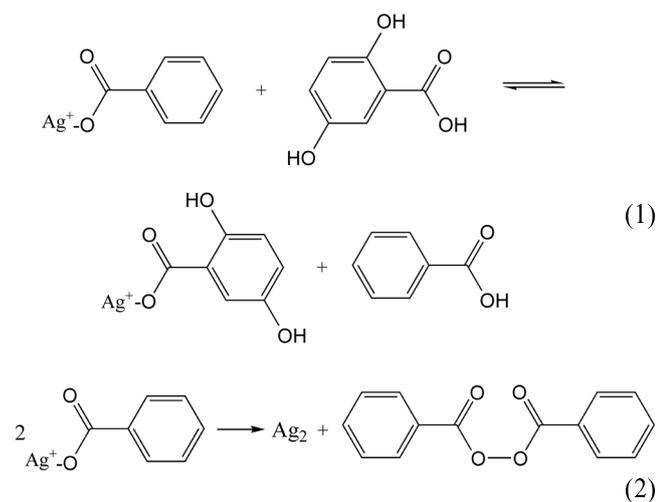


Figure 1. MALDI-TOF mass spectrum of 0.10 M DHB and 0.010 M AgBz.

with the jellium model theory.^{11,12} The intensity of Ag_{21}^+ is lower than that of Ag_{35}^+ . This is an unexpected result since the intensity of Ag_{21}^+ is generally much higher than that of Ag_{35}^+ .^{8,9,13} This implies that the mixture of AgBz and DHB generates higher silver clusters by absorbing laser energy. AgBz can directly produce silver cluster ions without matrix by absorbing laser energy and the LDI mass spectrum shows a clear silver cluster ion distribution which has much higher intensity of Ag_{21}^+ than those of Ag_n^+ ($n > 21$).¹³ Some AgBz molecules can react with the DHB molecules to produce silver 2,5-dihydroxybenzoates (AgDHB) and benzoic acids as shown in the reaction (1). This reaction is a kind of cation exchange reaction, where the cations are Ag^+ and H^+ . Since the acid dissociation constant of benzoic acid is lower than that of DHB, the reaction (1) can occur to some extent. The pK_a s of benzoic acid and DHB are 4.2 and 3.0, respectively. Thus, DHB is more acid than benzoic acid. The AgDHB can absorb the laser energy and generation of silver clusters will be activated. Another possibility is a disproportionation of AgBz as shown in the reaction (2). Synthesis of silver clusters using silver benzoate was reported.^{14,15}



AgTFA is a commonly used silver salt. The MALDI mass spectrum of the AgTFA shows a typical silver cluster ion intensity distribution as shown in Figure 2. The mass spec-

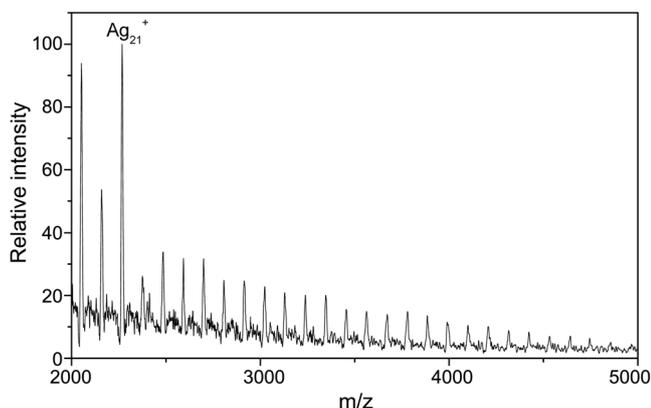
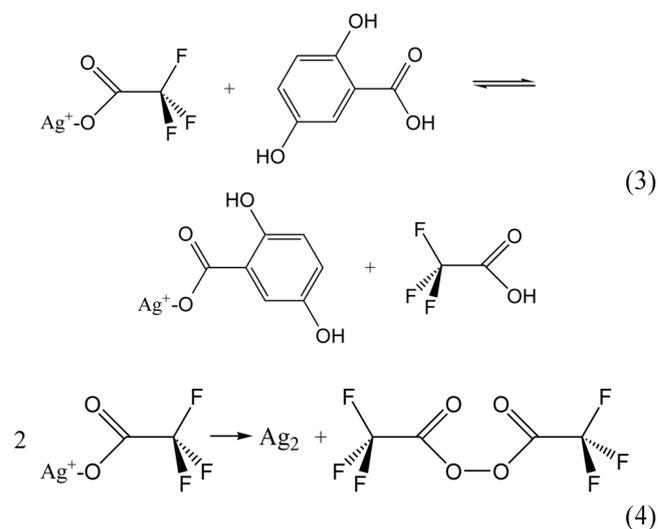


Figure 2. MALDI-TOF mass spectrum of 0.10 M DHB and 0.010 M AgTFA.

trum also shows the clear magic number of Ag_{21}^+ . Macha and coworkers¹⁶ investigated the formation of silver cluster ions under MALDI conditions using AgTFA together with various matrices and reported that less polar matrices such as anthracene, acenaphthene, and dithranol did not generate any silver clusters while polar matrices such as DHB, HABA, and RTA produced silver cluster ions. As similar to the case of AgBz, the AgTFA molecule can react with the DHB molecule to produce AgDHB as shown in the reaction (3) and a disproportionation of AgTFA will lead to generation of silver clusters as shown in the reaction (4). Since the pK_a of trifluoroacetic acid (TFA), the conjugate acid of AgTFA, is 0.3, TFA is more acid than DHB. Thus, the equilibrium constant of the reaction (3) of AgTFA may be much smaller than that of the reaction (1) of AgBz. This means the difference in the capabilities of AgBz and AgTFA to generate the silver cluster ions under MALDI conditions.



Figures 3 and 4 are the MALDI mass spectra of AgNO_3 and AgTS, respectively. They do not clearly show the silver cluster ions. For the MALDI mass spectrum of AgNO_3 , although the magic number of Ag_{21}^+ is observed but the silver cluster ion distribution is not clear after the Ag_{21}^+ . The MALDI mass spectrum of AgTS does not show any silver

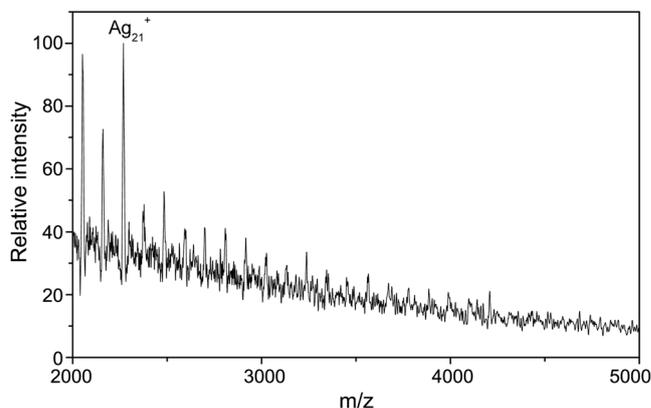


Figure 3. MALDI-TOF mass spectrum of 0.10 M DHB and 0.010 M AgNO_3 .

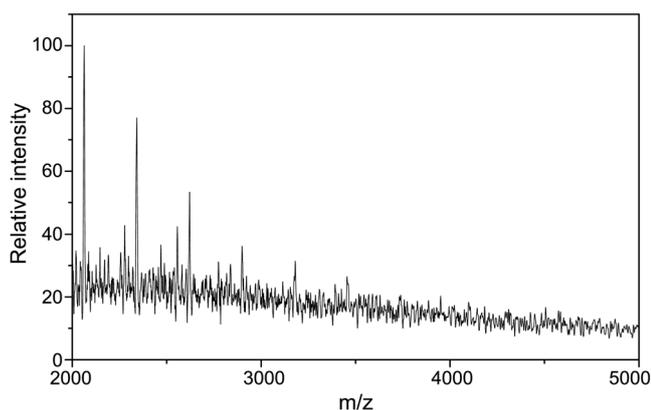


Figure 4. MALDI-TOF mass spectrum of 0.10 M DHB and 0.010 M AgTS.

cluster ions. These may be due to the inadequate properties of AgNO_3 and AgTS to the formation of silver cluster ions with DHB. Since the conjugate acids of AgNO_3 and AgTS , nitric acid and *p*-toluenesulfonic acids, respectively, are strong acids, the salts can not react with DHB to generate AgDHB and the conjugate acids.

The experimental results lead to a conclusion that formation of the silver cluster ions in MALDI conditions depends on the silver salt types and acidity of the conjugate acid of silver salt determines generation of the silver cluster ions since acidity of the conjugate acid affects the formation of AgDHB which activates generation of the silver cluster ions by absorbing the laser light.

Experimental Section

Silver benzoate (AgBz), silver trifluoroacetate (AgTFA), silver nitrate (AgNO_3), and silver *p*-toluenesulfonate (AgTS) of Aldrich Co. were employed as silver salts. 2,5-Dihydroxybenzoic acid (DHB) purchased from Aldrich Co. was used as matrix. The silver salts were dissolved in distilled water and the concentrations were 0.01 M. DHB was also

dissolved in distilled water and the concentration was 0.1 M. The silver salt and DHB solutions with the volume ratio of 1:5 were mixed. The mixture solution of 1 μL was spotted onto the sample plate and dried. Mass spectra were obtained with Axima-LNR MALDI-TOFMS (Kratos-Shimadzu Co. of Japan). Ions were produced by irradiation of the sample with nitrogen laser (337 nm). Profiling of product ions was achieved in the positive mode using linear TOF. The accelerating voltage was 20 kV. The sum of 50 shots was collected for each spectrum.

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