Studies of some Characters of Matrix Influencing Desorption Ionization Processes in Matrix-assisted Laser Desorption Ionization Mass Spectrometry

Shankai ZHAO*, Feng ZHONG, Jing Houng CHANG and Zhihua ZHU

Instrumental Analysis & Research Center, Zhongshan University, Guangzhou, P.R. China

Some results are introduced in studying characters of matrices influencing the desorption ionization processes in MALDI-MS, which have not been reported. By the use of low angle laser scattering photometer the association state of four matrices with lysozyme are measured. The results demonstrated that 3-amino-4-hydroxybenzoic acid (3A4HA) is the best one which can be calculated 224 molecules to associate with one molecule of lysozyme. The other three matrices, nicotinic acid (NA), 2,5 dihydroxybenzoic acid (DHB) and caffice acid (CA) are in the order of several tens of matrix molecules associated with one protein molecule. It is interesting to point out that 3-amino-4-hydroxybenzoic acid is the only one that cannot perform MALDI-MS spectrum and the reason is discussed. The results of the investigation in negative MALDI mass spectra of different matrices demonstrated that the desorption ionization processes are different with that of positive ions, and their mechanisms were proposed.

keywords: MALDI-MS, low angle laser light scattering photometer, negative ions

Matrix-assisted laser desorption ionization mass spectrometry has become a powerful tool for the analysis of high mass biopolymers since its introduction in 1987. It is known that matrix plays a key role in this method. Great efforts have been carried out for understanding what main characters of a good matrix. Other then nicotinic acid which initially used as matrix for 266 nm by Hillenkamp, more than hundreds of organic compounds had been tested, but just few of them are widely accepted. Such as sinapinic acid (SA), Caffeic acid (CA), 2,5-dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnamic acid (4HCCA) are successfully used in the analysis of polypeptides and proteins, 3-hydroxypicolinic acid (HPA) in the analysis of DNA. Among efforts in understanding effects of matrices, investigations for modeling the desorption ionization processes in MALDI-MS have been reported in large extent. However, search for new matrix candidates is still a trial-and-error procedure. In this paper some results are introduced in studying influence of matrices characters on desorption ionization processes in
MALDI-MS, which have not been reported.

A Study of Matrix-Protein Association by Low Angle Laser Light Scattering

In applying MALDI to the analysis of biopolymers, it was found that the ability of a matrix to form a solid solution of the analyte molecules is a prerequisite for successful desorption. For more understanding of the association manner of the matrix molecules with protein in MALDI, microscope and scanning electron microscope have been used to observe their co-crystallization. Because the viewing resolution of these technique is not enough to have a clear picture, further investigation has to be performed.

Low angle laser light scattering (LALLS) is a versatile technique for polymer and biopolymer characterization. Weight average molecular weight, polymerization kinetics, diffusion coefficients/particle size and etc. can be deduced by this technique. A solvent system have to be used in this technique, and the results are obtained in a solution state, which would not be the same as in solid crystallization state. But it could be assumed that when the solution is drying to form crystals in a smoothing way, drying in room temperature, their associated manner would not be changed tremendously. A set of common used matrices and few kinds of proteins were prepared to form the sample solution for the measurement. The method for the LALLS measurement has been stated in the literature. Some interesting results are described in the following.

The instrument used for measurement of average molecular weight $M_w$ and Second Virial Coefficient $A_2$ is a low angle laser light scattering photometer (KMX-6, Milton Roy Ltd. USA). The principle of the method is based on the following equation:

$$\frac{KC}{R_0} = \frac{1}{M_w} + 2A_2C$$

where $K = \text{The polymer optical constant}$

$C = \text{The solute concentration in g/mL}$

$R_0 = \text{Difference in the Raleigh Factor of the solution and that of the pure solvent}$

$A_2 = \text{the Second Virial Coefficient}$

Solution of five different concentrations of lysozyme in a solvent system of 0.15 M NaCl with four matrices (caffeic acid, 3-amino-4-hydroxybenzoic acid, nicotinic acid and 2,5-dihydroxybenzoic acid) were measured. The value of $R_0$ was obtained, and then a plot of $K / R_0$ vs $C$ was constructed. The reciprocal y-intercept yields $M_w$ and the slope is equal to $2A_2$.

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>$M_w$(g/mL)</th>
<th>$A_2$(mol/mL/°$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 0.15M NaCl</td>
<td>$1.39 \times 10^4$</td>
<td>$-3.64 \times 10^2$</td>
</tr>
<tr>
<td>2. 0.15M NaCl/0.015M CA</td>
<td>$2.49 \times 10^4$</td>
<td>$9.53 \times 10^2$</td>
</tr>
<tr>
<td>3. 0.15M NaCl/0.015M 3A4HA</td>
<td>$5.10 \times 10^4$</td>
<td>$1.89 \times 10^2$</td>
</tr>
<tr>
<td>4. 0.15M NaCl/0.015M NA</td>
<td>$1.52 \times 10^4$</td>
<td>$2.59 \times 10^3$</td>
</tr>
<tr>
<td>5. 0.15M NaCl/0.015M DHB</td>
<td>$1.88 \times 10^4$</td>
<td>$5.03 \times 10^3$</td>
</tr>
</tbody>
</table>

From the above results, without matrix the measured weight average molecular weight of lysozyme is $1.39 \times 10^4$. That is nearly the same of its molecular weight, but the value of $A_2$ is a negative value, $-3.54 \times 10^2$. This value means that the solution system is unstable and the solute solved badly in the solvent. By adding matrix, all the four matrices presented stabilization effects and solve well in solvent system. The results demonstrated that in such a solvent system matrices associated well with protein samples, and among them, 3A4HA appeared to have a far more high $M_w$ and value of $A_2$. The measured $M_w$ of Lysozyme is $5.10 \times 10^4$, it is 3.5 time of its true molecular weight. The $A_2$ is nearly one order higher than that of the other matrices. It means 3-amino-4-hydroxybenzoic acid tends to associate with the sample molecule much intensely than the other matrices use in this experiment. If one molecule of lysozyme is assumed to associate with $N_1$ molecules of 3A4HA, then $N_1$ can be calculated according to the following way.
\[ N_1 = (5.10 \times 10^4 - 1.39 \times 10^4) + 153 = 242 \]

where \( 1.39 \times 10^4 \) is the measured Mw of lysozyme without matrix, and 153 is the molecular weight of 3A4HA. If we assumed that two molecules of lysozyme associated with \( N_2 \) molecules of 3A4HA, then it can be calculated as follow:

\[ N_2 = (5.10 \times 10^4 - 1.39 \times 10^4 \times 2) + 153 = 154 \]

Following the above calculation the \( N \) value of the other three matrices were calculated. They are: caffeic acid: \( N=61 \), nicotinic Acid: \( N=11 \), and DHB: \( N=32 \).

It would be interesting to point out that among these four matrices 3A4HA is the only one that could not assist laser desorption ionization of proteins, and the others are widely used in MALDI-MS. What would be the explanation of this somehow curious phenomenon? We have measured that all the four matrices are highly UV absorbing compounds. It may be assumed that if large amount of matrix molecules aggregated tightly surrounding the whole protein molecule, the desorption would still happen but matrix molecules are not easy to loose from the analyte after desorption, and ionization, such as proton transfer, would hardly occur too.

**A Study of Matrices Influencing the Formation of Multicharged Ions in MALDI-MS**

Matrices influencing the formation of positive multicharged ions have been investigated by the author.\(^2\) It was found that \( \alpha \)-cyano-4-hydroxycinnamic acid (4HACA) tended to form more multicharged ions than the other matrices, such as sinapinic acid (SA) and DHB. The explanation is that their proton affinity (PA) is different. For example, the PA of SA is 212 Kcal/mol and 4HACA is 201 Kcal/mol. It means that 4HACA can more easily donate proton to the analyte than SA. Negative ion spectra of the same analyte with SA and 4HACA were obtained with the same instrument, same parameters and same sample preparation that have been described in the above mentioned paper. Again, we found that 4HACA still tended to form more negative multicharged ions. For comparison, the spectra are shown in Figure 1 (a) and (b). Where (a) is the positive ion spectrum of cytochrome C and (b) is the negative ion spectrum.

![MALDI spectra of cytochrome C with 4HACA as matrix](image)

**Fig. 1** MALDI spectra of cytochrome C with 4HACA as matrix. (a) positive spectrum (b) negative spectrum

The ionization process of MALDI has been studied extensively.\(^12,15,21,22\) Mechanisms have been proposed, and it was found that no one mechanism can account for all the experiments. Two possible mechanisms for the formation of \((M+H)^+\) in MALDI proposed by J. Allison\(^15\) are as follow.

1. Electronically excited matrix molecule
   \[ mH + hv \rightarrow mH^* \]
   \[ mH^* + M \rightarrow m^- + [M+H]^+ \]

2. Proton transfer following matrix photoionization
   \[ mH + nHv \rightarrow mH^+ + e^- \]
mH⁺ + M → m⁻ + [M + H⁺]

or mH⁺ + mH → m⁻ + [mH + H⁺]

[mH + H⁺] + M → mH + [M + H⁺]

and it has been pointed out that mechanism 1 should be thermodynamically favored over mechanisms 2. In these two mechanisms, the PA value of matrix may quantitatively influence analyte ionization. Obviously, when compare with the negative spectra formed with SA and 4HACA, it cannot be explained by the difference of their PA value as that for positive ion spectra. Furthermore, it has been reported that aromatic acids tended to show increased pKₐ values when they are electronically excited, and it will make the excited matrix molecules easier to donate protons. The above mentioned effects do not make contribution to form negative ions. So that, what would be assumed is that during the MALDI process, highly reactive negative radical (M⁻ or F⁻) were formed by photoelectron captured neutral molecule or neutral fragment, and the following mechanism is proposed for the formation of negative ions.

m + nhν → m⁻ - e

m⁻ + M → [M-H⁻] + [m+H]

or m⁻ + m → [m-H⁻] + [m+H]

[m-H⁻] + M → [M-H⁻] + m⁺

or F⁻ + M → [M-H⁻] + [F+H]

The mechanism for the formation of multicharged negative ions is proposed to be

2m⁻ + M → (M-2H)²⁺ + 2(m+H)

2F⁻ + M → (M-2H)²⁺ + 2(F+H)

or (M-H⁻)²⁻ + F⁻ → (M-2H)²⁻ + (F+H)

References


