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Investigation of the mechanism of matrix adduct formation in MALDI at elevated pressure

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Abstract

Experiments were carried out to gain insight into the mechanism of matrix adduct formation in matrix-assisted laser desorption/ionization (MALDI) conducted at elevated pressures. Two theories of matrix adduct formation were considered: incomplete cluster evaporation and the so called 'poisoning' model. Both theories can qualitatively explain the influence of the ionization conditions, such as laser fluence, the buffer gas pressure and declustering voltage on the matrix adduct formation. The new set of experiments differentiates between the two models, and the results strongly support the 'poisoning' model.

Keywords: MALDI; Adducts; Collisional cooling; Ion-molecule reactions; Clusters

1. Introduction

Matrix-assisted laser desorption/ionization (MALDI) [1,2] has become a popular method for studying biopolymers; it is used widely to acquire mass spectra of peptides, proteins, DNA's and other organic molecules. Adduct formation (attachment of matrix molecules to analyte ions) was observed in MALDI since its early days [3,4]. Matrix adducts represent a problem in MALDI as they complicate spectra and reduce intensities of the ions of interest. One of the first MALDI matrixes, nicotinic acid exhibited significant adduct formation increasing the peak width for protein ions [3]. At present, common matrixes, such as 4-hydroxy-α-cyanocinnamic acid (α-CHC) and 2,5-dihydroxybenzoic acid (DHB) are less prone to adduct formation under most typical MALDI conditions. However, the problem has recently reappeared for these matrixes when researchers started to use MALDI in combination with collisional cooling at pressures above 100 mTorr [5,6]. MALDI with collisional cooling turned out to be advantageous for many applications because it reduces the likelihood of metastable fragmentation of analyte ions. Since, the rate of cooling is directly proportional to the buffer gas density, it seemed natural to increase pressure in the source. However, severe adduct formation is often observed at pressures above 1 Torr. Understanding the mechanism of matrix adduct formation may, therefore, give directions for further improvement of MALDI at elevated pressures. The present work discusses two theories that describe the mechanism of matrix adduct formation.

1.1. Two theories of matrix adduct formation in MALDI at elevated pressures

The first theory is based on the assumption of incomplete evaporation of analyte–matrix clusters created during the MALDI process. This model states that the clusters represent an essential step in the desorption/ionization mechanism. In the case of vacuum MALDI, the clusters do eventually evaporate since they remain "hot" in the absence of cooling gas. Contrary to that, once the buffer gas is added to the ionization region, it cools down the ions and freezes the clusters. In other words, the analyte–matrix clusters that are

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Table 1
Qualitative explanation of the matrix adducts behavior in MALDI in response to changes in experimental conditions for two theories of adduct formation

Experimental observations	Theories of the matrix adduct formation	
	Incomplete evaporation of desorbed clusters	'Poisoning' in desorption cloud
Adducts increase with pressure	Faster cooling 'freezes' evaporation	Plume is more confined at higher pressure resulting in longer exposure time
Adducts increase with fluence	Larger analyte–matrix clusters are desorbed at higher fluence	More material is desorbed at higher fluence resulting in a higher number of collisions
Higher declustering voltage reduces adducts	Higher declustering voltage results in 'heating' of ions and evaporation of matrix	At higher declustering voltage ions travel faster and quickly leave the 'poisoning' area; in addition, 'heating' helps with matrix evaporation

observed in the spectra represent a snapshot of the ions that are present at the earlier stage of the MALDI process.

Another theory explains adduct formation via 'poisoning' of analyte ions as they go through the dense plume of matrix molecules. When MALDI is conducted at elevated pressures, two factors may contribute to an increase in the adduct formation: gas dynamic confinement and rapid cooling of the confined matrix molecules. Gas dynamic confinement of the plume extends the analyte ion exposure time to collisions with matrix molecules. Regarding the influence of rapid cooling of matrix molecules, one can speculate that colder matrix molecules attach more readily to analyte ions forming adducts.

Although the two theories are distinctly different, they both can qualitatively explain experimental observations of adduct formation in response to changes in the ion source conditions, such as laser fluence, buffer gas pressure and declustering voltage. These explanations are summarized in Table 1.

To distinguish between the two theories, we have employed a method similar to the one described in reference [7]. The essence of the method is that two types of samples are deposited nearby and then desorbed simultaneously by an overlapping laser spot. Such experiments make it possible to draw a distinction between the two theories mentioned above. Indeed, the incomplete evaporation theory predicts that adduct formation will not be affected by the presence of the second nearby sample; while according to the 'poisoning' theory, particles desorbed from the adjacent sample may contribute to adduct formation.

2. Experimental

2.1. Materials

Matrixes 2,5-dihydroxybenzoic acid (DHB) and ferrulic acid were obtained from Fluka; 4-hydroxy-α-cyanocinnamic acid (α-CHC) was purchased from Agilent. [Glu^1]-fibrinopeptide B, insulin B-chain and insulin (both bovine) were purchased from Sigma–Aldrich. Fifty-micrometers thick stainless steel foil (Spaenauer) was used in experiment with DHB and ferrulic acid; and 25-μm thick stainless steel foil was used in experiment with α-CHC. Holes in the 25 μm foil were made by laser drilling; the diameter of the holes was approximately 80 μm. Scotch tape (3 M) was used to attach pieces of stainless steel foil to targets.

2.2. Instrumentation

The matrix adduct studies with DHB and ferrulic acid were performed on a prototype QqTOF instrument equipped with an oMALDI source [8]. Analysis of masked samples with α-CHC matrix was done on a prototype MALDI TOF instrument described in [9]. In both instruments, the MALDI target was placed at an elevated pressure of about 1 Torr (N_2) in front of the cone with a 4 mm diameter opening. The cone was followed by a collisional focusing quadrupole ion guide, which operated at a pressure of several millitorrs.

The laser light was introduced through a 200 μm diameter optical fiber (InnovaQuartz) to ensure spatially homogeneous energy deposition. In the case of oMALDI-QqTOF, the laser spot size had an elliptical shape of 200 μm × 400 μm on the target, and the angle of incidence of the laser beam was 60° with respect to the axis of the quadrupole ion path. In the second instrument (MALDI TOF), the beam delivery system had the incidence angle of 30° and the resulting spot dimensions of 200 μm × 230 μm.

In both experiments, the pressure in the ionization region was not measured directly (due to a small size of the region), but was estimated to be approximately 1 Torr, based on the size of the gas inlet orifice, pumping speed of the front-end turbopump and the pressure in the quadrupole region. Low declustering voltage (potential difference between the target plate and the cone) of 3 V was used unless stated otherwise to facilitate adduct formation.

In both studies presented below, experiments were repeated multiple times to ensure that the observed effects were reproducible.

3. Results and discussion

3.1. Cross matrix adducts with DHB and ferrulic acid

In the first experiment, two foil strips, containing samples with different matrixes, were placed on the target side by side, as shown on Fig. 1. Scotch tape was used to secure the

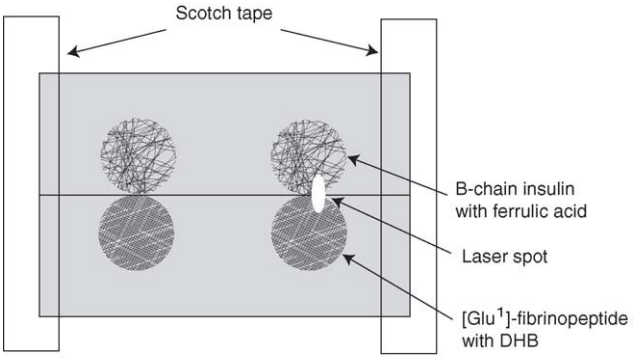

Fig. 1. Schematic view of the MALDI target plate with two types of sample placed side by side. Samples were deposited along the long edge of the stainless steel foil strip; two of those were then attached to the target plate by scotch tape.

foil strips in place. The first sample spot contained insulin B-chain with ferrulic acid as a matrix, and the second sample spot contained [Glu^1]-fibrinopeptide B with DHB as a matrix. DHB and ferrulic acid were chosen because they require similar laser fluences for an optimal operation of MALDI. Spectra were first collected for each individual sample separately, and then in the region, where the laser spot was covering both samples (as shown in Fig. 1). The same experimental conditions were used for all spectra acquisitions. Fig. 2A shows a spectrum recorded from the spot that contained B-chain insulin with ferrulic acid, where peaks of insulin B-chain ions and those of its matrix adducts (labeled) are clearly visible. Note that adducts show up as peaks, which contain the intact matrix molecule(s) and/or its fragment(s). When the spectrum was recorded on the border between the two samples, additional DHB matrix adducts (labeled in panel B) appeared. Even the peaks that contained hybrid adducts (with one or more DHB and ferrulic acid molecules) are observed in the 'overlapping' experiments. This behavior strongly supports the idea that the ions of interest pick up matrix adducts as they travel through the plume, i.e. the 'poisoning' model.

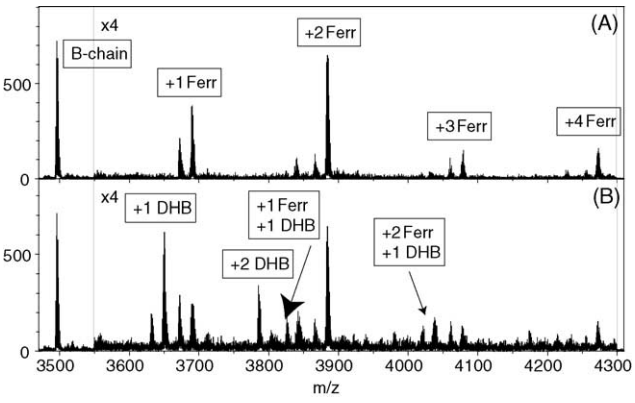

Fig. 2. MALDI spectra acquired from the target plate shown in Fig. 1. (A) recorded when laser illuminated the middle of a spot, containing insulin B-chain with ferrulic acid and (B) recorded when laser illuminated the border between the two different samples.

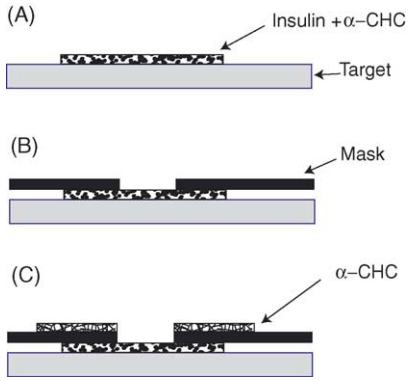

Fig. 3. Cross-section of the MALDI target for the 'masked sample' experiment. (A) regular sample spot of insulin with α-CHC matrix; (B) the same regular sample spot covered with the stainless steel foil with 80 μm diameter openings; (C) sample spot similar to (B), but the upper side of the foil precoated with α-CHC matrix prior to installation.

At the same time, the incomplete evaporation theory cannot explain why adducts of DHB molecules are present on the analyte ion that was desorbed from the spot where ferrulic acid was used as a matrix. It should be noted that similar 'cross-pollinated' adducts were observed for [Glu^1]-fibrinopeptide present in the DHB sample, but the degree of clustering was lower (data not shown). The fact that [Glu^1]-fibrinopeptide exhibited less pronounced adduct formation is not a surprise, the intensity of adduct formation observed in MALDI at elevated pressure usually correlates with the size of the molecule and also depends on its composition.

3.2. *Masked sample with α-CHC*

In the second experiment adduct formation was investigated for α-CHC matrix. Samples, containing insulin, were prepared on the MALDI target as shown in Fig. 3. One set (panel A) contained regular sample spots (insulin with α-CHC matrix); the second set (B) had the same regular samples but they were covered with the stainless steel foil with 80 μm diameter openings; and the last set (C) contained samples that were similar to B, but the upper side of the foil was precoated with α-CHC matrix prior to installation. Images of the samples taken prior to spectra acquisition are shown in Fig. 4. An approximate shape of the laser spot is drawn for reference in Fig. 4B.

Mass spectra collected under those experimental conditions from the three samples are presented in Fig. 5. Observed behavior of adduct formation agrees well with the 'poisoning' model. Relative intensity of matrix adducts correlates with the number of matrix molecules released into the plume. Lower relative intensity of adduct ions is observed for sample B where the amount of matrix desorbed into the plume is reduced. Samples A and C have approximately the same number of matrix molecules released into the plume, but the adduct formation is more pronounced for sample C. The likely explanation for this effect is that in sample C insulin ions are formed only in the orifice area, and therefore,

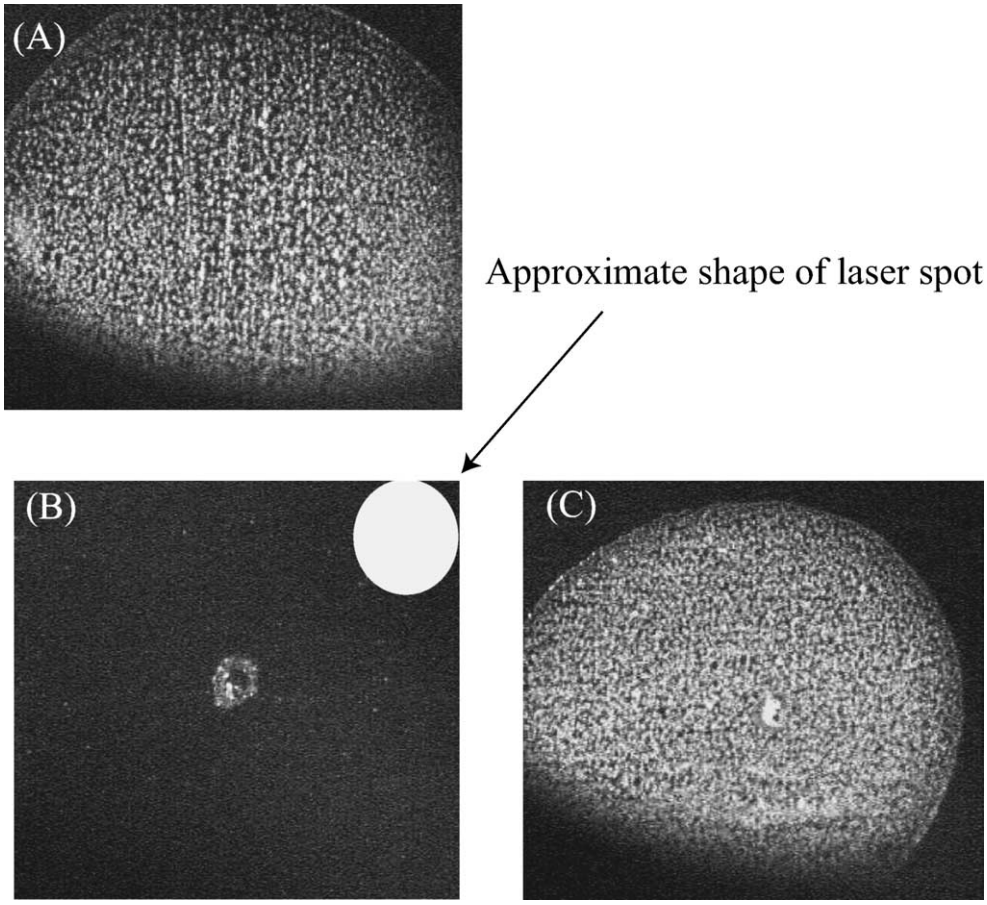

Fig. 4. Photographs of the samples schematically shown in Fig. 3 taken prior to spectra acquisition.

are more abundant in the core part of the plume where density of the matrix molecules is higher and exposure to 'poisoning' is longer. Note that integrated ion yield, i.e. the sum of intensities of all peaks, containing insulin, correlates well with the desorbed area, containing insulin sample. Therefore, the integrated yield for insulin in Fig. 5A is approximately 10 times larger than the integrated yields in Fig. 5B and C.

Mass spectra from samples A–C, obtained at higher declustering voltage (40 V), are shown in Fig. 6. The adduct peaks, containing matrix molecules, are virtually eliminated. Note that similarly to the integrated ion yield in Fig. 5 intensity of the insulin peak in each spectrum is related to the area of the desorbed spot, containing insulin. This is quite remarkable since the ions as well as neutral molecules of insulin should experience a significantly higher number of col-

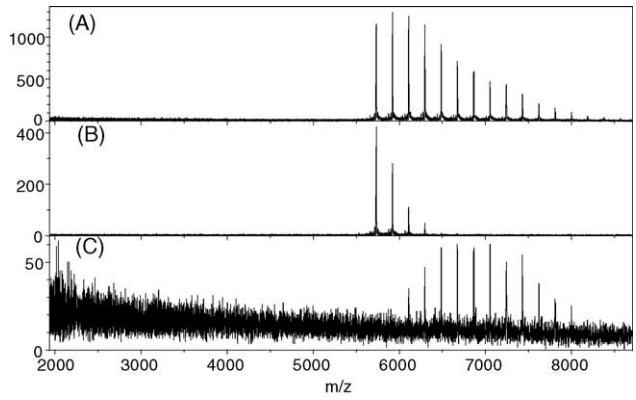

Fig. 5. MALDI spectra recorded from the three samples shown in Figs. 3 and 4. Spectra in panels B and C were acquired when laser spot was centered around the 80 μm diameter holes in the foil.

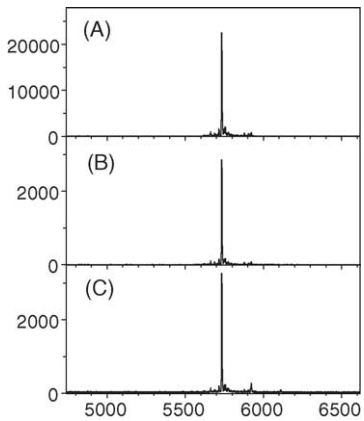

Fig. 6. MALDI spectra recorded under the same conditions as in Fig. 5 except declustering voltage, which was increased from 3 to 40 V.

lisions with matrix ions/molecules and clusters in the plume of sample C than in the plume of sample B.

Since experimental data do not show a significant change in the ion yield due to the presence of additional matrix particles introduced into the plume at a later stage, we may conclude that ionization in MALDI is happening on the time scale shorter than the time it takes for the material from the inner part of the plume to mix with the material from the outer part of the plume. Under the assumption that the mixing occurs when the plume expansion is stopped due to collisional dampening by the buffer gas, the mixing time can be estimated as ∼1 μs based on the characteristic collisional dampening time at the given pressure of ∼1 Torr [10]. A similar conclusion that ions appear in the plume at the early stages of MALDI can be drawn from results of [11], where mixing of solid samples with matrix was used to study the mechanism of MALDI. This conclusion is also supported by observation of ions in the first MALDI TOF instruments where ions were rapidly extracted from the plume by the strong electric fields present in the ion source region [1–4]. However, the time scale estimates in the latter cases would be different, since in these experiments, the plume was expanding into high vacuum region.

4. Conclusion

We have demonstrated that an experimental method where laser spot simultaneously covers two or more different samples can be useful for studying mechanism of adduct formation. The method can be also applied to study other fundamental aspects of MALDI.

We have gathered experimental evidence that matrix adduct formation in MALDI at elevated pressure is happening due to collisions of analyte ions with a dense plume of matrix molecules (the ‘poisoning’ model). The ‘poisoning’ model also suggests that by using smaller laser spots one can generate smaller plumes and reduce matrix adduct formation without changing other experimental conditions.

It is useful to note that adduct formation is affected by the density of matrix crystals on the target. For example, if the matrix crystals are scattered sparsely over the sample spot, the plume density will be lower than in the case of tight crystal packing. Therefore, the sample with scattered matrix crystals will generate fewer matrix adducts under the same experimental conditions.

Our experiments show that the insulin ion yield does not change when additional plume, containing matrix particles only, was merged with the primary plume, containing analyte and matrix particles. Thus, one can speculate that ionization in MALDI occurs on the time scale, that is, shorter than 1 μs or that the analyte ions already exist in the sample prior to desorption.

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