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New matrix of MALDI-TOF MS for analysis of small molecules

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Abstract

New matrix, metal-phthalocyanine (MPc), of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used for analysis of small molecules (usually <500 Da). By using MPcs as matrices, small molecular samples were moved to high mass-to-charge region where there was no interference caused by the traditional matrices. The mass of the target analyte was obtained by simple calculation.

Keywords: MALDI-TOF MS; Small molecules; Metal-phthalocyanines; New matrix

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The introduction of matrices to assist laser desorption/ionization [1,2] has enabled mass spectrometry to determine macromolecules, e.g., synthetic polymers [3] and biomolecules [4]. However, the unwanted peaks caused by matrices in low mass-to-charge (*m*/*z*) region limited the detection of small molecules (commonly <500 Da). In order to abate the challenges, quite some efforts have been focused on reducing the background peaks, such as matrix-free method [5], matrix suppression [6] and exploring graphite matrix [7]. These methods cannot eliminate the interference peaks completely. In this study, metal-phthalocyanines (MPcs) were used as new matrices of MALDI-TOF MS for analysis of small molecules. The analyte was moved upwards and measured in a sufficiently high mass region by using this mew matrix. Thus, the unavoidable interference derived from traditional matrix is kept away from the measuring window.

1. Experimental

The MALDI-TOF MS experiments were performed on an autoflex III instrument (Bruker Daltonics, Inc.), equipped with a 355-nm nitrogen laser. MPc and samples were dissolved in tetrahydrofuran (THF) separately. Then they were mixed together with different molar ratio. The mixture was deposited on the SCOUT MTP 384 MALDI target plate. After crystallization in air, the target plate was placed onto the tray and inserted into the mass spectrometer

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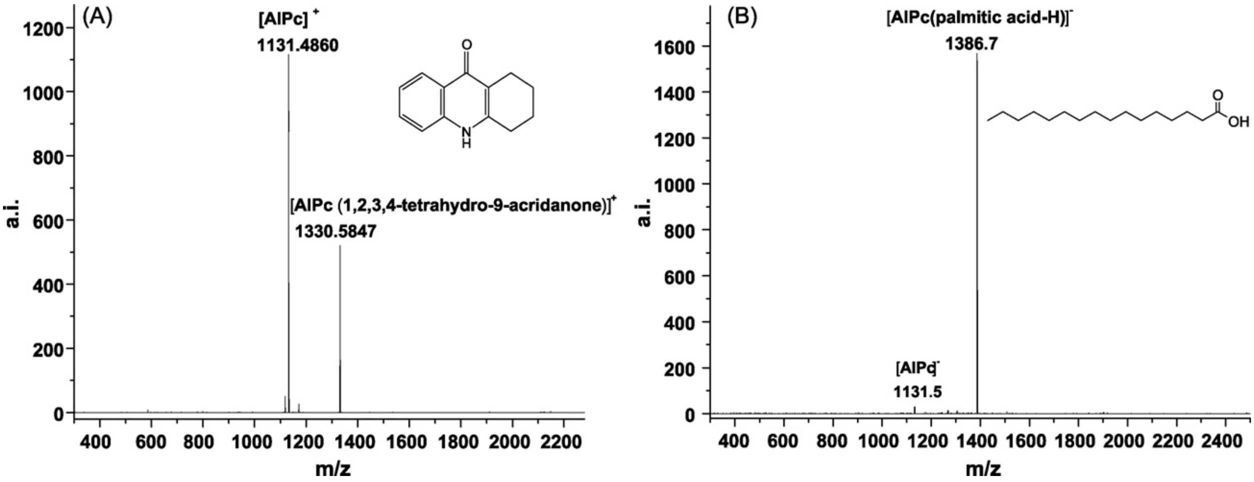

Fig. 1. Mass spectra of (A) 1,2,3,4-tetrahydro-9-acridanone in positive mode and (B) palmitic acid in negative mode detected by MALDI-TOF MS using the AlPc as matrix.

for analysis. The instrument parameters adopted in the experiment were calibrated by a peptide mixture using external standard method. All the mass spectra were acquired using reflection modes.

2. Results and discussion

With a conjugated π system, MPcs have a Soret absorption band between 290 nm and 450 nm attributed to the $4a_{2u} \rightarrow 6e_g$ π–π orbit transition. Except absorbing laser energy, MPcs were able to form clean and confidential peaks of MPc–analyte adducts in a mass region of >1000 Da, free of interference. Clear mass spectra with strong signals were obtained (Fig. 1) when small molecules were analyzed by MALDI-TOF MS using aluminum phthalocyanine (AlPc) as matrix. In positive mode (Fig. 1A), 1,2,3,4-tetrahydro-9-acridanone was detected in the form of $[\text{AlPc}(1,2,3,4\text{-tetrahydro-9-acridanone})]^+$. Interestingly, AlPc was also measurable, in the form of $[\text{AlPc}]^+$. The mass of the analyte is $1330.5847 - 1131.4860 = 199.0987$, which agrees very well with the theoretical value of 192.0997. Here, the peak of MPc can be used as internal standard for accurate mass measurement. To this analyte, the calculated error is 5 ppm. In this error range, the data can be used to determine element composition. What is attractive in negative mode is the much clearer spectrum (Fig. 1B). Palmitic acid was detected in the form of $[\text{AlPc(palmitic acid-H)}]^-$. The peak of $[\text{AlPc}]^-$ was also detected but very weak. The mass of palmitic acid can be calculated as follows: $1386.7 - 1131.5 + 1.0 = 256.2$, which equals to the expected value.

The factors, which influenced peak intensity of MPc–analyte adducts detected in MALDI, were investigated in our experiment. These factors included matrix/analyte molar ratio, substituent groups and central metal ions of MPcs.

Usually, when samples are analyzed by MALDI-TOF MS, the normal molar ratio of matrix to sample is from 1500:1 to 3000:1. As to some concrete samples, the ratio has to be changed on the basis of their species and molecular weight of samples. In this experiment, the molar ratio of matrix to sample was from 10:1 to 1:10. If the ratio was higher than 10:1, the peak of AlPc was too high to find the complex. When lower than 1:10, the analyte crystals hindered the effective absorption of laser energy and thus it was very hard for the complex to desorb/ionize. Also a lot of samples were wasted.

The introduction of different substituent groups on the Pc ring was mainly to increase the solubility [8]. Here, we found that MPc with phenyl groups commonly produced stronger MPc–analyte peak than MPc with alkyl groups. Moreover, an aromatic analyte often yielded higher target signals than an aliphatic analyte, implying that there might be a π–π interaction between analyte and MPc. The central metal ion was shown to have much stronger impact on the signals than the substituent groups. At present, only trivalent metal ions from the Element Group III gave quite ideal target peaks. Metal ion with too high a valence like Sn^{4+} or Ti^{4+} and too low a valence such as Zn^{2+} or Mg^{2+} produced no target signals. Within the Element Group III, the size of the ions also influenced the signals. The MPc–analyte adduct intensity was measured to have an order of $Al^{3+} >> Ga^{3+} > In^{3+}$, which was parallel to their ability to form a complex.

3. Conclusion

In summary, we reported MPcs were utilized as new matrices of MALDI-TOF MS to detect small molecules. It has been successfully applied to the analysis of various small molecules, including peptide, fatty acid, phenol and so on, being flexible and extendable.

Acknowledgments

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