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Novel effect of fullerene as an additive in IR-MALDI and UV-MALDI

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Overview

✓ Fullerene and graphite as a matrix additive increase the durability of ion signal in IR-MALDI or UV-MALDI.

✓ Fullerene and graphite enhance MALDI in-source decay (ISD) of glycan related materials (glycan and glycopeptide) in UV-MALDI.

1. Introduction

In MALDI-MS, an ultra violet (UV) laser is widely used, but an infrared (IR) laser is also used due to its features, such as a soft ionization effect, a wide selectivity of matrix materials, high sensitivity of macro molecules and so on. Fullerene is a molecule composed entirely of carbon (allotrope of carbon), in the form of a hollow sphere. It has been reported that the fullerene could be used as a matrix due to its energy-absorbing properties similar to those of conventional MALDI matrix. Furthermore, it was reported that the addition of graphite, which also is allotrope of carbon like the fullerene, into glycerol matrix improved the sensitivity in IR-/ UV-MALDI.[1,2] In this study, we show a new aspect of the fullerene as an additive in IR-/ UV-MALDI MS.

(Ref. 1. Anal. Chem. 1995, 67 (23), 4335–4342 2. Anal. Chem. 1998, 70, 4890-4895)

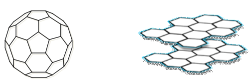


Figure 1 Structure of fullerene (C_{60}) and graphite

2. Methods and Materials

[Matrix] 2M Urea (in 50% ACN) was used as a matrix for IR-MALDI. 10mg/mL DHB and CHCA (in 50% ACN/ 0.1% TFA) were used as a matrix for UV-MALDI. A mirror-polished stainless sample target was used.

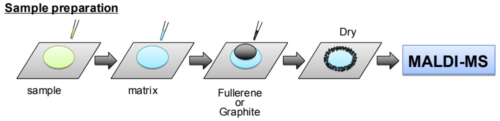
[Additive]. A saturated solution of fullerene (C_{60}) was prepared with toluene and then its supernatant was used as an additive. As for graphite, suspended solution in toluene was used as an additive.

[MALDI-MS] IR-MALDI mass spectra were acquired with an in-house built MALDI-digital ion trap TOF MS equipped with a tunable IR laser (5.5 - 10 μm), the wavelength of which was fixed to 5.9 μm, generated by difference frequency generation (DFG) (Kiss-Laser, Kawasaki Heavy Industries).

UV-MALDI mass spectra were acquired with AXIMA-Performance MALDI-TOF MS linear mode (Shimadzu/Kratos, UK) equipped with a ultraviolet laser (337nm).

All mass spectra were acquired in positive ion mode.

Sample preparation



3. Result

3-1. Fullerene and graphite effect in IR-MALDI

3-1-1. Durability of ion signal

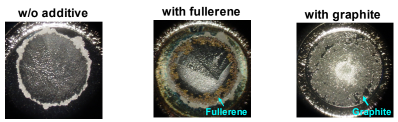
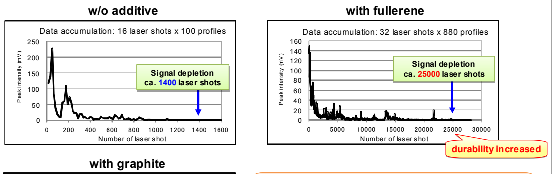
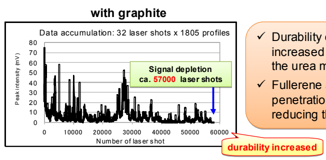


Figure 2. Stereoscopic microscope photographs of the crystallization of urea matrix and angiotensin II with/ without the fullerene or graphite.





✓ Durability of the ion signal was significantly increased by adding fullerene and graphite into the urea matrix.

✓ Fullerene and graphite may reduce the penetration of an IR wavelength, resulting in reducing the laser ablation.

Figure 3. Durability of angiotensin II (1pmol) ion signal on an arbitrary sweet spot.

3-1-2. Sensitivity

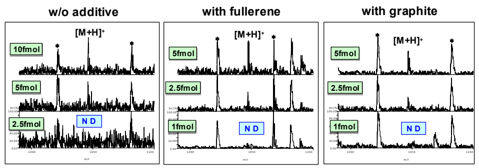


Figure 4. Limit of detection of angiotensin II with urea matrix and additives. The asterisks indicate a cluster ion of urea matrix.

✓ Fullerene and graphite as a matrix additive slightly improved the sensitivity.

3-2. Fullerene effect in UV-MALDI

3-2-1. Durability of ion signal

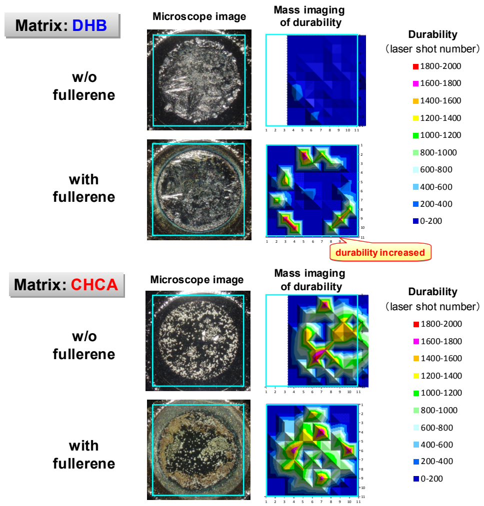


Figure 5. Mass imaging of durability of angiotensin II (10fmol) ion signal. Fullerene tended to be distributed along the periphery of the matrix crystallization.

For fullerene

✓Durability of the ion signal was increased by adding the fullerene into the DHB matrix but not CHCA in UV-MALDI. It is considered that a matrix property affect this result, but further investigation is needed.

✓Sensitivity was almost the same as without fullerene. (data not shown)

For graphite (data not shown)

✓Durability and sensitivity was not improved for both DHB and CHCA. The graphite had a tendency to inhibit the ionization by a UV-laser.

Acknowledgments

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3-2-2. Fullerene and graphite effect on in-source decay in UV-MALDI

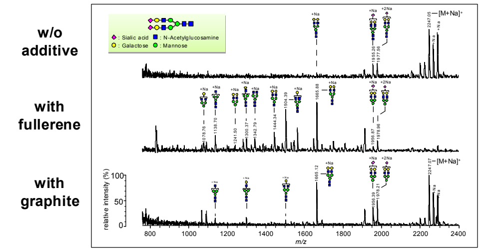

Figure 6. ISD spectra of A2-glycan with DHB matrix in UV-MALDI.

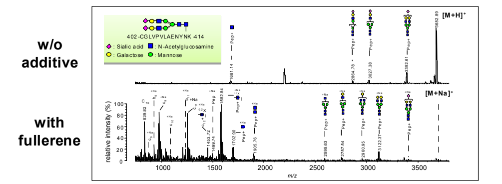

Figure 7. ISD spectra of glycopeptide (from human transferrin) with DHB matrix in UV-MALDI.

✓ ISD efficiency for glycan related-materials improved by adding the fullerene and graphite.

✓ ISD enhancement effect was not observed for peptides. (data not shown)

4. Conclusions

- Addition of fullerene and graphite into the matrix increased the durability of the ion signal in IR-MALDI (urea matrix) and UV-MALDI (DHB matrix).
  - ✓ It is considered that fullerene and graphite made a ablation area small by masking the matrix surface partially and a sample consumption was decreased. As a result, the signal durability would be increased.
- Addition of fullerene and graphite did not decrease the sensitivity in IR-MALDI.
  - ✓ It is anticipated that fullerene and graphite enhance the ionization efficiency considering that the sensitivity was not decreased despite increasing the signal durability.
- Fullerene and graphite enhanced ISD for glycan related-materials in UV-MALDI.
  - ✓ Fullerene and graphite might efficiently transmit their absorbed laser energy to the glycan and therefore induce the thermal fragmentation.