Using Molecular Recognition of β -Cyclodextrin to Determine Molecular Weights of Low-Molecular-Weight Explosives by MALDI-TOF Mass Spectrometry

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This study presents a novel method for determining the molecular weights of low molecular weight (MW) energetic compounds through their complexes of β -cyclodextrin (β -CD) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) in a mass range of 500 to 1700 Da, avoiding matrix interference. The MWs of one composite explosive composed of 2,6-DNT, TNT, and RDX, one propellant with unknown components, and 14 single-compound explosives (RDX, HMX, 3,4-DNT, 2,6-DNT, 2,5-DNT, 2,4,6-TNT, TNAZ, DNI, BTTN, NG, TO, NTO, NP, and 662) were measured. The molecular recognition and inclusion behavior of β -CD to energetic materials (EMs) were investigated. The results show that (1) the established method is sensitive, simple, accurate, and suitable for determining the MWs of low-MW single-compound explosives and energetic components in composite explosives and propellants; and (2) β -CD has good inclusion and modular recognition abilities to the above EMs. (J Am Soc Mass Spectrom 2006, 17, 189–193) © 2006 American Society for Mass Spectrometry

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has become a powerful instrument for analyzing large synthetic polymers and biomacromolecules. However, for low-mass analytes—especially compounds that have a mass-to-charge of less than 500 Da—it has been a challenge. Because most of the molecular weights (MWs) of the currently used matrixes are less than 500 Da and because, during laser irradiation, they also act as their own matrixes, a variety of matrix-related ions are produced. Therefore, the use of MALDI-TOF-MS has been limited to the analysis of low-mass analytes. As a consequence, many analysts and experts of MALDI-TOF-MS have made considerable efforts and approaches to overcome this problem [1–5].

Cyclodextrins are cyclic oligosaccharides that have α -D-glucose units that are connected through (1 $\rightarrow$ 4) linkages. The most common forms of cyclodextrin are α -, β -, and γ -cyclodextrin (cyclomaltohexa-, hepta-, and octaoses), which contain six, seven, and eight glucose units, respectively. The structures of these molecules are toroi-

dal, containing an apolar cavity with primary hydroxyl groups lying on the outside and the secondary hydroxyl groups being located inside [6, 7]. The guest molecules that have a suitable size and shape can be incorporated into the cyclodextrin cavity to form inclusion complexes. The formation of these complexes has been attributed to weak interactions, including van der Waals interactions, hydrophobic effects, and hydrogen bonding. The ability of cyclodextrins to form inclusion complexes with a variety of guest molecules has attracted widespread interest in academic research and industrial applications. Cyclodextrin complexes have been studied by using different physical methods and spectroscopic methods. However, the analysis of low-mass analytes by MALDI-TOF-MS, especially those in the mass range below 500 Da, has been a major challenge [8–15].

In this paper, a novel method of using the molecular recognition of β -cyclodextrin (β -CD) to determine the mass of low-MW explosives by MALDI-TOF-MS is established.

Experimental

Materials

Fourteen explosives, as outlined in Table 1, were used as guest molecules. These were provided by the Xi'an Modern Chemistry Research Institute (Xi'an, China).

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Table 1. The main ions of 14 kinds of low-MW single-compound explosives and one composite explosive determined by MALDI-TOF-MS

No.	Chemical name	Molecular code	Molecular formula	MW	Ion form	<i>m/z</i>	Base peak	Mensurated MW
1	Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	C ₃ H ₆ N ₆ O ₈	222.12	[β-CD + H] ⁺ [β-CD + RDX + H] ⁺	1135.29 1357.33	1357.33	222.04
2	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	C ₄ H ₈ N ₈ O ₈	296.16	[β-CD + H] ⁺ [β-CD + HMX + H] ⁺	1135.17 1431.26	1431.26	296.09
3	1-methyl-2,5-dinitro-benzene	3,4-DNT	C ₇ H ₆ N ₂ O ₄	182.14	[β-CD + H] ⁺ [β-CD + 3,4-DNT + H] ⁺	1135.18 1317.26	1317.26	182.08
4	1-methyl-2,5-dinitro-benzene	2,6-DNT	C ₇ H ₆ N ₂ O ₄	182.14	[β-CD + H] ⁺ [β-CD + 2,6-DNT + H] ⁺	1135.23 1317.33	1317.33	182.10
5	1-methyl-2,6-dinitro-benzene	2,5-DNT	C ₇ H ₈ N ₂ O ₄	182.14	[β-CD + H] ⁺ [β-CD + 2,5-DNT + H] ⁺	1135.16 1317.32	1317.32	182.16
6	1-methyl-2,4,6-trinitro-benzene	2,4,6-TNT	C ₇ H ₅ N ₃ O ₆	227.13	[β-CD + H] ⁺ [β-CD + 2,4,6-TNT + H] ⁺	1135.22 1362.31	1362.31	227.09
7	1,3,3-trinitro-azetidine	TNAZ	C ₃ H ₄ N ₄ O ₆	192.09	[-CD + H] ⁺ [-CD + TNAZ + H] ⁺	1135.19 1327.26	1327.26	192.07
8	1,3-dinitro-2-imidazolidone	DNI	C ₃ H ₄ N ₄ O ₅	176.09	[-CD + H] ⁺ [-CD + DNI + H] ⁺	1135.22 1311.33	1311.33	176.11
9	1,2,4-trinitrobutanetriol	BTTN	C ₄ H ₇ N ₃ O ₉	241.11	[-CD + H] ⁺ [-CD + BTTN + H] ⁺	1135.31 1376.45	1376.45	241.14
10	1,2,3-trinitro-propantriol	NG	C ₃ H ₅ N ₃ O ₉	227.09	[-CD + H] ⁺ [-CD + NG + H] ⁺	1135.18 1362.29	1362.29	227.11
11	1,2-dihydro-3H-1,2,4-triazol-3-one	TO	C ₂ H ₃ N ₃ O	85.07	[-CD + H] ⁺ [-CD + TO + H] ⁺	1135.29 1220.37	1220.37	85.08
12	1,2-dihydro-5-nitro-3H-1,2,4-triazol-5-one	NTO	C ₂ H ₂ N ₄ O ₃	130.06	[-CD + H] ⁺ [-CD + NTO + H] ⁺	1135.07 1265.31	1265.31	130.24
13	1,4-dinitro-piperazine	DNP:	C ₄ H ₈ N ₄ O ₄	176.13	[-CD + H] ⁺ [-CD + DNP + H] ⁺	1135.16 1311.23	1311.23	176.07
14	Tetrahydro-1,3,5-trinitro-1,3,5-triazin-2(1H)-one	662	C ₃ H ₄ N ₆ O ₇	236.10	[-CD + H] ⁺ [-CD + 662 + H] ⁺	1135.16 1371.25	1371.25	236.09
15	A composite explosive	2,6-DNT, TNT, RDX	C ₇ H ₆ N ₂ O ₄ C ₇ H ₅ N ₃ O ₆ C ₃ H ₆ N ₆ O ₈	182.14 227.13 222.12	[-CD + H] ⁺ [-CD + 2,6-DNT + H] ⁺ [-CD + 2,4,6-TNT + H] ⁺ [-CD + RDX + H] ⁺	1135.16 1317.21 362.38 1357.28	1357.28	182.05 227.22 222.12

Figure 1. MALDI-TOF mass spectra of RDX (a), the inclusion complex of β -CD and RDX (b), and the complex with NaCl added (c).

Their purities were more than 99.5%. Sinapinic acid (SA) was used as the matrix; this was acquired from the Sino-American Biotechnology Co. (Beijing, China) and was recrystallized from hot methanol and stored in the dark before use. β -Cyclodextrin was used as the host and was purchased from Sigma Chemical Co. (Fullerton, CA) and used without further purification. Water was obtained using a Milli-Q water purification system (Millipore, Milford, MA). Acetone used was of analytical purity.

Preparation of Inclusion Complexes

The inclusion complexes were prepared as follows: a mixture of 0.1 mmol/L guest acetone–water (50:50, vol/vol) solution and an excess of 0.1 mmol/L host aqueous solution was stirred vigorously for 72 h at 50 °C. It was then maintained for 48 h at 0 °C. The inclusion complexes in the solution state obtained were filtered, washed with water three times, and dried in an oven. The inclusion complexes used in the MALDI-TOF-MS analysis were obtained by this method.

MALDI-TOF Mass Spectrometry

The MALDI-TOF mass spectra were recorded in the positive-ion reflection mode on a Biflex II type time-of-flight mass spectrometer (Bruker, Bremen, Germany). The operating conditions for MALDI-TOF-MS were: using a nitrogen laser ($\lambda = 337$ nm) to desorb and ionize the samples; accelerating voltage, 22 kV; delayed extraction voltage, 15.5 kV; time of delayed extraction, 80 ns; scan mass range, 900–1700 Da. A stainless steel target was used as the MALDI substrate. The spectra were externally calibrated by angiotensin II and sodium ions. A total of 8 to 16 laser shots were averaged to generate a spectrum.

Sample Preparation

The matrix microcrystal layer was first densely packed on the stainless steel probe by fast solvent evaporation of the 0.1 mmol/L matrix acetone–water solution. The sample solution was prepared with water and then deposited onto the layer and fast evaporated by vacuum to form the second layer. The molar ratio of SA and its complex was 1000:1. The sample plate was finally loaded into the ion source for analysis.

Results and Discussion

The MALDI-TOF mass spectra of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), the inclusion complex of β -CD and RDX, the complex with added NaCl, and the inclusion complex of β -CD and propellant with unknown components, are shown in Figures 1 and 2. The main ions of 14 kinds of low-MW single-compound explosives and one composite explosive of known components are listed in Table 1. From Figures 1 and 2 and Table 1, the following observations can be made.

- Figure 1a shows that when only conventional matrix SA is present, the MALDI-TOF mass spectrum of RDX contains many strong peaks that correspond to matrix-related ions, including a protonated matrix molecule $[SA + H]^+$ ion at m/z 339.39, a hydrous matrix molecule $[SA + H_2O + H]^+$ ion at m/z 357.42 and a fragment $[SA - H_2O + H]^+$ ion at m/z 321.27. Only two peaks are displayed at m/z 1135.29 and 1357.33, which relate to the complex of β -CD with RDX in the mass range of 900 to 1700 Da in Figure 1b. Clearly, the matrix-related ions of SA do not interfere with these peaks, and they can be ascribed to the molecular ions: $[\beta\text{-CD} + H]^+$ and $[\beta\text{-CD} + RDX + H]^+$, respectively. Figure 1c is the spectrum of complex containing NaCl. Instead of the protonated complex (m/z 1357.33), the base peak in the

Figure 2. MALDI-TOF mass spectra of the inclusion complex of β -CD and propellant with unknown components.

spectrum corresponds to that of the NaCl complex ion of m/z 1379.35 (Figure 1c). So, the intense peak in Figure 1b is caused by $[\beta\text{-CD} + \text{RDX} + \text{H}]^+$. Other spectra are similar to those seen in Figure 1b, indicating that no background and no matrix-related signals interfere in these spectra.

- The protonated complex molecular ion ($\beta\text{-CD} + \text{EMs} + \text{H}$) emerged in all of the mass spectra, as base peaks show that $\beta\text{-CD}$ has a strong inclusion ability to the 16 kinds of EMs.
- By means of subtracting the m/z value of the protonated molecular ion $[\beta\text{-CD} + \text{H}]^+$ from that of the protonated complex molecular ion $[\beta\text{-CD} + \text{guest} + \text{H}]^+$, the following MWs are obtained: 222.04 for RDX, 296.09 for HMX, 182.08 for 3,4-DNT, 182.10 for 2,6-DNT, 182.16 for 2,5-DNT, 227.09 for TNT, 192.07 for TNAZ, 176.11 for DNI, 241.14 for BTTN, 227.11 for NG, 85.07 for TO, 130.24 for NTO, 176.07 for DNP, and 236.09 for 662.
- In the spectrum of the inclusion complex of $\beta\text{-CD}$ with a composite explosive composed of 2,6-DNT, TNT, and RDX, there are no matrix-related ions with interference, as shown in Table 1. The signals at m/z 1317.21, 1362.28, and 1357.38 are ascribed to the protonated complex molecular ions that are formed by $\beta\text{-CD}$ with 2,6-DNT, TNT, and RDX, respectively. The MWs of components obtained by the difference in m/z values of $[\beta\text{-CD} + \text{energetic component} + \text{H}]^+$ and $[\beta\text{-CD} + \text{H}]^+$ ions are 182.05 for 2,6-DNT, 222.12 for RDX, and 227.22 for TNT. This is in good agreement with the MW values of the corresponding components in Table 1.
- For the inclusion complex of $\beta\text{-CD}$ and the propellant with unknown components, the ion peaks at m/z 1135.16, 1245.23, 1362.27, 1375.33, and 1431.41 in Figure 2 are assigned to $[\beta\text{-CD} + \text{H}]^+$, $[\beta\text{-CD} + \text{resorcinol} + \text{H}]^+$, $[\beta\text{-CD} + \text{NG} + \text{H}]^+$, $[\beta\text{-CD} + \text{DINA} + \text{H}]^+$, and $[\beta\text{-CD} + \text{HMX} + \text{H}]^+$, respectively. The MWs of components were obtained by the difference in m/z values of $[\beta\text{-CD} + \text{component} + \text{H}]^+$ and $[\beta\text{-CD} + \text{H}]^+$, and the deduced compo-

nents were 110.07 for resorcinol, 227.11 for NG, 240.17 for DINA, and 296.25 for HMX, respectively. These components agree with the results obtained by analyzing the components of the propellant by high-performance liquid chromatography. Therefore, we deduce that this propellant is an ST-35 propellant that is composed of resorcinol, NG, DINA, and HMX.

Conclusions

The method determined for assessing the MWs of low-MW energetic materials in a mass range, avoiding matrix interference, under the inclusion conditions of $\beta\text{-CD}$ to EMs by MALDI-TOF-MS is satisfactory in accuracy. The results of 14 low-MW single-compound explosives, one composite explosive, and one propellant determined by using the MALDI-TOF-MS method agree with the data in the literature.

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References

- Kinumi, T.; Saisu, T.; Takayama, M.; Niwa, H. MALDI TOF Mass Spectrometry Using an Inorganic Particle Matrix for Small Molecule Analysis. *J. Mass Spectrom.* **2000**, *35*, 417–422.
- Chen, Y.-C.; Wu, J.-Y. Analysis of Small Organics on Planar Silica Surfaces Using Surface-Assisted Laser Desorption/Ionization Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1899–1903.
- Cohen, L. H.; Gusev, A. I. Small Molecule Analysis by MALDI Mass Spectrometry. *Anal. Bioanal. Chem.* **2002**, *373*, 571–586.
- McCombie, G.; Knochenmuss, R. Small-Molecule MALDI Using the Matrix Suppression. Effect to Reduce or Eliminate Matrix Background Interferences. *Anal. Chem.* **2004**, *76*, 4990–4997.
- Soltzberg, L. J.; Patel, P. Small Molecule Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Using a Polymer Matrix. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 1455–1458.
- Szejtli, J. Cyclodextrins and their Inclusion Complexes; Akademiai Kiado: Budapest, 1982, pp 5–12.

7. Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: New York, 1978, pp 2–9.
8. Dotsikas, Y.; Loukas, Y. L. Efficient Determination and Evaluation of Model Cyclodextrin Complex Binding Constants by Electrospray Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2003**, *14*, 1123–1129.
9. Cheng, Y.; Hercules, D. M. Measurement of Chiral Complexes of Cyclodextrins and Amino Acids by Electrospray Ionization Time-of-Flight Mass Spectrometry. *J. Mass Spectrom.* **2001**, *36*, 834–836.
10. Bakhtiar, R.; Hop, C. E. C. A. A Study of the Complexation Between Dimethyl Cyclodextrin and Steroid Hormones Using Electrospray Ionization Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1478–1481.
11. Janshoff, A.; Steinem, C.; Michalke, A.; Henke, C.; Galla, H. J. Mono-functionalized β -Cyclodextrins as Sensor Elements for the Detection of Small Molecules. *Sens. Actuators B* **2000**, *70*, 243–253.
12. Kobetic, R.; Jursic, B. S.; Bonnette, S.; Tsai, J. S. C.; Salvatore, S. J. Study of the Lorazepam:Cyclodextrin Inclusion Complexes Using Electrospray Ionization Mass Spectrometry. *Tetrahedron Lett.* **2001**, *42*, 6077–6082.
13. Guo, Z.; Zhang, Q.-C.; Zou, H.-F.; Guo, B.-C.; Ni, J.-Y. A Method for the Analysis of Low Mass Molecules by MALDI-TOF Mass Spectrometry. *Anal. Chem.* **2002**, *74*, 1637–1641.
14. Lehmann, E.; Salih, B.; Gomez-Lopez, M.; Diederich, F.; Zenobi, R. Matrix-Assisted Laser Desorption/Ionization Mass Spectra Reflect Solution-Phase Formation of Cyclodextrin Inclusion Complexes. *Analyst* **2000**, *125*, 849–854.
15. Mele, A.; Malpezzi, L. Noncovalent Association Phenomena of 2,5-Dihydroxybenzoic Acid with Cyclic and Linear Oligosaccharides. A MALDI-TOF Mass Spectrometric and X-ray Crystallographic Study. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 228–236.