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Molecular Weight Determination of Polymers by Matrix Assisted Laser Desorption Ionization in Mass Spectrometry

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Abstract : Matrix assisted laser desorption ionization in mass spectrometry is a fast and accurate method to determine the molecular weight of natural and synthetic polymers. Unknown peptides such as elastase inhibitor and D-hydantoinase were analyzed using sinapinic acid as matrix and their molecular weights were compared with the results from protein sequencer and gel filtration chomatography, respectively. Synthetic polymers such as polyethyleneglycol, polypropyleneglycol, polydimethylsiloxane, and polystyrene were analyzed using matrices such as 2,5-dihydroxybenzoic acid, 4-hdroxy-azobenzenecarboxylic acid, and 2-nitrophenyl octyl ether. Average molecular weights of polystyrene were compared with molecular weights by gel permeation chromatography.

Keywords : MALDI, Matrix, Peptide, Protein, Synthetic Polymer, Average Molecular Weight, Gel Permeation Chromatography

I. Introduction

Matrix assisted laser desorption ionization (MALDI) in mass spectrometry is a soft ionization technique[1] and a powerful tool[2] for the mass determination and sequence analysis of natural polymers such as peptides, proteins and oligonucleotides as well as synthetic polymers such as polyethyleneglycol and polystyrene. The molecular weight distribution of oligomers has also been obtained by field desorption[3], fast atom bombardment[4] and electrospray ionization mass spectrometry[5]. Because MALDI method allows these nonvolatile and high molecular weight polymers to be transferred into the gas phase and to be ionized with matrix, it is possible to determine molecular weights of biopolymers and synthetic polymers with high accuracy and sensitivity. The role of matrix is an important factor in order to assist the ionization of these polymers. Efficient matrices generate high yield of ions from analyte relative to the matrix. Usually, the matrices such as sinapinic acid(SPA)[6], 2,5-dihydroxybenzoic acid (DHB)[7], and 4-hydroxyazobenzene carboxylic acid (HABA)[8] are solid compounds. In this case, the matrix is crystallized directly on the tip of the insertion probe. Liquid matrices such as 2-nitro phenyl octyl ether (NPOE)[9] are also used. In this study the MALDI method was applied to determine the molecular weight of the unknown biopolymers such as elastase inhibitor

(EI) from Korean leech *H. nipponia*[10] and D-hydantoinase from *Bacillus stearothermophilus* SD-1(HBS)[11], and the synthetic polymers such as polyethyleneglycol (PEG), polypropyleneglycol (PPG), polydimethylsiloxane (PDMS), and polystyrene (PS). This new MALDI method was compared to the classical techniques such as amino acid analysis and gel permeation chromatography (GPC).

2. Experimental

2.1. Instrumentation

A Kompact MALDI II mass spectrometer (Kratos company, U.K. Manchester) was used to obtain the MALDI mass spectra. The mass spectrometer was consisted of a nitrogen laser with 5 ns pulse width at 337 nm. The laser power was attenuated about 70% of the full power. Ion accelerating voltage was set at 20 kV. The laser was focused onto the sample spot of about 30-μm diameter. Mass spectra shown was typically accumulated from 30 laser shots.

2.2. Sample preparation

New elastase inhibitor was extracted from Korean leech *H. nipponia*[10]. Natural D-hydantoinase, was isolated by screening the hydantoinase-producing thermophiles from soil, and was purified before analysis[11]. Each 1 μL of EI and HBS dissolved in aqueous 0.1 % TFA solution, and 2 μL of matrix SPA solution in water/acetonitrile(1:1), were mixed together on an inert stainless steel probe. The solvent was removed in warm air stream and the sample was then inserted into the mass spectrometer. EI and HBS, of which the typical sample amount used for analysis was in the range of about 10 pmol, were detected by MALDI mass spectrometer. The internal references such as substance P, insulin, cytochrome C, and albumin were used to calibrate the molecular weights of EI and HBS exactly.

The synthetic polymers such as PEG, PPG and PS were dissolved in H_2O, H_2O/EtOH (v/v=1:1), and tetrahydrofuran, respectively. The matrix DHB was used for the analysis of PEG and PPG analysis at the concentration of 10 g/L in H_2O/EtOH (v/v=9:1), and 0.5 μL sample of the polymer solution was mixed on a polished stainless steel probe with 2 μL of matrix solution. The matrices HABA and NPOE were used for the analysis of PS and PDMS. For the analysis of PS, 5 g/L of the sample was dissolved in NPOE as matrix. In some cases, 5 g/L silver trifluoroacetate and 1μL of alkaline salt solution(NaCl, KCl, about 0.1 M) were added to increase the yield of cationized polymers.

For the determination of average molecular weight of synthetic polymer, the integrated peak areas, reflecting the number of ions (N_i), are used with the isotope-averaged molecular weight M_i to calculate the number average (M_n) and weight average (M_w) of polymer using the following equations:

$$M_n = \sum N_i M_i / \sum N_i, \qquad M_w = \sum N_i M_i^2 / \sum N_i M_i$$

And the average molecular weights of synthetic polymers were compared with the gel permeation data. The ratio of M_w and M_n represents the polydispersity.

3. Result and Discussion

3.1. Analysis of biopolymers

The compositions of elastase inhibitor from Korean leech *H. nipponia* and subunit of D-hydantoinase of *Bacillus stearothermophilus* SD-1 were confirmed to be a 57-residues polypeptide[10] and a 466-residues potein[11] by amino acid analysis, respectively. The results from MALDI MS for EI and HBS were different from those of SDS-PAGE and gel filtration chromatography according to the typical MALDI mass spectra shown in *Fig. 1* and *Fig. 2*. The molecular weight 6,119 Da of native EI determined by MALDI method in *Fig. 1* was

much smaller than 7,800 Da by SDS-PAGE, but was very similar to 6,122 Da by amino acid sequencing method. So the total amino acid sequence of EI could be confirmed by comparing 6,122 Da based on amino acid sequencing with 6,199 Da obtained from MALDI method within mass accuracy of 0.01 %.

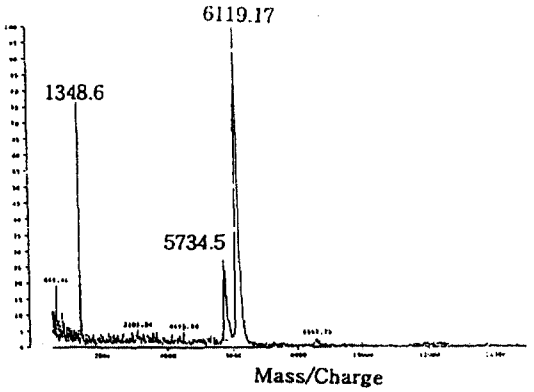

Fig. 1. MALDI mass spectrum of elastase inhibitor, substance P and insulin

The molecular weight 102.1 kDa of native HBS by MALDI method shown in *Fig. 2* was smaller than 133.9 kDa by gel filtration chromatography. The 51 kDa of subunit of HBS by MALDI method was also smaller than 54 kDa by SDS-PAGE. Thus HBS could be presumed to be organized as a dimer of subunit. Because twice of molecular weight of subunit from gel filteration chromatography and SDS-PAGE data was not similar to that of native HBS within error range, MALDI MS application to the analysis of the protein having a molecular weight above 100 kDa was preferable to other methods.

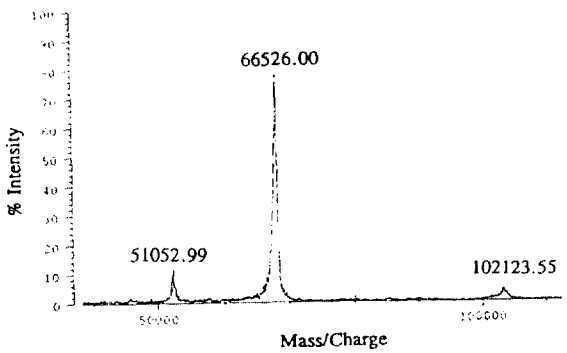

Fig. 2. MALDI spectrum of D-hydantoinase and albumin

3.2. Analysis of synthetic polymers

Table I shows the average molecular weight and polydiversity of PEG 2000, PPG 4000, PS 2000, PS 1200, and PDMS 2000.

Table I. Molecular Weights and Polydispersity of PEG, PPG, PDMS, and PSs

Polymer	Average Mass	Monomer Mass	M_w/M_n
PEG 2000	1943.5	44.29	1.01
PPG 4000	3992.5	57.38	1.00
PDMS2000	1362.3	74.22	1.08
PS 1200	1333.4	104.84	1.04
PS 2000	1928.4	102.22	1.02

The values gave a deviation of 5-10 % from those of manufacturer's specification. But the average molecular weight of PPG 4000 in *Table I* was equal to that of supplier. *Fig. 3* shows the MALDI spectra of polystyrenes from PS 580 to PS 2000. *Table II* summerizes the molecular weights M_w, M_n and polydispersity of these polymers by MALDI method compared to those of supplier determined by GPC. It could be seen that the mass differences between MALDI and GPC methods for PEG, PPG, PDMS and PS

Table II. Comparison betweeen Molecular Weights and Polydispersity of PSs

Polymer	M_w(E)	M_w(S)	M_w/M_n(E)	M_w/M_n(S)
PS 580	929.6	580	1.023	1.13
PS 970	1176.0	970	1.025	1.09
PS 1400	1549.5	1400	1.021	1.05
PS 2000	1965.1	2000	1.014	1.04

[E]Values by MALDI mass spectra in *Fig. 3*
[S]Supplier'values by GPC method

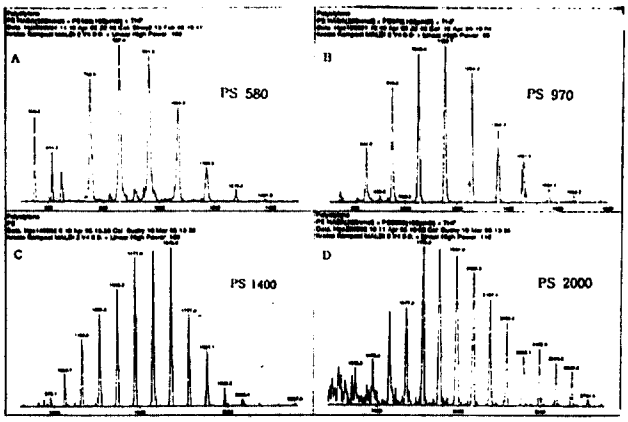

Fig. 3. MALDI spectra of PS 580(A), PS 970(B), PS 1400(C), and 2000(D) in HABA as a matrix

above 2 kDa were small. When average molecular weights of PS by MALDI method were compared with those measured by GPC method, the deviations of molecular weight and polydispersity were reduced with increasing the average molecular weight of PS.

4. Reference

1. M. Karas, and F. Hillenkamp, *Anal. Chem.* **60**, 2299(1988); R. C. Beavis, B. T. Chait, *Rapid Commun. Mass Spectrom.*, **3**, 233 (1989).
2. O. Vorm, and P. Roepstorff, *Biological Mass Spectrometry*, **23**, 734(1994).
3. G. Shaulsky, R. L. Johnson, and A. A. Stark, *Carcinogenesis*, **11**, 519(1990).
4. V. T. Vu, C. C. Fenselau, and O. M. Colvin, *J. Am. Chem. Soc.* **103**, 7362(1981).
5. J. B. Fenn, C. Meng, and C. M. Whitehouse *Mass. Spec. Rev.*, **9**, 37(1990).
6. R. C. Beavis and B. T. Chait, *Rapid Commun. Mass Spectrom.*, **2**, 151(1989).
7. K. Strupat, M. Karas and F. Hillenkamp, *Int. J. Mass Spectro. Ion Processes*, **111**, 89(1991)
8. G. Montaudo, C. Puglisi and F. Samperi, *Rapid Commun. Mass Spectrom.*, **8**, 1011 (1994)
9. U. Bahr, A. Deppe, and F. Hillenkamp, *Anal. Chem.*, **64**, 2866(1992).
10. H. I. Jung, S. I. Kim, K. Ha, C. O. Joe, and K. W. Kang, *J. Biol. Chem.*, **270**, 1379(1995)
11. S. G. Lee, D. C. Lee, M. H. Sung, and H. S Kim, *Biotechnol. Lett.*, **16**, 461(1994)