Electrospray Mass Spectrometry: Variation of Standard Deviation of Experimentally Determined Molecular Weights with Average Charge Number

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The variation of the standard deviation of the molecular weights determined by electrospray with the average charge number of multiply charged ions is investigated for peptides and proteins with molecular weights ranging from 1000 to 20000. This result shows the tendency that the standard deviation of experimentally determined molecular weights increases with the average charge number of multiply charged ions. It is found that the molecular weight determination by electrospray accompanies the error of at least the product of 0.06 and the average charge number in the experiment.

Keywords Electrospray mass spectrometry, molecular weight, average mass, peptide, protein

Electrospray mass spectrometry has received much attention during the last few years. This unique feature is its ability to generate multiply charged ions from peptides, proteins and nucleotides, and to detect them with high sensitivity by a quadrupole mass spectrometer.¹⁻⁴

The algorithms for calculating molecular weights from the peaks of multiply charged ions observed in electrospray mass spectra have been proposed by Mann *et al.*⁵ Recently, several groups used an electrospray ionization method in conjunction with a quadrupole mass spectrometer to obtain accurate molecular weights of a variety of proteins which could not be previously measured by mass spectrometry.^{6,7} They reported that experimentally determined molecular weights by electrospray almost correspond to average masses. However, the variation of the standard deviation of experimentally determined molecular weights by electrospray with the charge numbers of multiply charged ions has never been quantitatively discussed for a series of peptides and proteins. This variation datum may be useful for evaluating the extent of the precision of the molecular weight determination by electrospray for ions with different charges.

Experimental

The electrospray ion source used here was almost the same as one described elsewhere.⁸ The electrospray probe consisted of an approximately 1 inch stainless steel tube with 100 μm i.d. attached by epoxy glue to a 4 inch $\times$ 1/16 inch o.d. stainless steel tube. The probe tip

was positioned approximately 15 mm from the sample aperture and was typically biased to 3 kV with respect to ground. Sample solutions were continuously infused by a syringe pump at a flow rate of 0.5 $\mu\text{l}/\text{min}$. The quadrupole mass spectrometer (mass range: m/z 1–1500) was used as a mass analyzer.

In this study, a total of 9 peptides and proteins (bradykinin, melittin, bovine glucagon, human parathyroid hormone, bovine insulin, thioredoxin, horse heart cytochrome *c*, horse heart myoglobin and sperm whale myoglobin) were investigated.

Sample solutions were 10^{-5} M in concentration prepared in deionized distilled water acidified with 2.5% acetic acid. The electrospray mass spectra were obtained by taking averages of 5 scans acquired in 30 s from sample solutions. All the peptides and proteins were purchased from Sigma Chemical Company (St. Louis, MO, USA). These samples were purified with a liquid chromatograph.

Results and Discussion

We have investigated 9 different peptides and proteins with molecular weights ranging from 1000 to 20000 daltons. Figure 1 shows the typical electrospray mass spectrum of horse heart cytochrome *c* (nominal, mono-isotopic and average masses are 12346, 12352.3 and 12360.1, respectively) in the mass range of m/z 400 and 1350.

The mass spectrum exhibits the distribution of multiply charged ion peaks resulting from the attachment of 10–21 protons to horse heart cytochrome *c* molecules.

Each of the ionic species in the electrospray mass spectrum provides an independent determination of the molecular weight of the protein. The calculation method of an average molecular weight from measured m/z values of multiply charged ions in an electrospray mass spectrum has been described previously.⁵ Any pair of ions can be used to calculate the charge state and molecular weight.^{6,7} The molecular weight (M) can be determined by the next equations:

$$n_2 = (m_1 - 1)/(m_2 - m_1), \quad (1)$$

$$M = n_2(m_2 - 1), \quad (2)$$

where n_2 is the charge number of a peak, and m_1 and m_2 are the mass numbers of a pair of peaks. The value of n_2 must be the whole number which is the nearest to the real value calculated by Eq. (1). The numbers of peaks used for molecular weight determinations, their charge numbers and the experimentally determined molecular weights of the peptides and proteins calculated by Eqs. (1) and (2) are shown in Table 1. In these molecular weight determinations, all multiply charged ions were used for each sample. In addition, the

calculation results of nominal, monoisotopic and average masses are shown in the same table. The calculation was carried out with the program developed by Yergey.⁹ As other groups reported, the result here supports that the experimentally determined molecular weights are in close agreement with the average masses. In this experiment, the difference is 0.4 daltons even in the case of sperm whale myoglobin with molecular weight of about 20000 daltons. This reveals that the high accuracy of molecular weight determination by electrospray is achieved in the experiment.

Under the condition that the high accuracy was achieved, we investigated the variation of the standard deviation of experimentally determined molecular weights with charge number. Since the standard deviation of experimentally determined molecular weights shows the precision of molecular weight determination by electrospray, the variation datum is thought to be important for estimating the extent of the precision of molecular weight determinations by electrospray for ions with different charges. Figure 2 shows the variation of the standard deviation with the average charge number of multiply charged ions. We can easily find the tendency that the standard deviation

Fig. 1 The electrospray mass spectrum of horse heart cytochrome c.

Fig. 2 The variation of the standard deviation of the experimentally determined molecular weights with the average charge number.

Table 1 The experimentally determined molecular weights with the numbers of peaks, the average charge numbers and the calculation results of nominal, monoisotopic and average masses for the peptides and proteins used in the experiment

Sample	Number of peaks	Number of charges	Molecular weight $\pm$ SD	Nominal mass	Monoisotopic mass	Average mass
Bradykinin	3	3+ - 1+	1060.2 $\pm$ 0.1	1059	1059.6	1060.2
Melittin	4	6+ - 3+	2846.4 $\pm$ 0.2	2843	2844.8	2846.5
Bovine glucagon	3	5+ - 3+	3482.8 $\pm$ 0.2	3479	3480.6	3482.8
Human parathyroid hormone	5	8+ - 4+	5063.7 $\pm$ 0.4	5058	5060.7	5063.9
Bovine insulin	3	7+ - 5+	5733.4 $\pm$ 0.3	5727	5729.6	5733.6
Thioredoxin	7	15+ - 9+	11673.2 $\pm$ 0.6	11660	11666.1	11673.4
Cytochrome c	12	21+ - 10+	12360.2 $\pm$ 0.7	12346	12352.3	12360.1
Myoglobin (horse heart)	15	27+ - 13+	16950.9 $\pm$ 1.4	16931	16940	16950.5
Myoglobin (sperm whale)	12	27+ - 16+	17198.6 $\pm$ 1.3	17179	17188.2	17199

increases with the average charge number. This phenomenon may be explained as follows. Since small errors in observed masses result in large errors in molecular weight determinations in the case of highly charged ions. The deviation of an observed mass by 0.1 leads to the deviation of a molecular weight at least by $0.1 \times n_i$ for the ion with the charge number of n_i . The deviation of an observed mass by 0.1 may occur due to the low mass resolution of a quadrupole mass spectrometer, or the peak broadening resulting from solvent molecule attachment to sample molecules, or other reason such as impurities. The charge numbers of several multiply charged ions are represented by the average charge number of n_a . Therefore, the standard deviation of the experimentally determined molecular weights from the multiply charged ions is expected to follow the value estimated by $0.1 \times n_a$. In our experiment, the standard deviation change can be approximately explained by the straight line of $0.06 \times n_a$, as shown in Fig. 2. This result reveals that the molecular weight determination by electrospray accompanies the error of at least $0.06 \times n_a$. In other words, the value of $0.06 \times n_a$ may be convenient for estimating the extent of the precision of molecular weight determination by electrospray.

In order to get the higher accuracy and precision of molecular weight determination by electrospray, we must use a high resolution mass spectrometer such as a sector type mass spectrometer or a Fourier transform mass spectrometer. More study is necessary to obtain the

high accuracy and precision of molecular weight determination by electrospray.

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