Mass Spectrometry Quantification of Protein Mixtures

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Overview

Mass spectrometry has been widely used for quantitation. Under ESI-MS conditions, intact proteins are presented as a charge envelope that is composed of protein peaks at different charge states. Due to the overlap of different protein envelopes, the mass spectra of protein mixtures can be very complicated. The quantitative analysis of intact proteins with MS presents special problems because of the distribution of multiply charged species. The interpretation can be further complicated if the sample is a mixture of proteins. Therefore, identification of components of the mixture and measurement of the relative amount in the mixture is challenging for data processing software. In this study, we successfully differentiated cytochrome c species from different sources and obtained quantitative information from the mass spectrum of the mixture by using maximum entropy software.

System Components

Waters® BioSuite™ Intact Protein High Resolution MS System

Waters® 2796 Bioseparations Module
Waters® Micromass® Q-Tof 2™ Mass Spectrometer

Experimental

MS Conditions:

-Source = ESI(+)
-Capillary (kV) = 3.3
-Cone (V) = 35
-Temperature (°C)
  -Source = 150
  -Desolvation = 400
-Gas Flow (L/Hr)
  -Cone = 50
  -Desolvation = 500
-Scan Mode
  -MS Mode
-Collision Energy (V) = 10
-Infusion Rate (µL/min) = 20

Software:

-Waters® MassLynx™ 4.0
-MaxEnt™ 1 Program

Results and Discussion

Figure 1. Quantitation of 1:1 Horse and Chicken Cytochrome c Mixture by Mass Spectrometry

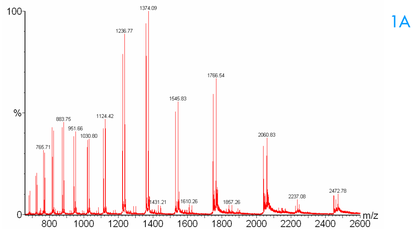

Figure 1A: ESI Mass Spectrum of 1:1 Cytochrome c Mixture

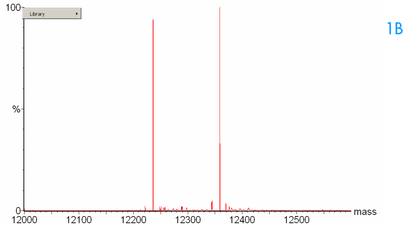

Figure 1B: MaxEnt 1 Deconvoluted Spectrum of 1:1 Mixture

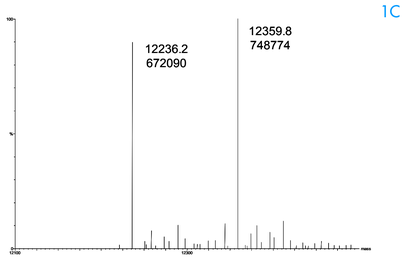

Figure 1C: Quantitation Chart of 1:1 Cytochrome c Mixture

Figure 2. Quantitation of 1:10 Horse and Chicken Cytochrome c Mixture by Mass Spectrometry

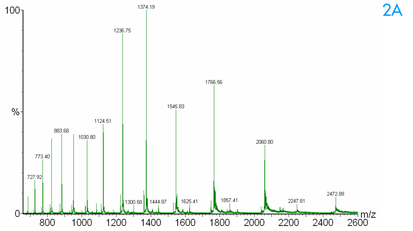

Figure 2A: ESI Mass Spectrum of 1:10 Cytochrome c Mixture

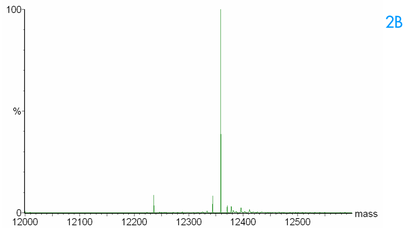

Figure 2B: MaxEnt 1 Deconvoluted Spectrum of 1:10 Mixture

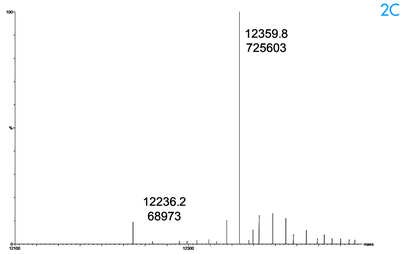

Figure 2C: Quantitation Chart of 1:10 Cytochrome c Mixture

Figure 3. Quantitation of 1:100 Horse and Chicken Cytochrome c Mixture by Mass Spectrometry

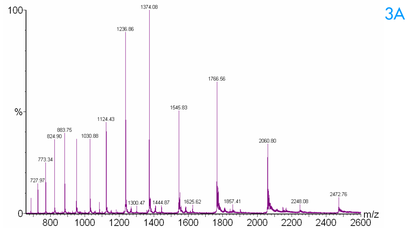

Figure 3A: ESI Mass Spectrum of 1:100 Cytochrome c Mixture

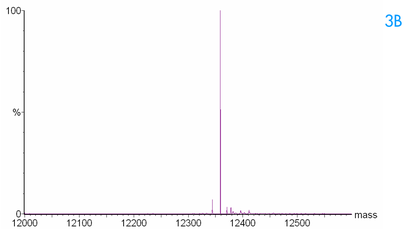

Figure 3B: MaxEnt 1 Deconvoluted Spectrum of 1:100 Mixture

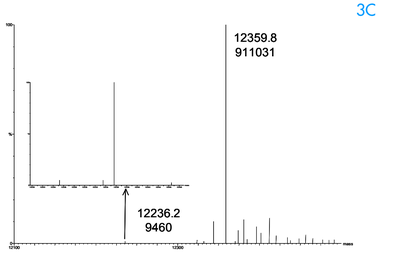

Figure 3C: Quantitation Chart of 1:100 Cytochrome c Mixture

Three horse cytochrome c samples (1 µg/µl each) were mixed with 1 µg/µl , 0.1 µg/µl, and 0.01 µg/µl chicken cytochrome c samples respectively in solution before infusing into a mass spectrometer. Mass spectra shown in Figure 1A, Figure 2A and Figure 3A, were acquired in MS mode with identical parameter settings for all three experiments.

MaxEnt 1 software was used for deconvolution of ESI mass spectra with the same processing values. After deconvolution, each component in the protein mixtures with 1:1, 1:10, and 1:100 ratios were all identified at the right masses (Figure 1B, Figure 2B, and Figure 3B). Deconvoluted spectra were centered to get peak intensity information which were used for quantitation plot.

In Figure 1C, Figure 2C, and Figure 3C, the deconvoluted peaks on the left are chicken cytochrome c peaks and the peaks on the right are from horse cytochrome c. The numbers on the top are the molecular weight of cytochrome c from each component and the numbers on the bottom are the peak areas of deconvoluted peaks containing quantitation information. By calculating the ratios of peak area, 0.90:1, 0.95:10, and 1.04:100 intensity ratios are obtained from 1:1, 1:10, and 1:100 cytochrome c mixtures. The quantitation information of three mixtures was successfully retrieved from the ESI mass spectra of three mixtures within an acceptable error range by utilizing MaxEnt 1 program.

Conclusions

- MaxEnt™ 1 can be used for deconvolution of complex ESI intact protein mass spectra without prior information
- Quantitative information of intact protein spectra is preserved during MaxEnt™ 1 deconvolution and can readily be retrieved for quantification calculations
- Relative quantitation of protein mixtures by mass spectrometry is achievable if the structures of these proteins are similar to each other such as proteins and their post translational modification products
- Identification and relative quantitation can be done on protein mixtures at 100:1 ratio