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 mixtures 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® 2794 Bioseparations Module
Waters® Micromass® Q-Tof 2™ Mass Spectrometer

Experimental
MS Conditions:
- Source = ESI+
- Capillary (V) = 3.3
- Cone (V) = 35
- Temperature (°C) = 350
- Source = 150
- Desolvation = 400
- Gas Flow (L/Hr) = 20
- 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
MaxEnt™ 1 software was used for deconvolution of ESI mass spectra with the same processing values. After deconvolution, each component in the protein mixtures at 1:1, 1:10, and 1:100 ratios was 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 calculations. 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 horse cytochrome c. The numbers on the top are the molecular mass and the numbers on the bottom are the peak areas of deconvoluted peaks containing quantitation information.

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 for protein mixtures at 1:100 ratio.

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 at 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 calculations.