A METHOD FOR ESTIMATING THE ABSOLUTE CONCENTRATION OF PROTEINS IN COMPLEX MIXTURES

Jeffrey C. Silva¹, Craig A. Dorschel¹, Martin Gilar¹, Marc V. Gorenstein¹, Petra Olivova¹, Johannes P.C. Vissers², Guo-Zhong Li¹, Scott J. Geromanos¹ and LeRoy B. Martin¹

¹Waters Corporation, Milford, Massachusetts 01757-3696, USA and ²Waters Corporation, Transistorstraat 18, 1322 CE Almere, The Netherlands

OVERVIEW

We describe a new method of absolute quantification of proteins based on the discovery of a relationship between MS signal response and the absolute protein concentration, wherein the mass MS signal response for the three most intense tryptic peptides is constant per mole of protein.

RESULTS AND DISCUSSION

Figure 3 illustrates those peptides identified to the five standard proteins in Sample 1, sorted by descending intensity. The three most intense tryptic peptides from each protein have been colored blue. The average intensities of the top three peptides are indicated in Table 2. Using ADH1 as the internal standard, the relative ratio of each protein has been determined from the average intensity of the top three ionizing tryptic peptides. The correlation of the relative ratio of the proteins provided an indication of the relationship between the absolute quantity of a protein and the average signal response of the three most intense tryptic peptides. Knowing that there was 10 pmoles of ADH1, the relative ratios have been converted to the absolute quantity of each protein. The results from this analysis indicated that approximately 26,121 counts corresponds to 1 pmole of protein.

Table 1 summarizes the protein sequence coverage and number of peptides identified to each standard protein obtained from the analysis of the sample mixtures without human serum.

Table 2

Table 2. The correlation between the average signal response of the three most intense tryptic peptides and the molar quantity of protein is valid based on the low coefficient of variation of 4.9% (Table 2). To further test this relationship, the peptide/protein results from the remaining samples were organized in a similar fashion to obtain the average intensity of the three most intense tryptic peptides for each of the constituent proteins as a function of their absolute concentration (Table 1). The average signal response for the three most intense tryptic peptides was plotted against the absolute concentration for all proteins among the six dilution series. A linear correlation was obtained from 125 to 15,000 femtols of all the proteins as illustrated in Figure 4. A linear fit to the data produces an R² value of 0.9939. These results prove that the average MS signal response of the three most intense tryptic peptides is constant for all proteins. As further validation, the signal response from ADH1 spiked into serum was used to determine the absolute concentration of 11 securely identified serum proteins (blue circles and whiskers) from seven individual patient samples (Figure 5). These results were compared to the average concentration values obtained from Specialty Laboratories (red circle), which included their expected minimum and maximum values (red whiskers).

CONCLUSION

The ability to determine the absolute concentration of proteins in complex mixtures provides a means to study stoichiometric relationships among proteins within a sample. The label-free LC-MS method described in this work is ideally suited for determining the absolute concentration of proteins present in both simple and complex mixtures. The ability to determine the absolute concentration of proteins in complex mixtures provides a means to study stoichiometric relationships among proteins within a sample.