Fourier Transform Ion Cyclotron Resonance Mass Spectrometry with NanoLC/Microelectrospray Ionization and Matrix-Assisted Laser Desorption/Ionization: Analytical Performance in Peptide Mass Fingerprint Analysis

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Protein identifications by peptide mass fingerprint analyses with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) were performed using microelectrospray ionization coupled to nano liquid chromatography (NanoLC), as well as using matrix-assisted laser desorption/ionization (MALDI). Tryptic digests of bovine serum albumin (BSA), diluted down to femtomole quantities, have been desalted by fast NanoLC under isocratic elution conditions as the high resolving power of FT-ICR MS enables peptides to be separated during the mass analysis stage of the experiment. The high mass accuracy achieved with FT-ICR MS (a few ppm with external calibration) facilitated unambiguous protein identification from protein database searches, even when only a few tryptic peptides of a protein were detected. Statistical confidence in the database search results was further improved by internal calibration due to increased mass accuracy. Matrix-assisted laser desorption/ionization and micro electrospray ionization (ESI) FT-ICR showed good mass accuracies in the low femtomole range, yet a better sensitivity was observed with MALDI. However, in higher femtomole ranges slightly lower mass accuracies were observed with MALDI FT-ICR than with microESI FT-ICR due to scan-to-scan variations of the ion population in the ICR cell. Database search results and protein sequence coverage results from NanoLC FT-ICR MS and MALDI FT-ICR MS, as well as the effect of mass accuracy on protein identification for the peptide mass fingerprint analysis are evaluated. (J Am Soc Mass Spectrom 2003, 14, 553–561) © 2003 American Society for Mass Spectrometry

Proteins expressed by the genome, distribution of proteins in cells, and post-translational modifications of proteins are known to have direct links with cell function. One of the major aims of the relatively new area of proteomics is to gain a deeper insight into the correlations between the proteome and the course of diseases. As such insight will reveal new drug targets, the development of fast and reliable techniques for protein identification will be the key to developing innovative medications [1, 2].

Mass spectrometry has become one of the fastest and most important techniques in proteome analysis [3]. Peptide mass fingerprint analysis is the method for protein identification based on the mass measurement of enzymatically digested proteins by mass spectro-

metry and the comparison of such data with theoretical fingerprints from protein databases [4, 5]. During the last decade this has become the preferred method of choice for fast and high throughput protein identification [6]. The introduction of the soft ionization methods like matrix-assisted laser desorption/ionization (MALDI) [7] and electrospray ionization (ESI) [8] as well as microelectrospray (microESI) [9] qualifies mass spectrometry as a useful tool for proteome analysis as MALDI and ESI/microESI are capable of ionizing biological compounds such as peptides and proteins without inducing fragmentation [3]. Furthermore, due to the sensitivity of these techniques, both MALDI and ESI/microESI mass spectrometry can be used to analyze very small amounts of samples, often in the low femtomole range. The combination of high-performance liquid chromatography (HPLC) in the microcapillary-scale (NanoLC) with microESI have even made it possible to analyze complex biological mixtures, such as cell extracts, online [10, 11].

Published online April 17, 2003

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Highly accurate mass assignment for observed peptides plays a major role in the reliability and statistical confidence of database results [12]. The number of observed peptides necessary for unambiguous protein identification is greatly reduced when mass accuracy is high [13]. This is beneficial as often only parts of the protein sequence can be found in the database mostly due to post-translational modifications changing a peptide mass from the expected value.

Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) [14, 15] is known for very high mass accuracy and high sensitivity [16]. The development of external ion sources provided a way in which to couple ESI to FT-ICR [17]. ESI is one of the best ionization methods for the study of intact proteins as well as protein/protein interactions [18]. External ion accumulation with ESI FT-ICR was developed to help control the total number of ions introduced into the ICR cell. This proved to be especially useful for LC coupling [19]. External MALDI sources coupled to FT-ICR provided the means to measure protein digests very rapidly by peptide mass fingerprinting [20]. Hundreds of samples can be deposited on a single MALDI target and each sample spot can be measured in a few seconds [21]. Medium and high pressure MALDI sources are external low voltage MALDI sources with ion accumulation and collisional cooling. MALDI-produced ions from several laser shots are cooled (by pulsed collision gas) and collected in a multipole. This enables a very sensitive ionization of labile biomolecules. Unwanted fragment peaks are effectively reduced and, therefore, the parent peak's intensity is improved resulting in a better sensitivity [22].

In view of these technical advances, FT-ICR MS has become one of the leading methods for proteome analysis [23–25]. Using external mass calibration by a completely independent experiment (external calibration), mass measurements with accuracies of a few ppm can be achieved routinely. For datasets calibrated from known peaks within the spectrum (internal calibration) the level of mass accuracy is improved significantly. Ions generated by MALDI do not have reproducible abundances from scan to scan [26]. Averaging mass spectra, therefore, may result in lower mass accuracy due to space charge effects causing small frequency shifts [27]. In-source accumulation of ions from multiple laser shots prior to their transfer into the ICR cell reduces the effect of ion number variation from scan to scan. Furthermore, MALDI predominantly creates singly charged ions that have a higher m/z ratio, thus a lower resonance frequency. Therefore, lower mass accuracies (a few ppm) are achieved even with internal mass calibration. In comparison to MALDI, electrospray methods (ESI, microESI and nanospray) can provide sub-ppm levels of mass accuracy because of the rather uniform ion intensity from scan to scan. Additionally, electrospray methods produce multiply charged ions that are in the low m/z region and have higher cyclotron frequencies than singly charged equivalents.

In this work, MALDI and NanoLC-microESI sources are used with FT-ICR for evaluation of the performance of FT-ICR mass spectrometry for proteome analysis. Our objective is based on the utilization of high mass accuracy and high resolution of FT-ICR to improve on protein identification and to increase the level of confidence for this identification. A tryptic digest of bovine serum albumin (BSA) was measured at different concentrations. Based on the well known mass spectrum of BSA digest, this sample has been used for the method evaluation. The mass spectra have been calibrated externally with a capillary skimmer dissociation spectrum of LHRH free acid and internally using defined peaks of the BSA digest. Other methods for internal calibration for NanoLC are the introduction of calibrants into the external storage multipole [28] and for MALDI the rapid switching of MALDI target positions [29].

Experimental

Instrumentation

Mass spectra were acquired with an APEX II FT-ICR mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with a 7 tesla superconducting magnet (Bruker Biospin GmbH, Karlsruhe, Germany) and a cylindrical ICR cell ("infinity cell") [30] that has equipotential-line-segmented trapping plates. A SCOUT 100 low-voltage MALDI source (Bruker Daltonics) with pulsed in-source collision gas and in-source ion-accumulation capability was used for the MALDI measurements. This external ion source uses collision gas (argon) for cooling the ions, introduced during ion formation and ion accumulation. The basic construction of this ion source has been described elsewhere [20, 31]. Mass spectra were acquired from m/z 790 to 3000 with 512 k data points. The NanoLC-microESI FT-ICR measurements were performed using a NanoLC system Ultimate (LC Packings, Amsterdam, The Netherlands) fitted with the Famos autosampler (LC Packings). The online-nanospray ESI source was equipped with an angled off-axis spraying system, that used a Picotip adapter (New Objective, Woburn MA) to connect the tubing of the NanoLC column to a distal-coated fused silica needle that had a 10 μm inner diameter (New Objective). A spring in the needle holder provided the connection from the needle to ground potential. The tip of the needle was placed a few mm in front of the orifice of the glass capillary at an angle of approximately 70°. The voltage applied to the metal-coated capillary entrance of the electrospray source was set between –1200 V and –1800 V. The NanoLC column PepMap C-18 (75 μm i.d., 15 cm length, 5 μm particle size, LC Packings) was used for online-desalting only. The NanoLC FT-ICR measurements were performed with isocratic elution (acetonitrile:water 50:50 vol:vol, 0.1% formic acid) at a flow rate of 200 nL/min. The average time for the fast isocratic elution was about 5 min. Mass spectra of NanoLC measurements were acquired in the mass

range from m/z 200 to 3000 with 512 k data points. The FT-ICR mass spectra were deconvoluted and automatically mass labeled using the software XMASS 5.0 (Bruker Daltonics).

Sample Preparation

Bovine serum albumin (BSA) (Gerbu Biotechnik GmbH, Gaiberg, Germany) was digested by trypsin (Roche Diagnostics GmbH, Mannheim, Germany) using a standard script [32] with carbamidomethyl (NanoLC-microESI) and S-pyridylethyl (MALDI) modification of the SH groups of cysteine.

2,5-dihydroxybenzoic acid (DHB) (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was used as the matrix for all MALDI measurements described in this work. A 50 mg/ml solution of DHB in acetonitrile, water (30:70 vol:vol), with the addition of 0.2% trifluoroacetic acid was prepared. A stock solution of BSA digest (10 pmol/ μ L in water) was diluted with the matrix solution mentioned before. 0.25 μ L of the resultant solutions were applied on the MALDI target for each sample spot in amounts down to 1 fmol. For the NanoLC-microESI FT-ICR experiments, between 0.2 and 1.0 μ L of BSA digest solution were injected onto the NanoLC column.

Data Analysis

The mass spectra were processed using Biotoools 2.2 software (Bruker Daltonik GmbH) as a gateway to set up database searches. The MASCOT search engine (Matrix Science, London, UK) [33, 34] was used to search the OWL database. MOWSE (molecular weight search) scores, assigned by MASCOT, were calculated based on the algorithm described by Pappin et al. [5]. Search criteria for all experiments included one missed cleavage, no preference for taxonomy and oxidation of methionine as a variable modification. The fixed modifications carbamidomethyl for NanoLC-microESI and S-pyridylethyl for MALDI were set according to the experiment. Peptide mass search tolerances between 2 and 10 ppm were also varied depending on experiment: NanoLC, external calibration, mass tolerance ± 5 ppm; NanoLC, internal calibration, mass tolerance ± 2 ppm; MALDI, external calibration, mass tolerance ± 10 ppm; MALDI, internal calibration, mass tolerance ± 5 ppm. The mass spectra were internally calibrated with the following four peptides from the BSA digest: YLYEIAR (M: 926.48612), HLVDEPQNILK (M: 1304.70880), LGEYGFQNALIVR (M: 1478.78807), KVPQVSTPTLVEVSR (M: 1638.93041); $[M + H]^+$ for MALDI and $[M + 2H]^{2+}$ for NanoLC-ESI spectra. The external calibration has been performed by capillary skimmer dissociation of the peptide LHRH free acid using mass peaks between m/z 250 and 1200. For internal and external calibration a two parameter calibration has been used [35].

Results and Discussion

The evaluation of the techniques, MALDI FT-ICR MS and NanoLC-microESI FT-ICR MS, for protein analysis was carried out with focus on sensitivity. Success was determined by the level of confidence associated with the identification of a protein. Peptide mass fingerprints were generated for the tryptic digest of BSA. Figure 1 shows the spectrum obtained for 6 fmol BSA digest deposited on the MALDI target. More than 20 peptides could be identified covering 41% of the total protein sequence. A list of the top 10 database results is also depicted in Figure 1. The top "hit" suggests BSA as the most likely candidate. This result has been given a MOWSE score of 262 by MASCOT. MOWSE scores are defined as the absolute probability that the observed match is a random event using a negative logarithm scaling [5]. For example, in the search shown in Figure 1, a score of 67 indicates a random event with a probability less than 5% using the database OWL with about 313,000 sequences. With a MOWSE score of 262, therefore, BSA is identified unambiguously. Further confidence can be gained by considering the remaining candidates in the list of search results. For the example given in Figure 1, poor MOWSE scores were obtained for all other proteins other than BSA.

BSA was identified from peptide mass fingerprints at concentrations down to about 1 fmol with both MALDI FT-ICR MS and NanoLC-microESI FT-ICR MS. An overview showing the effect of concentration on percentage of protein sequence coverage is given in Figure 2. The results of MALDI FT-ICR MS and NanoLC-microESI FT-ICR MS are comparable. However, with low femtomole amounts of digest better sequence coverages were observed with MALDI. MOWSE scores obtained from the database search of the MALDI experiments and NanoLC-microESI experiments are shown in Figure 3a and b, respectively. To emphasize the significance of the MOWSE scores for BSA, the MOWSE scores for other albumins and other proteins not related to BSA have been plotted in the diagrams separately. Figure 3a shows results from the MALDI experiments where BSA could be identified down to sample amounts in the low femtomole range. A MOWSE score of 129 was obtained with only 1.5 fmol BSA digest on the MALDI target. A MOWSE score of more than 250 was obtained from only 6 fmol. Analysis of the data from NanoLC-microESI experiments gave very similar results: Figure 3b shows data from fast NanoLC-microESI performed using isocratic conditions. Very high MOWSE scores, 500 and higher, were achieved when 20 fmol and more BSA digest were analyzed. This could be explained by the detection of different peptides using NanoLC-microESI in contrast to MALDI. The MOWSE score obtained when 5 fmol digest were deposited on the micro-capillary column is comparable to the MALDI result of a similar amount of sample (6 fmol). When only 1 or 2 fmol of BSA digest were analyzed by NanoLC-microESI experiments, MOWSE scores below

Accession	Mass	Score	Description
1. <u>ALBU_BOVIN</u>	71244	262	SERUM ALBUMIN PRECURSOR. - BOS TAURUS (BOVINE).
2. <u>VF17_VACCC</u>	11614	32	DNA-BINDING 11 KD PHOSPHOPROTEIN. - VACCINIA VIRUS (STRAIN COPENHAGEN),...
3. <u>S59187</u>	18979	28	S59187 NID: g299792 - Mus sp. Balb/c thymus.
4. <u>ALBU_SHEEP</u>	71139	28	SERUM ALBUMIN PRECURSOR. - OVIS ARIES (SHEEP).
5. <u>ALBU_FELCA</u>	70611	27	SERUM ALBUMIN PRECURSOR. - FELIS SILVESTRIS CATUS (CAT).
6. <u>ALBU_PIG</u>	71362	27	SERUM ALBUMIN PRECURSOR (FRAGMENT). - SUS SCROFA (PIG).
7. <u>ALBU_MACMU</u>	69832	27	SERUM ALBUMIN PRECURSOR (FRAGMENT). - MACACA MULATTA (RHESUS MACAQUE).
8. <u>ASFV55KB9</u>	7842	24	ASFV55KB NID: g450694 - African swine fever virus.
9. <u>DSA2247651</u>	6540	24	DSA224765 NID: g3127819 - Drosophila subobscura.
10. <u>DSAJ50331</u>	6588	24	DSAJ5033 NID: g3549850 - Drosophila subobscura.

Figure 1. Mass spectrum and MASCOT database results (MOWSE score and hit list) from the MALDI FT-ICR MS measurement of 6 fmol BSA digest.

60 were obtained. The protein BSA was still listed as the first "hit" in the database results, suggesting this as the most likely candidate.

The effect of internal and external calibration concerning the database results is depicted in Figure 3c and d for MALDI- and NanoLC-microESI experiments, respectively. In these diagrams, the ratios of the MOWSE score for BSA to the MOWSE score for the best non-BSA related protein, and the ratios for BSA to the best non-bovine albumin (note the difference) are shown as a function of concentration. In this script MOWSE score ratios BSA/best non-BSA related protein will be abbre-

viated MOWSE-SR1, whereas MOWSE score ratios BSA/the best non-bovine albumin will be named MOWSE-SR2. The ratio of the MOWSE scores represents the level of confidence in BSA identification as it stresses the difference between the top "hit" and the next possible candidate (e.g., in Figure 1 BSA has a MOWSE score of 262, the best non-BSA related protein is the 11 kDa DNA-binding phosphoprotein with a MOWSE score of 32, hence the MOWSE-SR1 is 262/32 and the best BSA related protein is sheep serum albumin with a MOWSE score of 28, hence the MOWSE-SR2 is 262/28). The MOWSE-SR1 is dependent on the amount of sample used for analysis, whereas this effect is negligible for MOWSE-SR2: Figure 3c shows the results from MALDI experiments. With both external and internal calibration, the MOWSE-SR1 increase as the amount of sample increases. With low amounts of digest of only 1.5 and 3 fmol the MOWSE-SR1 is greater than 4 and 6, respectively, with internal calibration. The effect of setting different mass tolerance limits for database searches, 5 ppm for internal calibration and 10 ppm for external calibration, is relatively small. Nevertheless, a huge effect was observed for the measurement of 6 fmol digest. In this experiment the MOWSE-SR1 increased from about 8 to more than 13 with internal calibration.

NanoLC-microESI experiments (results given in Figure 3d) show similar trends as observed with the MALDI experiments. High amounts of sample resulted in high MOWSE-SR1. Changing from external to internal calibration led to a dramatic increase in MOWSE-

Figure 2. Comparison of percentage protein sequence coverage obtained from NanoLC-microESI and MALDI experiments using different amounts of BSA digest.

Figure 3. MOWSE scores and ratio of MOWSE scores [BSA/non-BSA related proteins (MOWSE-SR1) and BSA/other albumin (MOWSE-SR2)] from MALDI and NanoLC-microESI measurements. (a) MOWSE scores from MALDI experiments using external calibration, (b) MOWSE scores from NanoLC-microESI experiments using external calibration, (c) MOWSE-SR1 and MOWSE-SR2 from MALDI experiments using internal and external calibration, and (d) MOWSE-SR1 and MOWSE-SR2 from NanoLC-microESI experiments using internal and external calibration.

SR1 for the analysis of 6 fmol of digest. For very low amounts of digest (1 and 2 fmol) low ratios of about 1.5 and 3 were observed, respectively. Internal calibration, however, improved MOWSE-SR1s. Despite the poor MOWSE scores obtained from NanoLC-microESI experiments with low femtomole amounts of sample, the protein BSA was still identified with a significant level of confidence.

Now consider the MOWSE-SR2s: This ratio is nearly constant for high amounts of sample, reaches a maximum at 5 fmol of sample then drops quite sharply for less than 5 fmol of BSA digest. The trend is common to both the MALDI and NanoLC-microESI experiments. This phenomenon can be explained by detecting more impurities with higher amounts of sample which then lowers the MOWSE-SR2. The calibration (internal and external) has no effect on MOWSE-SR2s because of the relationship between the amino acid sequence for BSA and other albumins. Close similarity between the amino acid sequences for proteins means a number of peptides

produced from tryptic digests of BSA are exactly the same as those produced from tryptic digests of other albumins.

Deviation of measured masses from theoretical masses for peptides identified as BSA fragments are shown in Figure 4a and b. The distribution of these mass errors fits a Gaussian function. A correlation between the measured m/z value and the mass error using internal and external calibration has not been found. The center of the Gaussian function is shifted a few ppm when MALDI or NanoLC-microESI data are externally calibrated. This is due to space charge effects of ion populations of different abundance, a factor not significant for internally calibrated data. The standard deviation (σ) for the distribution of mass errors for the NanoLC-microESI experiments gave similar σ -values of 1.2 ppm for externally calibrated data and 1.3 ppm for internally calibrated data. These values are much smaller than the σ -values obtained from MALDI experiments. In that case $\sigma = 3.4$ ppm and 2.6 ppm resulted

Figure 4. Distribution of mass errors with applied Gaussian function for internally and externally calibrated data for (a) MALDI measurements and (b) NanoLC-microESI measurements. Mass errors were calculated from all spectra obtained with (a) 1.5–50 fmol of analyte and (b) 1–50 fmol of analyte.

from external and internal calibration, respectively. The standard deviation for the distribution of mass errors and the center of the Gaussian functions are listed in Table 1. Higher mass accuracy was obtained when using the ionization method microESI as opposed to MALDI. This can be explained by the higher resonance frequency of multiple charged ions produced by microESI, compared to mainly singly charged ions of lower resonance frequency produced by MALDI. Further-

Table 1. Standard deviation of mass errors and center of Gaussian function of mass errors for MALDI and NanoLC-microESI experiments using internal and external calibration

Ionization method	Calibration	σ -value	Center of mass error distribution fit
NanoLC-microESI	External	1.2 ppm	0.0 ppm
NanoLC-microESI	Internal	1.3 ppm	+0.9 ppm
MALDI	External	3.4 ppm	-0.3 ppm
MALDI	Internal	2.6 ppm	+1.3 ppm

more, variation in ion population from scan to scan cause space charge effects which in turn lead to a reduction in mass accuracy. This is more relevant for MALDI than for electrospray ionization.

The influence of mass accuracy on database results has been studied by analyzing the MOWSE-SR1 and MOWSE-SR2 using different peptide mass tolerances set for the MASCOT search. Figure 5a shows the variation of the MOWSE-SR1 when the peptide mass tolerance is changed for a MALDI measurement of 6 fmol BSA digest. Peptide mass tolerances set between 5 and 200 ppm were used. The MOWSE-SR2 was nearly independent of the chosen peptide mass tolerance at higher tolerance settings. Nevertheless, a small increase in this ratio from 7 to more than 9 is observed when peptide mass tolerances were set to 10 ppm or less. The effect of the allowed peptide mass tolerance on the MOWSE-SR1 is more impressive: An increase of this ratio from 3 to more than 6 is observed by reducing the peptide mass tolerance from 100 to 20 ppm. This ratio increases dramatically to more than 13 if a peptide mass tolerance is set to 5 ppm for the database search. Therefore, the level of confidence for the protein identification can be improved significantly. The same effect on the MOWSE-SR1 has been observed for the NanoLC-microESI experiments with 5 fmol BSA digest (Figure 5b). The MOWSE-SR2 remains nearly constant for the higher peptide mass tolerance settings. The MOWSE-SR1 increased from 3.5 to more than 16 by reducing the peptide mass tolerance from 200 ppm to 2 ppm. Setting very low peptide mass tolerances leads to a remarkable effect as the ratio changes from 9 to more than 16 when using a mass tolerance of 2 ppm instead of 5 ppm. This clearly shows that the chances of unambiguous protein identification via peptide mass fingerprinting can be significantly improved by the very high mass accuracy provided by FT-ICR MS. The MOWSE-SR1 obtained from the measurement of 1 fmol BSA digest using NanoLC-microESI are depicted in Figure 5c. Analysis of low sample amounts typically results in smaller numbers of observed peptides. This subsequently results in poor MOWSE scores and MOWSE-SR1, making it difficult to identify a protein. Nevertheless, the correct protein identification is still possible by allowing very stringent peptide mass tolerances for the database search. Reducing the peptide mass tolerance from 10 ppm to 2 ppm the MOWSE-SR1 increased from 1.5 to about 2.3 for only 1 fmol of digest analyzed. The reduction of peptide mass tolerance settings for optimized protein identification is shown in detail in Figure 6.

The protein BSA could not be identified with a mass tolerance of 200 ppm (Figure 6a). A mass tolerance of 50 ppm lifted BSA to the position of the most likely candidate, nevertheless unambiguous identification is not possible as other proteins with similar MOWSE scores have been found (Figure 6b). Reducing the mass tolerance allowed for the search from 50 ppm to 10 ppm did not improve the MOWSE score of BSA, but the

Figure 5. Effect of peptide mass tolerance for the database search. Results are given as MOWSE-SR1 (BSA/non-BSA related proteins) and MOWSE-SR2 (BSA/other albumin) using internally calibrated data for (a) MALDI measurement of 6 fmol BSA digest, (b) NanoLC-microESI measurement of 5 fmol BSA digest, and (c) NanoLC-microESI measurement of 1 fmol BSA digest.

scores for other proteins have been reduced substantially leading to a higher level of significance for BSA (Figure 6c). The confidence level for BSA identification can be further improved by reducing the peptide mass tolerance setting to only 2 ppm (Figure 6d). Now, the protein BSA is significantly identified and the second "hit" is another albumin, lending support to the top candidate. This example demonstrates that despite a small number of peptides observed proteins can be clearly identified by setting strict mass tolerance limits for peptide mass fingerprint analyses.

Conclusion

Fast NanoLC-microESI FT-ICR MS using isocratic elution as well as MALDI FT-ICR MS are both suitable methods for protein identification by peptide mass fingerprinting: The protein bovine serum albumin (BSA) could be identified unambiguously using a NanoLC-microESI with fast isocratic elution with an

amount of only 5 fmol resulting in a very high MOWSE score of nearly 300. The MOWSE score for the next candidate was 77, the closely related sheep albumin. However, MOWSE scores of non-BSA related protein were below 32 based on the high mass accuracy used for database search. Despite a low MOWSE score below the limit for unambiguous protein identification for the measurement of 1 fmol BSA by NanoLC-microESI, BSA could still be identified based on a very low peptide mass tolerance set for the database search. MALDI experiments showed that BSA could be identified unambiguously with low femtomole amounts of protein digest, very comparable to the NanoLC-microESI experiments. A correct identification has been observed by MALDI-FT-ICR MS using only 1.5 fmol BSA digest on the MALDI target. The MOWSE score of BSA was a factor of more than 3 higher than all other proteins found.

The high mass accuracy attained by NanoLC-microESI FT-ICR MS and MALDI FT-ICR MS enabled

Figure 6. Probability based MOWSE scores from the NanoLC-microESI experiment with 1 fmol BSA digest. Data were internally calibrated. Peptide mass tolerances were set to (a) 200 ppm, (b) 50 ppm, (c) 10 ppm, and (d) 2 ppm for database searches.

database searches using very strict peptide mass tolerances. This substantially improved the chance of correct protein identification, and the significance of any identification. The advantage of highly accurate mass measurement for peptide fingerprints is demonstrated by successful protein identification even with low percentages of protein sequence coverage. The high mass resolution of FT-ICR mass spectrometry also accelerates and simplifies peptide fingerprint analyses.

Acknowledgments

The authors thank Ulrike Schweiger-Hufnagel for the preparation of the tryptic digests of bovine serum albumin and Jackie Jarvis for applications discussions.

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