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Communication

Micro-capillary liquid chromatography Fourier transform ion cyclotron resonance mass spectrometry — a powerful tool for peptide and protein identification★✪

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In order to be able to study complex biological samples, a micro-capillary liquid chromatography system was coupled to a 9.4 T Fourier transform ion cyclotron resonance mass spectrometer. The setup was tested on a tryptic digest of bovine serum albumin, which resulted in high sequence coverage (> 92 %) of the protein.

The identification and characterization of peptides and proteins are of great importance in medical and biochemical research. There is a strong need for tools that enable the identification of proteins in biological samples such as body fluids and tissue extracts. Electrospray ionization in combination with Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) has proved to be a valuable tool in the analysis of biomolecules due to the soft ionization technique, high mass accuracy (less than 1 ppm error possible), ultra

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Abbreviations: ACN, acetonitrile; ECD, electron capture dissociation; FTICR MS, Fourier transform ion cyclotron resonanace mass spectrometry; HAc, acetic acid; LC, liquid chromatography.

high resolving power (10^5–10^6 mass/ Δmass) and sensitivity of this mass spectrometer (in the attomole range).

Generally, biological samples are complex and the salt concentrations are high. Some of the components in the samples are present in very low concentrations. If the samples are infused directly into the mass spectrometer there will be overlapping isotopic clusters in the spectrum, and signals from compounds of high concentrations might obscure signals from less abundant components. Salts will interfere with the electrospray ionization process. Liquid-chromatography mass spectrometry, LC-MS, facilitates the handling of biological samples since this approach results in less ion suppression due to separation and a concentration of the analytes, in combination with desalting of the sample. The coupling of LC-systems to FTICR-MS has proved to be useful in peptide analysis of more complex mixtures [1, 2]. Here, we report on the instrumentation and results of such experiments from our laboratory.

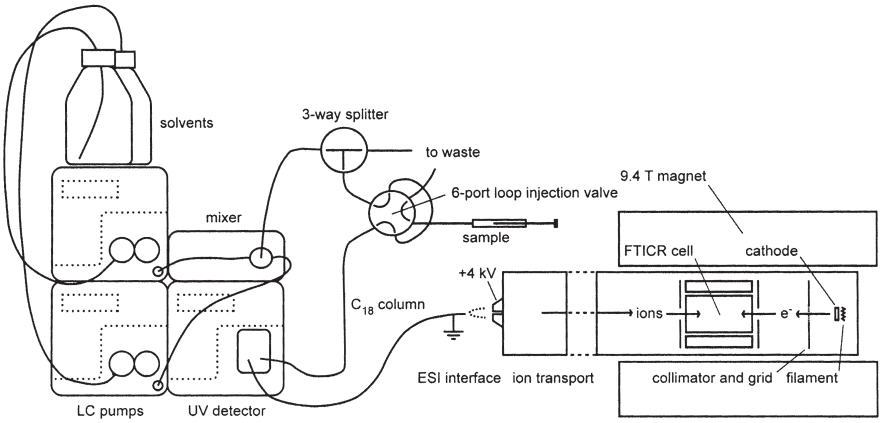

Figure 1. The micro-capillary liquid chromatography FTICR mass spectrometric experimental set-up.

The sample is injected to a 6-port injection valve with a 10 μl loop (injected amount about 10 pmol), separated on a C-18 column, passing a UV-detector and electrosprayed on-line to a 9.4 T FTICR mass spectrometer. The mobile phase flow is delivered by two LC-pumps and split in a 3-way splitter before entering the valve in order to reduce the flow rate to about 200 nl/min.

MATERIALS AND METHODS

In our laboratory, a micro-LC system has been coupled to a Bruker Daltonics (Billerica, MA, U.S.A.) BioAPEX-94e 9.4 T FTICR mass spectrometer. The chromatographic separations were carried out on an in-house packed 10 cm long C_{18}-column, I.D. 200 μm. A mobile phase gradient (20–99.5% ACN, 0.5% HAc) was delivered by two HPLC-pumps (JASCO, Japan) (Fig. 1). The sample was electrosprayed using an in-house built sheathless interface [3].

As a model system, bovine serum albumin was digested with trypsin, separated on-line and detected in the mass spectrometer. The experimental masses of the fragments were compared to theoretical masses of tryptic fragments calculated from the protein sequence.

RESULTS

An overview of the results from the LC-FTICR MS experiment of the tryptic digest of bovine serum albumin is seen in Fig. 2. The identified peptides cover more than 92% of the protein sequence. Compared to direct infusion experiments [4], the spectra are less complex, and thus easier to interpret. Additional information on other properties of the peptides, e.g. charge and hydrophobicity, is also given in the chromatographic step.

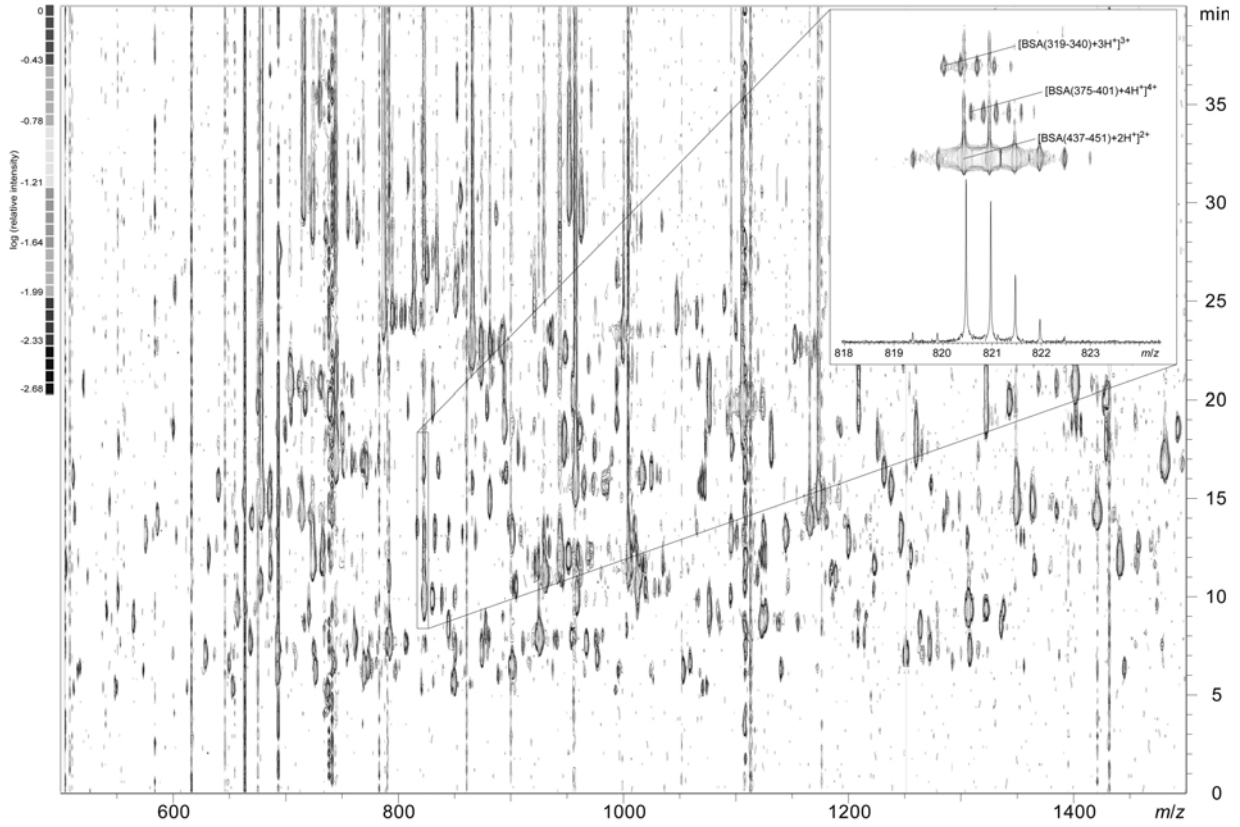

Figure 2. A contour plot of the result from an LC-FTICR MS experiment of a tryptic digest of bovine serum albumin.

The mass-to-charge ratios of the bovine serum albumin fragments are given on the x-axis and the time of elution on the y-axis. In the upper right corner one example is shown where three fragments that were not easily detected by direct infusion mass spectrometric experiments are identified.

CONCLUSIONS

The results look promising for future applications. Initial μLC-FTICR experiments on tryptic digests of much more complex samples such as various body fluids have been performed. The method is under development, and the separation and electrospray conditions need to be further optimized. Post-translational modifications make peptide identification more complicated. A complementary approach to the above mentioned procedure is to run MS/MS-experiments on unidentified peptides to generate short sequence tags, do *de novo* sequencing or to identify post-translational modifications. In our laboratory, electron capture dissociation (ECD) has recently been combined with μLC-FTICR MS. Peptide mixtures were analyzed alternating ordinary and ECD-fragment spectra (unpublished data). The success of these experiment open up even more possibilities and future applications for liquid chromatography-Fourier transform ion cyclotron resonance mass spectrometry.

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