On-Chip Mesoporous Functionalized Magnetic Microspheres for Protein Sequencing by Extended Bottom-up Mass Spectrometry

Datenbank

CEABA, Copyright WTI-Frankfurt eG

Deskriptoren

Fraktionierung; Anreicherung; Mikrosphäre; Protein; monoklonale Antikörper; Massenspektrometrie; Mikrofluidik; Protease; magnetisches Feld; Aufkonzentrierung; Strukturanalyse; Antikörper; Mikrochip; Proteolyse; Lab-on-a-Chip-Technologie

FRACTIONATION; ENRICHMENT; MICROBEADS; PROTEINS; MONOCLONAL-ANTIBODIES; MASS-SPECTROMETRY; MICROFLUIDICS; PROTEOLYTIC-ENZYME; MAGNETIC-FIELD; PRECONCENTRATION; STRUCTURAL-ANALYSIS; AB:ANTIBODY; MICROCHIPS; PROTEOLYSIS; LAB-ON-A-CHIP

Abstract

A limited amount and extreme concentration variability of proteomic-related samples require efficient analyte preconcentration and purification prior to the mass spectrometry (MS)-based analysis. Preferably, these steps should be coupled online with chosen fractionation and detection techniques for the minimization of the sample loss. To realize such sample pretreatment, herein, an on-chip solid-phase extraction-gradient elution-tandem mass spectrometry (SPE-GEMS/MS) is introduced. This technique combines in a microfluidic format online sample preconcentration/purification on SPE sorbent with further fractionation and MS/MS analysis. C8-functionalized mesoporous magnetic microspheres are chosen as a sorbent, spatially confined with an applied magnetic field. They ensure a selective enrichment and analysis of large hydrophobic peptides (2.5-7 kDa), matching the desired mass bin of the extended bottom-up proteomic (eBUP, 3-7 kDa) approach. Within less than 35 min and without additional sample purification, SPE-GEMS/MS provided 66.5% of protein sequence coverage from 75 fmol of BSA tryptic digest. Analysis of only 33 fmol of a single monoclonal antibody, digested with secreted aspartic protease 9 (Sap9) to large peptides, yielded 80% of its sequence coverage. A more complex equimolar mixture of six antibodies (55 fmol each), submitted to Sap9 proteolysis, was also successfully processed by SPE-GEMS/MS, resulting in 50-67% of the total antibody sequence coverage. Importantly, for all antibodies, unique peptides containing complementarity determining regions were detected for both heavy and light chains, leading to a correct identification of mixture components despite their high sequence homology. Moreover, SPE-GEMS/MS microchip and chosen magnetic sorbent are cost-effective and can be produced and operated in a disposable manner. Therefore, the present technique could be potentially suitable for a high throughput sequencing of monoclonal antibodies and rapid eBUP-based structural protein analysis, especially when only a limited sample amount is available.

Autor
Gasilova, Natalia; Srzentic, Kristina; Qiao, Liang; Liu, Baohong; Beck, Alain; Tsybin, Yury O.; Girault, Hubert H.

Institution
Ecole Polytechnique Federale de Lausanne (EPFL), Sion, CH; Fudan University, Shanghai, CN; Centre d'Immunologie Pierre Fabre (CIPF), Saint-Julien-en-Genevois, FR

Quelle
Analytical Chemistry * Band 88 (2016) Heft 3, Seite 1775-1784 (10 Seiten, 41 Quellen)

Sprache
EN Englisch

Dokumentart
J Zeitschrift
ED Digital Object Identifier (DOI)

Erscheinungsjahr
2016