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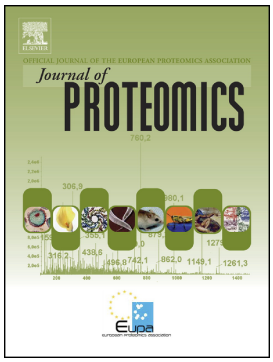

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A flow-through nanoporous alumina trypsin bioreactor for mass spectrometry peptide fingerprinting

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Abstract

Mass spectrometry-based proteomics benefits from efficient digestion of protein samples. In this study, trypsin was immobilized on nanoporous anodized alumina membranes to create an enzyme reactor suitable for peptide mass fingerprinting. The membranes were derivatized with 3-aminopropyltriethoxysilane and the amino groups were activated with carbonyldiimidazole to allow coupling of porcine trypsin via ε-amino groups. The function was assessed using the artificial substrate Nα-Benzoyl-L-arginine 4-nitroanilide hydrochloride, bovine ribonuclease A and a human plasma sample. A 10-membrane flow-through reactor was used for fragmentation and MS analysis after a single pass of substrate both by collection of product and subsequent off-line analysis, and by coupling on-line to the instrument. The peptide pattern allowed correct identification of the single target protein in both cases, and of >70 plasma proteins in single pass mode followed by LC-MS analysis. The reactor retained 76% of the initial activity after 14 days of storage and repeated use at room temperature.

SIGNIFICANCE

This manuscript describes the design of a stable enzyme reactor that allows efficient and fast digestion with negligible leakage of enzyme and enzyme fragments. The high stability facilitates the use in an online-setup with MS detection since it allows the processing of multiple samples within an extended period of time without replacement.

KEYWORDS: nanoporous aluminum oxide, immobilization, trypsin, peptide mass fingerprinting, on-line digestion, ESI-TOF-MS, enzyme stability