Design and Application of an Immobilized-enzyme Microfluidic Reactor for Rapid Online Digestion of Various Natural Macromolecules

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Enzymatic degradation of macromolecules can provide insight into their chemical structure by studying the constituting fragments. The use of an immobilized-enzyme reactor offers a number of advantages compared to in-solution digestion. Higher enzyme-to-substrate ratios can be achieved, which leads to higher efficiencies and thus shorter digestion times in the range of minutes to seconds. Miniaturization of the system offers additional advantages such as low substrate and enzyme consumption, and better control of reaction conditions and time. Finally, immobilized-enzyme reactors allow for long-term operational and storage stability of the enzymes.

A critical step in the successful analysis of proteomes is the conversion of proteins to peptides, via endoproteases. Herein, long digestion times (up to 18 hours) remain an important bottleneck for high-throughput protein analysis. Alternatively, proteins can be digested using an immobilized-enzyme reactor (IMER), enabling shorter digestion times in the order of minutes to seconds. For this contribution, a cyclic-olefin-copolymer microfluidic reactor has been constructed, containing trypsin immobilized on a polymer monolithic material via a 2-vinyl-4,4-dimethylazlactone linker. The IMER was applied for the rapid offline digestion of both individual model proteins and complex protein mixtures prior to LC-MS analysis. The effects of protein concentration and residence time in the IMER were assessed for model proteins of varying molecular weight (up to 240 kDa). Compared to in-solution digestion for 18 hours at 37 °C, IMER-assisted protein digestion at room temperature yielded similar results in terms of sequence coverage and number of identified peptides. Additionally, the IMER showed good repeatability with relative standard deviation of 6.3 % (n = 9) for sequence coverage. Finally, the potential of the IMER was demonstrated for the analysis of complex protein mixtures in dried blood spots (DBS). Compared to a conventional workflow a comparable number of protein identifications could be achieved, while reducing the digestion time from 16 hours to 5.6 minutes and omitting the sample pretreatment steps (denaturation, reduction, and alkylation). No notable differences were observed in terms of molecular weight or hydropathicity of the proteins identified by the workflows.

For future research, flow-through immobilized-enzyme reactors could be implemented inline in a high-throughput LC×LC workflow and the proposed microfluidic platform could be further extended to contain heating elements, a mixer, and a trapping unit. By incorporation of specific enzymes, possible applications could also include analysis of biopolymers (e.g. cellulose and lignocellulosic materials) or synthetic polymers (e.g. polyesters).