Screening for Proteases by Means of LC with Biochemical Detection

Nils Helge Schebb, Martin Vogel, and Uwe Karst

Institute of Inorganic and Analytical Chemistry, University of Münster,
Corrensstr. 30, 48149 Münster, Germany
schebb@uni-muenster.de

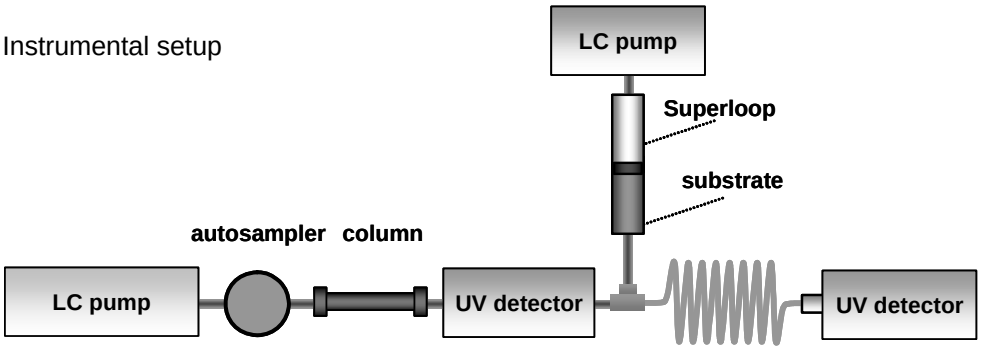
Enzymes originating from natural sources are getting more and more important as biocatalysts for industrial processes or as targets in drug discovery. In particular, proteases are involved in many physiological regulation reactions. Prominent examples are well characterized proteases like thrombin, coagulation factors or angiotensin converting enzyme (ACE), playing a key role in the regulation of blood coagulation or blood pressure. Other proteases are of clinical importance as well, e.g. distinct protease activity in tissue can be used as a cancer biomarker, or an increase in the proteolytic activity of serine-protease in the digestive system is related to the irritable bowel syndrome (IBS). The human genome codes for approximately 600 proteases and many of them have not been characterized yet. On this poster, we present a new methodology to screen for protease activities:

In the first step the proteases are characterized with respect to their activity in an at-line ESI-MS based multi-substrate enzyme assay[1]. Here, the protease-fraction is incubated with eight *p*-nitro aniline (pNA)-labeled peptides, and product formation and substrate consumption are monitored by repeated flow-injection analysis in multiple reaction monitoring (MRM) mode. The most active pNA-peptide is applied as substrate in the LC with biochemical detector (BCD)subsequently.

Here, the proteases are separated under non-denaturing conditions by size-exclusion chromatography and ion-exchange chromatography, respectively. As it has already been described for inhibitor-screening methods[2] the eluate is directed to a continuous flow reactor serving as a BCD (Fig.1). Here, the eluting proteins initially pass a UV detector (280 nm), and subsequently are subjected to a reaction with the pNA-peptide. In a knitted reaction coil, the proteases catalyze the cleavage of the model substrate, thus releasing the coloured pNA. Measurement of the enzymatic activity is performed using a second UV detector at 405 nm, detecting the pNA after the reaction coil.

The separation and the post column reaction were systematically validated with purified enzymes and the method was applied on snake venom and the supernatant of amoeba cultures than. In both of these complex mixtures we were able to identify proteolytically active fractions with the developed method.

Fig.1: Instrumental setup



(1) Liesener, A.; Karst, U. *Analyst* **2005**, *130*, 850-854.
(2) Schebb, N. H.; Heus, F.; Saenger, T.; Karst, U.; Irth, H.; Kool, J. *Anal Chem* **2008,** (in press).