Screening for Protease Inhibitors Using LC/ESI-MS with a Continuous-Flow Enzyme Assay

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Introduction

Enzymatic conversions play a crucial role in the regulation of all processes of life. In particular, proteases like thrombin and coagulation factors play a major role in the regulation of blood coagulation and are important targets for the development of drugs and diagnostic applications. Hence, it is a goal of pharmaceutical research to screen for new specific inhibitors, which can be used as drugs. Natural mixtures such as extracts from plants and bacterial suspensions or snake venom are promising sources for new inhibitors. To identify inhibitors in these complex mixtures, a fast and robust screening system containing a separation step is needed. Coupling a continuous flow enzymatic assay directly to an LC separation is a promising strategy to screen for new inhibitors.

Method

Therefore we are developing an LC method with biochemical detection (LC-BCD) for the separation and detection of protease inhibitors. Potential inhibitors are separated by RP-chromatography. Constant flows of peptides (as substrate) and protease solution are added to the eluate by two additional pumps. In a reaction coil, the substrate is cleaved by the protease. The substrate as well as both products are continuously monitored by ESI-MS. If an inhibitory compound elutes, less product is formed, resulting in a negative peak in the product signal and a positive peak in the substrate signal. Additionally, the mass of the inhibitor is directly detected by ESI-MS and further characterized by MS/MS experiments.

Preliminary data

The method was optimized in flow injection analysis (FIA) mode, without chromatographic separation. In a first step, the system was used to analyze known inhibitors for thrombin and the mammalian digestion enzymes trypsin and chymotrypsin, based on fluorescence detection. The determined IC_{50} values were compared to convential plate reader assays. Next, ESI-MS was used for detection, allowing the use of different substrates in parallel. Finally, with gradient LC separation (RP-18 column) prior to the developed BCD system, the activity of a mixture of 7 different inhibitors can be measured in one single run. This system will be used to screen for new inhibitors of thrombin in complex natural samples like snake venom. Furthermore, the system will be miniaturized to reduce the consumption of substrate, enzyme and sample.

Novel Aspect

A novel ESI-MS based biochemical detector is presented, allowing the application of different substrates in parallel and direct characterization of inhibitors.

Schebb, N. H.; Heus, F.; Saenger, T.; Karst, U.; Irth, H.; Kool, J., Development of a countergradient parking system for gradient liquid chromatography with online bio-chemical detection of serine protease inhibitors. Anal Chem **2008**, 80, (17), 6764-72.