OP 5.5

Online coupling of 2D-HPLC and CN-PAGE for native separation of Metalloproteins with subsequent detection by LA-ICP-MS

Frank Hasenäcker , Janine M. Toffel, Sebastian D. Müller and Alfred V. Hirner

Institute of Environmental Analytical Chemistry, University Duisburg-Essen, Universitätsstr. 3-5, D-45141 Essen, Germany

The metabolism of trace elements and in particular their binding to proteins in living systems is of great importance in toxicological and biochemical studies. The main problem of the analysis of non-covalently bound metal-protein complexes is the low stability of the three-dimensional structure of the protein, which needs to be preserved during sample preparation. In order to conserve the integrity of as many metalloproteins as possible during separation, native separation conditions are required. Due to lower resolution of native separation techniques in comparison to denaturing methods, only multi-dimensional combination of gentle separation techniques provides both preservation of native protein conformation and good resolution.

In this work, a semi automated, three dimensional, native separation technique was developed. Two dimensional high performance liquid chromatography (2D-HPLC) was coupled to native polyacrylamide gel electrophoresis (PAGE). For the first HPLC dimension a Superdex-200 exclusion column with a separation range between 10 kDa and 600 kDa was used. Peaks, which are detected by an online coupled ultra violet/visible detector, were trapped on short (2,1mm x 30 mm) mixed bed ion exchange columns. After switching the trap column into a second flow path the next separation step was realised by passage of the sample through a mixed-bed ion-exchanger (2,1mm x 50 mm). Peaks were detected inline by an ultra-violet diode array detector. These are directly applied to a slot of native gel using a fraction collector after adapting the efflux to the conditions required for gel electrophoresis by adding an electrophoresis sample buffer using a t-split. After gel electrophoresis, detection of protein-bound metals by laser ablation inductively coupled plasma mass spectrometry is possible. The state of native conformation of metalloproteins such as alkaline phosphatase (AP) and superoxide dismutase (SOD) following size exclusion, ion exchange and native PAGE separation was investigated by enzymatic assays. The applicability of this method for non-standard samples was demonstrated by determination of protein-bound metals in human serum.