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Biochemical neutron activation analysis (BNAA) in pre- and post-genomic era of proteomics and metabolomics

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Neutron activation analysis (NAA) is a well-established analytical technique for the simultaneous measurement of multielement concentrations. The most common forms of NAA are instrumental (INAA), radiochemical (RNAA) and preconcentration (PNAA). We have previously reported the development of speciation NAA (SNAA). We are also interested in the development of biochemical NAA (BNAA) methods for the separation, purification, characterization, and measurement of metalloproteins and organic compounds containing trace elements which are of biological/biochemical importance. We take advantage of the unique features of both techniques. For example, we need an analytical technique capable of analyzing a few milligrams of solid as well as liquid samples non-destructively and independent of chemical species, and of determining trace levels of several elements simultaneously within a short time in a matrix largely composed of carbon, hydrogen, nitrogen, oxygen and sulfur. Of course, the technique should provide excellent precision, accuracy, sensitivity, and detection limits. NAA meets all of these requirements. The biochemical techniques of interest are dialysis, ammonium sulphate precipitation, gel filtration, ion exchange and hydroxyapatite chromatography, high-performance liquid chromatography, chromatofocusing, isoelectrofocusing, isotachophoresis, sedimentation equilibrium and enzymatic assay. We called this combination of two techniques BNAA which is ideally suited for studying metalloproteins, protein-bound trace elements, and organometallic compounds. We observed that most of the trace elements in bovine kidneys, for example, were largely concentrated in the cytosol fraction. More than 70% of As, Br, Cl, Co, K, Na and Rb, about 65% of Cd, and 30-35% of other elements except Se (14.4%), Cr (15.6%) and Mo (24.6%) were detected in this fraction. Elements such as Ca, Cr, and Se were more abundant in the nuclei fraction with concentrations of 34%, 75% and 73%, respectively. The dialysis experiments showed that more than 90% of Ca, Cd, Cu, Fe, Mg, Mn, Mo, Se, V and Zn, and possibly As, and I, and about 20% of Br were bound to macromolecules, mainly proteins. Most of these proteins were stable in the pH range of 3.5 to 10.5. We separated and purified several metalloprotein species. Examples of these species with particular emphasis on Se will be presented.

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