Quantitative Analysis of Various Metalloprotein Compositional Stoichiometries with Simultaneous PIXE and NRA

ANDREW MCCUBBIN, PAUL DEYOUNG, GRAHAM PEASLEE, Hope College, MEGAN SIBLEY, Clemson University, JOSHUA WARNER, Hope College — Stoichiometric characterization has been carried out on multiple metalloproteins using a combination of Ion Beam Analysis methods and a newly modified preparation technique. Particle Induced X-ray emission (PIXE) spectroscopy is a non-destructive ion beam analysis technique well suited to determine the concentrations of heavy elements. Nuclear Reaction Analysis (NRA) is a technique which measures the areal density of a thin target from scattering cross sections of 3.4 MeV protons. A combination of NRA and PIXE has been developed to provide a quantitative technique for the determination of stoichiometric metal ion ratios in metalloproteins. About one third of all proteins are metalloproteins, and most do not have well determined stoichiometric compositions for the metals they contain. Current work focuses on establishing a standard method in which to prepare protein samples. The method involves placing drops of protein solutions on aluminized polyethylene terephthalate (Mylar®) and allowing them to dry. This technique has been tested for several proteins of known stoichiometry to determine cofactor content and has proven to be a reliable analysis method, accurately determining metal stoichiometry in cytochrome c, superoxide dismutase, concanavalin A, vitamin B12, and hemoglobin.

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