Point of VIEW

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Gel Electrophoresis and X-ray Fluorescence: A Powerful Combination for the Analysis of Protein Metal Binding

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ABSTRACT Understanding the complex biochemical mechanisms that underlie the regulation, toxicity, and protein binding of metal ions requires the ability to analyze the metal content of individual proteins in complex mixtures. In this issue of *ACS Chemical Biology*, a technique combining gel electrophoresis with synchrotron X-ray fluorescence imaging demonstrates a rapid and powerful solution for simultaneously examining multiple proteins and metal ions of interest. The resulting technique is broadly applicable, does not require specialized equipment for sample preparation, and is likely to be extensible in the future.

Trace metal ions such as Fe, Ni, Co, Cu, and Zn play numerous essential roles in biology, contributing to the function of metalloproteins and metalloenzymes. Moreover, complex mechanisms regulate the available concentrations and subcellular localizations of these metal ions to ensure correct metal loading of proteins and avoid potential metal toxicity (*1*–*3*). Understanding these dynamic processes and the impact of environmental conditions, such as heavy metal toxicity, on the distributions of metals between different proteins is a central goal of bioinorganic chemistry. Biochemical investigations in this area often require the analysis of metal binding to specific proteins in complex mixtures. Traditionally, this has been accomplished through the isolation and characterization of individual proteins using techniques such as atomic absorption and inductively coupled plasma mass spectrometry. While advances have been made in combining separation and analysis systems, for systems with multiple proteins of interest carrying out individual isolations under conditions that do not remove or alter the identity of a bound metal cofactor can be a daunting task. In their article in this issue, Finney and coworkers present a method for facilitating this type of analysis by quantitatively imaging metals in nondenaturing protein gels (*4*). This approach combines the resolving power of gel electrophoresis with the sensitivity and specificity of X-ray fluorescence for metal detection. The end result is a simple yet powerful technique that can simultaneously examine multiple proteins and metal ions of interests, does not require specialized lab equipment or radioisotopes for sample preparation, and yields results in a universally familiar format.

X-ray fluorescence (XRF) is a well-established analytical technique that has recently seen a renaissance in biology as a method for mapping metal concentrations in tissues and cells (*5, 6*). Synchrotron-based instrumentation and improved focusing techniques have allowed XRF imaging of transition metals in a variety of biological samples ranging from tissues to individual cells, with sufficient sensitivity and spatial resolution to map transition metals at up to 100 nm spatial resolution (*e.g.*, refs 7−9). In their manuscript, Finney *et al.* take advantage of the low metal detection limits of synchrotron XRF imaging to map concentrations of biological trace metals and toxic metals in gel electrophoresis experiments. Specifically, they describe the use of nondenaturing gel electrophoresis, followed by electroblotting onto a protein binding membrane and XRF mapping (Figure 1). A major advance described in this manuscript is the use of electroblotting to immobilize separated proteins and bound metals onto a solid support that is amiable to X-ray analysis, in this case a PVDF membrane. Using this approach, the authors demonstrate detection limits for protein quantification in the

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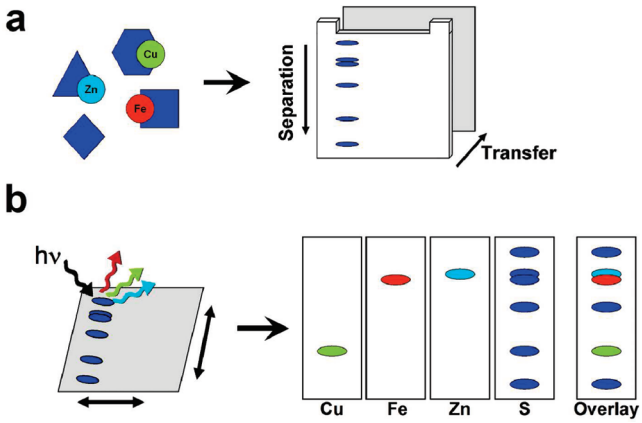

Figure 1. XRF imaging of protein gels. a) Sample preparation is carried out using standard gel electrophoresis and electroblotting techniques, such as those used for Western blotting. Protein mixtures are separated by nondenaturing PAGE, and samples are then transferred to a PVDF membrane, effectively fixing both proteins and metals. At this point, membranes may be dried and stored for analysis. b) Protein and metal quantification are carried out using synchrotron X-ray fluorescence. Focused, high-energy X-rays (*hν*) are used to excite elements ranging from P to Zn. Each element emits fluorescent X-rays at characteristic energies whose intensity is measured and used to produce quantitative maps. The positions of protein bands can be mapped on the basis of S content.

range of 200 ng using S as an elemental marker and XRF detection limit for the bound metal ions at picomolar levels. These protein detection limits are comparable to those of Coomassie protein staining.

The authors demonstrate the utility of this technique in two test cases. In the first case, they examine the patterns of protein binding for Cr(III) and Cr(VI) compounds added to blood plasma and identify the formation of oxidation-state-specific protein complexes. They then extend their approach to include X-ray absorbance near edge spectroscopy (XANES), which is sensitive to both the oxidation state and coordination environment of metal ions. Cr-protein complexes from Cr(III)-propionate supplemented plasma are shown to have XANES spectra resembling Cr(III)-protein adducts, rather than that of Cr(III)-propionate. A second example case demonstrates both the ability to map Fe in protein gels and the applicability of this approach to metalloproteomic studies. Cell lysates from the Gram-negative anaerobic bacterium *Shewanella oneidensis* were examined for differences in metalloprotein content under aerobic and anaerobic growth conditions; a strong Fe band was observed only during anaerobic growth. These experiments demonstrate the broad utility of the approach with potential applications extending beyond metal toxicity and metalloproteomics to other areas of bioinorganic chemistry.

Overall, the power and utility of this technique derives in large part from the incorporation of gel electrophoresis, a fundamental technique of molecular biology. The data generated are similar in many regards to those produced by phosphorimager analysis of radiolabeled samples, and groups interested in applying this approach are likely to already possess the necessary equipment and expertise for sample preparation and data analysis. Some level of optimization, particularly with regard to electrophoresis conditions, may be required for different experimental systems. For example, in their Cr experiments Finny and colleagues determined that inclusion of low levels of SDS during electrophoresis improved separation of plasma proteins. Additional optimizations may be required to maintain metal binding in protein/metal complexes with relatively fast dissociation rates that may lead to loss of metal during electrophoresis. Importantly, facilities and technical assistance for carrying out XRF analysis are available at synchrotron user facilities. A beamline facility (8-BM) tailored for this technique and available to the public has recently been built at the Advanced Photon Source of Argon National Laboratories.

Looking forward, this technique is likely to be applicable to multiple areas of study, both in and outside of bioinorganic chemistry. One exciting possibility is the ability to broadly assess changes in metalloprotein levels caused by conditions such as heavy metal toxicity, oxidative stress, and aging. The ability to examine multiple proteins and metals simultaneously will greatly facilitate our understanding of interrelationships in the regulation of different transition metals. XRF can also map nonmetallic elements including halogens, P, S, and Se and may therefore be applicable to the study of post-translational modifications that incorporate, or can be made to incorporate, one of these elements. Finally, imaging of gels is likely to represent a first step in the fusion of molecular biology and XRF analysis. For example, Western blotting of membranes may be useful in tracking individual proteins during or subsequent to XRF analysis. It may also be possible to incorporate 2D electrophoretic separations for increased protein resolution and mass spectroscopic identification of metal binding proteins. In sum, the marriage of these two techniques moves metal ion analysis in the direction of becoming a standard, broadly applicable and easily extensible benchtop technique.

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