O15

Nanogels for Multifunctional Biomolecular Electrophoresis Assays

Lisa A. Holland
C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, WV, USA, Lisa.Holland@mail.wvu.edu

Summary

Significance. Capillary electrophoresis is a powerful separation technique for volume-limited samples in biomedical and pharmaceutical research. An advanced method is developed for native enzyme analyses providing insight into protein structure and function.

Introduction. Protein-based assays are difficult to achieve with capillary electrophoresis under native conditions because of the interaction of proteins with the separation channel. By miniaturizing the enzymatic reactions are integrated in real-time with separation-based assays. Biocompatible gels advance nanoscale reactions by enabling new separation modalities critical to evaluating and harnessing enzyme activity. Self-assembled phospholipid nanogels are thermally reversible media that maintain the biological function of complex biomolecules and provide a means to create and embed multifunctional assays in capillary separations. In this presentation nanogel technologies are described to create nanoliter reactions zones to interrogate biomolecules in seconds.

Experimental Approach. To date, as many as 7 sequential processing steps have been performed in series in microscale channels. This is possible because the viscosity of nanogel is thermally dependent and thermally reversible. At temperatures below ~22°C nanogels have liquid-like viscosity. At higher temperatures nanogels have a gel-like viscosity. This property makes it easy to fill and pattern nanogels in narrow-bore capillaries at low temperatures using an automated capillary electrophoresis instrument. Once the nanogel is loaded into the capillary, the fluids are then locked in place by raising the temperature to gel the material. This enables the precise placement of 2-5 nanoliter enzyme reaction zones at the beginning of a capillary with a total liquid volume less than 1 microliter. In this way, a series of discreate reaction zones is created and integrated with a separation step. Enzyme reactors of this low volume are mixed electrophoretically and then the substrate and products, or products, are separated, detected, and quantified.

Results and Discussion. This approach is automated and reduces the time for enzymatic conversion from hours to seconds. The analyte resolution of biomolecules separated in nanogel yields efficient separation. Applications with hydrolase and transferase enzymes are demonstrated. This work is significant to separations because it transforms standard electrophoresis methods into sophisticated multifunctional separations that are programmed, erased, and repeatedly run.

Conclusions. An approach for nanoliter enzyme reactions under native conditions is demonstrated that leverages the low sample volume requirements of capillary electrophoresis. The technology outlined in this presentation provides new strategies to either leverage or evaluate enzyme specificity and activity.