Svetlana M Krylova et al., J Chromatogr Sep Tech 2016, 7:5(Suppl)
http://dx.doi.org/10.4172/2157-7064.C1.016

Aptamer-based affinity chromatography as a rapid, single step method for purification of native proteins

Svetlana M Krylova, J Bao, S Boloborodov, O Borisade and S N Krylov
York University, Canada

Isolation and purification of recombinant proteins is one of major tasks of modern biotechnology. Isolation of enzymes and antibodies requires conditions that could preserve biological activities of proteins. Often fusion of proteins with His-, GST-, and MBP-tags is used to facilitate their isolation by affinity chromatography. However, the tags, may interfere with the application of the protein while there removal is often accompanied by protein's loosing its biological activity. We developed aptamer-based affinity chromatography allowing isolation of the recombinant proteins from the crude cell lysate as a quick method yielding native biologically active enzymes. DNA aptamers to AlB protein were developed and characterized by Kinetic Capillary Electrophoresis (KCE). Synthetic DNA aptamers with K_d values in the nanomolar range were used for selective binding and isolation of AlkB from the cell lysate. Specifically, gold (DE3) bacterial culture of cells, expressing E. coli AlkB protein was loaded on aptamer-modified magnetic beads (immobilized though a biotin-streptavidin link). The unwanted components of the cell lysate were removed by washing the beads. AlkB was eluted using different solutions with high ionic strengths. The results were compared with the activity and yield of the enzyme purified using standard tag-based methods of protein purification. Our new method was also succesfully repeated for isolation and purification of MutS protein. In my presentation, I will discuss the CE based aptamer development technology, and I will demonstrate the potential of using aptamers for purification of enzymes from cell lysates in a single simple step, providing biologically active pure recombinant proteins.

Biography

Svetlana M Krylova completed her PhD from the Russian Academy of Sciences. She has over 10 years of research leadership experience in the area of Medical Diagnostics and Drug Development in biotechnology and pharmaceutical companies in Canada. She has been a contract faculty member at York University in Toronto since 2008. She is leading research projects in the area of Bioanalytical Chemistry as a Senior Research Associate in the Centre for Research on Biomolecular Interactions at York University.

krylova@yorku.ca

Notes: