STRUCTURAL CHARACTERIZATION OF HAEMOPHILUS INFLUENZAE FERRIC BINDIND PROTEIN IN DIFFERENT ENVIRONMENTAL CONDITIONS

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The importance of iron in the metabolic activity of living organisms creates a scientific interest to Ferric Binding Proteins. In unbound form, Fe^{+3} is not soluble in water whereas; Fe^{+2} form is highly toxic to freely move [1]. Therefore, biological systems have developed complex mechanisms to carry and host iron. Our main interest is on ferric binding protein (FBP) from pathogenic bacteria; Haemophilus Influenzae ferric binding protein (hFBP), which is the competitive transport protein of transferrin in human. The unique hijacking mechanism of hFBP, where one of the two irons is detached from human transferrin and transported through the periplasmic space and captured by hFBP, is believed to be regulated by pH and ionic strength [2]. In our present project, we are studying structural changes and related iron binding dynamics due to the modifications in the environmental conditions.

We have expressed and purified hFBP by using recombinant DNA technology. Characterization of the protein was performed by Size Exclusion Chromatography (SEC), Dynamic Light Scattering (DLS) and Small Angle X-Ray Scattering (SAXS). As a result, protein was obtained as monomer. We have also managed to observe structural changes due to the iron binding dynamics; radius of hFBP decreases when it binds to iron because of the closing up movement (Figure 1). Iron realizing dynamics were also monitored in different ionic strength conditions. The characteristic ligand-to-metal charge transfer absorption peak of hFBP-Iron complex at around 480 nm was observed by increasing concentration of citrate [3]. hFBP was found to be more stable in high salt concentrations and with the presence of phosphate since it is the synergistic anion in the binding pocket [2]. Stability of hFBP in physiological conditions vs. low ionic strength conditions was also compared with Molecular Dynamic simulations, which were correlated by our experimental results.

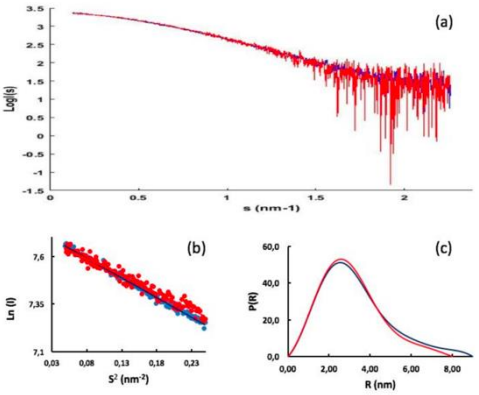

Figure 1: (a) SAXS profiles, (b) Guinier Plots and (c) P(R) distribution for apo (blue) and holo (red) hFBP.

We believe that the structural information during iron binding and release kinetics will help us to understand the unique iron acquisition of the bacterial protein, which may lead us further drug design and biosensor studies.

References:

[1] Crichton, Robert R., and Johan R. Boelaert. *Inorganic biochemistry of iron metabolism: from molecular mechanisms to clinical consequences*. John Wiley & Sons, 2001.

[2] Bruns, Christopher M., et al. "Crystallographic and biochemical analyses of the metal-free Haemophilus influenzae Fe3+-binding protein." *Biochemistry* 40.51 (2001): 15631-15637.

[3]Guo, Maolin, et al. "Synergistic anion and metal binding to the ferric ion-binding protein from Neisseria gonorrhoeae." *Journal of Biological Chemistry* 278.4 (2003): 2490-2502.

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