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Investigation of the Hsp70/J-Domain Protein ATPase dependent substrate binding cycle using small molecule modulators.

Hsp70s are ubiquitous, ATP dependent chaperone proteins involved in a wide range of cellular processes. The substrate binding and release cycle of Hsp70s is tightly controlled by J-domain proteins (JDP) and Nucleotide Exchange Factors (NEF). JDP proteins bind the substrate independently of Hsp70. The JDP-substrate complex interacts with Hsp70, stimulating its ATPase activity, which leads to conformational changes and the formation of a stable Hsp70-substrate complex. Nucleotide exchange factors stimulate the dissociation of ADP resulting from the hydrolysis of ATP by Hsp70. This results in the binding of a new ATP molecule, changing the conformation of Hsp70 and releasing the substrate.

Due to the participation of chaperones in many housekeeping processes, disturbances in their activity lead to deleterious effects on the cells. Literature data indicate their participation in the formation of tumors. Normal/high activity of Hsp70/JDP has an inhibitory effect on the apoptotic processes of cancer cells, and low activity of Hsp70/JDP affects the abnormal folding of proteins. The solution is to restore the normal activity of the chaperone proteins in the cell by using inhibitors and/or activators of these proteins. The goal of our project is to test and select small molecule modulators of chaperone activity. This requires the use of an automated screening test based on a simple measurement of ATPase activity.

My task was to optimize measuring of ATPase activity using a colorimetric test based on malachite green. This method detects the inorganic phosphate (Pi) formed during the hydrolysis of ATP in the ATPase Hsp70 cycle and uses the reaction of phosphomolybdate with a basic malachite green dye to form a colored complex that is measured spectrophotometrically. As a model system, we chose bacterial chaperone proteins involved in the iron-sulfur clusters [FeS] biogenesis composed of specialized Hsp70 - HscA, JDP protein - HscB and their protein substrate - IscU on which FeS centers are synthesized.

Optimizing the conditions for measuring ATPase Hsp70 stimulation will give us an experimental system for testing modulators of this reaction on a large scale. These works will be conducted in cooperation with prof. Jason Gestwicki (University of California San Francisco). To this end, I have purified the above-mentioned proteins, which I sent to the laboratory of prof. Jason Gestwicki, where screening tests will be carried out.

Using the aforementioned method, I determined the kinetic parameters of the ATP hydrolysis reaction by HscA in the presence and absence of JDP HscB protein and IscU substrate. The obtained results will help in the interpretation of the influence of selected modulators on the HscA/HscB/IscU ATPase dependent substrate binding cycle.