The Summer Undergraduate Research Fellowship (SURF) Symposium
6 August 2015
Purdue University, West Lafayette, Indiana, USA

Elucidating the Role of HAUSP Ubiquitin Like Domains in the Catalytic Function of USP7

Anuj Patel, Nicole Davis, and Dr. Andrew Mesecar
Department of Biological Sciences, Purdue University

ABSTRACT

Ubiquitin specific proteases (USPs) are a class of enzymes involved in myriad cellular processes. One USP of great interest due to its oncogenic properties is USP7. In normal conditions USP7 is closely regulated due to its responsibility for destabilizing the tumor suppressor, p53, through the deubiquitination of MDM2. In multiple myeloma cases, it appears the regulation of USP7 subsides, as it is largely overexpressed, leading to the inappropriate degradation of p53. Inhibition of USP7 could, therefore, prove a viable target for cancer therapy. A greater understanding of USP7's function and structure can lead to more insight into how this enzyme could be inhibited. USP7 is composed of the TRAF, catalytic and 5 HUBL domains. Previous work has shown that the catalytic activity of USP7 is greatly reduced in the absence of the HUBL 4 and 5 (H-45) domains. However, it is unclear if the other HUBL domains have specific roles in USP7 activity. To evaluate the individual HUBL domain roles in USP7s activity, constructs containing the full length HUBL domain, as well as just H-45 truncations were obtained. Each construct was expressed *in E. coli BL21 (DE3)* cells and purified by chromatography. These constructs were left with their respective histidine tags in order to evaluate the kinetics of their interactions *in trans* with the catalytic domain using the Fortebio Octet Red 384 system. Kinetic assays using the ubiquitin rhodamine substrate showed that the histidine tagged proteins are still able to activate the catalytic domain of USP7. Optimization of the Fortebio Octet Red 384 system suggested that the catalytic domain bound nonspecifically to the Anti-Penta-His (HIS1K) obscuring the off binding rates of the HUBL protein. Further truncations of the HUBL domains including H1, H2, H3, H1-2 were successfully subcloned using recombinant cloning techniques and will be analyzed using the Octet system.

KEYWORDS

USP7, HAUSP, HAUSP Ubiquitin Like Domains, HUBL, recombinant cloning, Fortebio Octet Red, Ubiquitin specific protease, USP,