Biophysical & Structure Properties of the UspA712 of Micrococcus Luteus

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Introduction

UspA712 is a 12kDa single domain protein. It is upregulated in exponentially growing wild type strain of Micrococcus luteus, one of the two models used in the study of bacterial dormancy. When UspA712 is knocked out, the mutant MI strain exhibits an extended lag phase. The mechanism of action of the Universal Stress Protein (USP) in relation to stress response are not currently known. Unlike the two other Usp’s in Micrococcus luteus (UspA616 & UspA184), UspA712 protein does not bind nucleic acids and so we hypothesized that it might have an allosteric property in modulating its regulatory functions and molecular interactions. UspA712 protein is purified as a dimer and structure of the protein shows an extended α-helix wrapped around the β sheets. UspA712 is quite unstable and degradation occurs after a number of days. We hypothesized that degradation starts at the α helices, which lacks most of its electron density. This is highlighted with HSQC experiment data gathered over a couple of days showing a collapse in the middle of the spectra (α-helices) indicative of degradations while showing much distinct peaks around the edges (β sheets). To study the proteins conformational changes, future experiments are directed towards NMR structural dynamics studies after the first step of assigning the protein backbone structure. To characterize the biophysical properties of the UspA712 protein, melting temperature and thermodynamic parameters were estimated using differential scanning fluorimetry experiments. The protein exhibits a one state unfolding mechanism as opposed to two state characteristic of a dimeric protein. At increasing concentrations of the protein, melting temperature and hence enthalpy and free energy changes have roughly the same values. This was also correlated with differential scanning calorimetry experiments showing an almost consistent enthalpy and free energy values with the DSF.

Protein expression and purification

UspA712 protein was expressed using the amplified UspA712 gene from Micrococcus luteus genome and ligated with pET14b expression vector. The ligated plasmid was transformed into BL21 PLYS bacteria cells. Experimentally growing cells were IPTG induced and grown for 4 – 5 hours at 37°C. Growing cells were spun down, homogenized and protein precipitation was done at 60% with ammonium sulphate. Precipitates were resuspended in buffer and run through the S200 and then S75 column respectively. Confirmation of dimeric protein was done at 2mg/ml and at 0.2mg/ml using the S75 analytical column.

Structure determination

Figure 4 – Five exclusion chromatography purification of the UspA712 with a concentration of its dimeric formation (2mg/ml) at high (black, 2mg/ml) and low (red, 0.2mg/ml) protein concentrations. Transparent line showing long tail of dimeric protein standard (2mg/ml)

Towards NMR backbone assignment

Our future goal is to complete a Sequential Backbone Resonance Assignments using 15N/13C and 15N/13C/2H labeled samples of the UspA712 protein. This will naturally allow for future NMR studies to understand the molecular motions and interactions of the residues of this protein in the presence of small molecules. We have carried out initial HSQC experiments with spectra peaks identified. This was set up over a series of days to identify among other things the rate of degradation some of the amino acid residues.

References


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