# Abstract

Tight regulation of the cellular protein content and make up are primary to accomplishing faultless execution normal cellular function. Secondly, the removal of unnecessary and misfolded protein is essential for cell survival. Cellular proteins are removed either by going through lysosomal degradation or proteasome mediated degradation. Selective elimination of proteins is done by Ubiquitin Proteasome System (UPS) which is a targeted degradation process. UPS accomplishes this function by conjugating a small covalent modifier protein called ubiquitin (Ub) to substrate protein to be degraded.

The Ubiquitin mediated proteasome degradation system is mainly controlled by a set of three enzymes. The first enzyme is Ubiquitin activating enzyme (E1/Uba1), which binds to ubiquitin at the cost of ATP via a covalent thioester bond, second enzyme ubiquitin-conjugating enzyme (E2) forms intermediate ubiquitin-E2 conjugates and third enzyme ubiquitin ligase (E3) transfers the ubiquitin molecules to its target protein. The target protein conjugated with polyubiquitination chain undergoes degradation by 26S proteasome.

The ubiquitin-activating enzyme Uba1 (E1) holds an important position being an activator enzyme for ubiquitin. It is a 1024 residues long monomeric protein having six structural domains namely, Inactive Adenylation Domain (IAD), Active Adenylation Domain (AAD), First Catalytic Cysteine Half-domain (FCCH), Second Catalytic Cysteine Half-domain (SCCH), Four Helix Bundles (4HB) and Ubiquitin Fold Domain (UFD), among which UFD, FCCH and SCCH domains are connected to their adjacent domains by long flexible linkers suggesting large-scale conformational changes of E1 enzyme during the ubiquitination process. The study of the domains is of interest because some anti-cancer drugs are targeted to this enzyme. In view of E1's importance, the study focuses mainly on

structural and functional characterization of the domains FCCH, 4HB, SCCH and UFD of E1 individually.

Observations from the structural characterization of the domains FCCH, 4HB, SCCH and UFD suggested their ability to retain their native-like secondary structure. Domain FCCH was observed to be primarily made up of β-sheets, whereas domains SCCH and 4HB displayed more of helical structure and UFD contained mixed α/β structure. These structural characteristics were comparable with structural characteristics of domains when they remain integral parts of the whole E1 enzyme. Therefore, the above observation suggested the peptides are capable of folding and attaining native-like secondary structure.

Denaturation of the domains by guanidine hydrochloride showed a red shift in wavelength, suggesting a change in solvent exposure of aromatic amino acids. Results obtained from the addition of extrinsic fluorophore ANS (1-Anilino 8-naphthalene sulphonic acid) suggested the presence of hydrophobic core in FCCH, 4HB and SCCH domains, whereas UFD showed the presence of hydrophobic residues on the surface of the peptide exposed to solvent.

Further, the peptides were tested for the effect of their presence in the cell as they were showing the ability to fold on their own and attain native like structures. The functional domains might have the potential to interact with their respective binding partners resulting in interference or slowing down cellular machinery by competing with the E1 enzyme. To test this possibility cells were monitored for their response under normal as well as stress conditions. Under conditions like antibiotic and thermal stress, the protein degradation rate would rise to get rid of protein aggregates formed. Domains FCCH and 4HB had no effect on cellular processes. Overexpression of the domains SCCH and UFD affected cell growth under stress conditions.

# Abstract

Ubiquitin tag as a posttranslational modifier regulates and affects many cellular processes. It is a small highly conserved 76 residues long globular protein. Residues Glu64, Ser65 and Gln2 make an unusual C-terminal parallel β-bulge. The residues Glu, Ser and Gln are less preferred and uncommon amino acids for their respective positions. These residues were replaced by more preferred amino acids resulting in β-bulge mutants E64G, S65D and Q2N. Previously the functional efficiency of these mutations was studied *in vivo* using an already standardized method, where wild type ubiquitin was present for house-keeping. The objective was to check the activation efficiency of β-bulge mutants by ubiquitin activating enzyme E1 *in vitro* in the absence of any wild type ubiquitin. All three mutants E64G, S65D and Q2N were found to be capable of forming a covalent bond with E1 with the same efficiency as wild type ubiquitin. The results established that mutant forms of ubiquitin could be bound to an active site of the E1 enzyme. This showed all three mutants were functionally active like the wild type ubiquitin. Further, far-UV CD spectra were recorded to check the stability of these β-bulge mutants with a range of different pH conditions. All three mutants were found to retain their structures with minor differences. The observation indicated the stability of β-bulge mutants even under extreme pH conditions.