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Elucidation of the molecular mechanism of heat shock proteins and its correlation with K722Q mutations in Lon protease

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ABSTRACT

Cells withstand the effects of temperature change with the help of small heat shock proteins IbpA and IbpB. The IbpAB protein complex interacts with Lon protease in their free form and gets degraded at physiological temperature when there is no temperature stress. However, the proteolytic degradation of IbpAB is diminished when Lon is mutated. The mutation K722Q in Lon brings about some structural changes so that the proteolytic interactions between the heat shock proteins with that of the mutated Lon protease are lost. However, the detailed molecular aspects of the interactions are not yet fully understood. In the present, we made an attempt to analyze the biochemical aspects of the interactions between the small heat shock proteins IbpAB with wild type and mutant Lon protease. We for the first time deciphered the molecular details of the mechanism of interaction of small heat shock proteins with Lon protease bearing K722Q mutation i.e. the interaction pattern of heat shock proteins with mutant Lon protease at physiological temperature in absence of proteolytic machinery. Our study may therefore be useful to elucidate the mechanistic details of the correlation with IbpA, IbpB and Lon protease.

1. Introduction

Cells live in a wide variety of environments and face several kinds of stresses. The stresses may be physical (temperature stress) or chemical (superoxide stress, anoxic stress, pH stress). As a result of cellular stresses, cellular proteins start to become unfolded or misfolded (Agutter, 2011). To maintain proper functioning, proper folding of the cellular misfolded or unfolded proteins is essential and to do this is a grand challenge of nature. (Mukhopadhyay and Bairagya, 2011) Cell has its own intrinsic mechanism to ensure the proper folding of misfolded or unfolded proteins (Bagchi and Ghosh, 2011; Mittal and Acharya, 2013). Cellular stresses are frequent and recurring which challenge the cells to elicit anti-stress response to combat it (Kitagawa et al., 2000). The anti-stress response mechanism involves a variety of molecules such as folding chaperones, Holdase, Disaggregases and Proteases whose level elevates at stressed condition. Most of the cellular proteins denature, aggregate and damage at elevated temperature. The folding chaperones maintain the proper native folding state of the misfolded or unfolded proteins, Holdases prevent the misfolded protein and Disaggregases prevent disaggregation whereas Proteases eliminate the damaged proteins. Thus, these anti-stress molecules comply with each other to maintain the cellular protein quality (Jiao et al., 2008).

Small heat −shock proteins (sHsps) belong to the families of chaperones with low molecular weight (12–30 kDa) so they are considered as molecular chaperones. Small heat shock proteins prevent misfolding or aggregation of stressed protein and then send it to folding chaperones to maintain the native state of the stressed protein. Two members of small heat shock family, IbpA and IbpB are initially found as inclusion body protein but later it is found that they show increased expression at heat-stressed condition and prevent heat-stressed proteins from being misfolded, so they usually function as holdase. IbpA and IbpB with 48% sequence similarity at amino acid level, have characteristic α-crystalin domain flanked N- and C- termini (Strózecka et al., 2012). Upon stress, the N and C termini of these two proteins interact with each other and oligomerize to induce anti-stress response or chaperone activity. The α-crystalin domain is highly conserved throughout the evolution. This conserved domain is recognized by protease and by interacting with this domain; the protease can initiate the proteolytic degradation of small heat shock proteins in absence of stress i.e. when IbpA & IbpB remain in their free form.

Lon is the homo-oligomeric ATP- dependent principal protease in *Escherichia coli* (Bissonnette et al., 2010) which is conserved throughout the course of evolution. It comprises of a protease

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domain and an ATPase domain. Homohexamers of Lon constitute an internal chamber which promotes degradation of misfolded as well as damaged proteins (Vieux et al., 2013). It is reported that IbpA and IbpB are substrates of Lon protease in their free form at physiological temperature when there is no cellular stress which suggest that there is an interrelation between sHsps and protein degradation machinery (Bissonnette et al., 2010). It is observed and reported that, within the proteolytic domain, Lon employs a unique conserved Ser^{679}–Lys^{722} dyad in active site to promote catalysis (Botos et al., 2004). This catalytic pocket of the protease domain with Ser-Lys dyad plays an important role in substrate binding.

In our previous work, the pattern of interactions between IbpA, IbpB and Lon protease is observed at physiological as well as cold and heat-shock temperature and it is found that at physiological temperature, Lon interacts more favourably with IbpAB than those of the stressed temperatures (Bhattacharjee et al., 2015). In the present work, a comparison at physiological temperature has been made to identify the pattern of interactions between IbpA and IbpB with wild type as well as mutant Lon protease to come up with a plausible model of molecular mechanism between sHsps and protein degradation system. So far this is the first report to predict and to compare the interaction schemes between these proteins.

2. Materials and methods

2.1. Sequence homology search and template selection for modeling of the proteins

The amino acid sequences of IbpA, IbpB and Lon protease (UniProt Accession ID: P0C054, P0C058 and P0A9M0 respectively) and their basic information such as sequence length, molecular mass, amino acid modifications were collected from UniProt (http://www.uniprot.org/) (Leinonen et al., 2004).

The sequences were used to search for suitable templates in Protein Data Bank repository or PDB (www.rcsb.org/) (Berman, 2008) using NCBI Position Specific Iteration-Basic Local Alignment Search Tool (PSI-BLAST) (Camacho et al., 2009). For scoring parameters BLOSUM62 matrix is used with the gap cost Existence: 11 Extension: 1. The PSI-BLAST threshold is 0.005. In case of both the IbpA and IbpB, the sequence similarity of the obtained PDB templates from BLAST search are less than 30 percent as there was no crystal structure of IbpA and IbpB of *Escherichia coli* in PDB. So, the FASTA sequences of IbpA and IbpB are deposited in RaptorX (raptorx.uchicago.edu/) (Källberg et al., 2012) modelling tools which generated automated models of the proteins. In case of IbpA, RaptorX selected the Heat shock protein 16.0 from *Schizosaccharomyces pombe* (PDB ID 3W1Z) (Hanazono et al., 2013) as best template from the database with the highest value of significance. To study the mode of interactions, as the entire structure of these proteins are necessary, these modelling servers are able to build the entire model of the protein. The generated model of IbpA by RaptorX shows an uGDT score of 89, a *p*-value of 1.17e-06 and no disordered regions. The modelled protein with high *p*-value and uGDT score is likely to be a good quality model, so this parameter values indicate that the generated model is a good quality model. In case of IbpB, to generate the model of IbpB protein RaptorX selected the crystal structure of eukaryotic small heat shock protein (PDB ID 1GME) (van Montfort et al., 2001) as best template from the database with the highest value of significance. The generated model of IbpB by RaptorX shows an uGDT score of 88, a *p*-value of 1.44e-06 and no disordered regions. The modelled protein with high *p*-value and uGDT score is likely to be a good quality model, so this parameter values indicate that the generated model is a good quality model.To authenticate the generated model secondary structure prediction is also done using PSIPRED secondary structure prediction server. For the proteolytic activity, the α-crystallin domain residues are important because Lon protease recognizes this α-crystallin domain and promote proteolysis. It is predicted by PSIPRED Version 3.3 (van der Lee et al., 2014) that the α-crystallin domain of IbpA and IbpB spans from amino acid residue 35 to amino acid residue 120 with seven β- sheets and the generated model is also showed the similar secondary structure. So the generated model significantly matches with the predicted structure (Fig. 1).

In case of Lon protease, the crystal structure of the catalytic domain of Lon protease was retrieved from PDB (PDB code: 1RRE) (Botos et al., 2004) that was found as a significant match to the target sequence of Lon protease. The crystal structure of Lon was considered as final structure. The template–target relationship was reconfirmed by multiple sequence alignment between template proteins and query sequences via Clustal X (Larkin et al., 2007). The stereo-chemical qualities and the conformations of the resultant structures of IbpA and IbpB as well as the crystal structure of Lon protease were checked with PROCHECK (Laskowski et al., 1996) and Verify3D (Lüthy et al., 1992) through Structural Analysis and Verification Server (SAVES) at http://nihserver.mbi.ucla.edu/SAVES/and Ramachandran plots were drawn. There were no residues present in the disallowed regions of Ramachandran plots. The Verify3D score of IbpA, IbpB and Lon protease pass the value of 1D-3D profile.

2.2. Site-directed mutagenesis of Lon protease

It is reported that if the catalytic dyad residue Lysine722 is mutated to Glutamine i.e. mutation K722Q, then it leads to total loss of proteolytic activity (Botos et al., 2004). So, site-directed mutagenesis was performed in Discovery Studio version 2.5 to mutate Lon protease at that particular position. The generated mutated structure was energy minimized and the stereo-chemical properties were tested with PROCHECK and Verify3D through SAVES.

2.3. Molecular docking with IbpA, IbpB and Lon protease

The models of IbpA and IbpB were docked using Zdock server at http://zdock.umassmed.edu (Pierce et al., 2014). ZDOCK is a rigid-body docking algorithm. It uses Fast Fourier Transform (FFT) method for an exhaustive six-dimensional grid-based search in the translational and rotational space between the two molecules. ZDOCK performs the search for orientation space by keeping the receptor protein fixed in space and rotating the ligand around its geometric centre. The best 10 docked complex which showed best docking score was selected and then was checked with PROCHECK and Verify3D. The docked complex with highest value of PROCHECK and Verify 3D was selected as final working model.

Next, the above mentioned docking procedures were again performed to dock the IbpAB docked complex with the crystal structure of wild type Lon protease as well as mutant Lon protease separately in Z-Dock protein–protein docking server. Again top 10 docked complexes with best docking scores are selected and in each of these docked complexes are minimized and in each case, the binding free energy and interaction energy of these docked complexes are calculated. The stereo-chemical properties of this docked complex were tested using PROCHECK and Verify3D. The docked complex with least energy scores and best PROCHECK and Verify3D score are selected as final docked complex (IbpAB-Lon protease) (Tables 1 and 2).

2.4. Molecular dynamics simulation of the IbpA-IbpB-Lon protease docked complex

In total, two systems (one wild type docked complex and another mutant type docked complex) were prepared for sim-