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A structural insight into the prokaryotic heat shock transcription regulatory protein σ^{32}: an implication of σ^{32}-DnaK interaction

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Abstract:
The heat shock response mechanism is a very vital biochemical process and is mainly controlled by σ^{32} protein. The function of σ^{32} is temperature dependent and at lower temperatures σ^{32} is inactivated by its interactions with DnaK. This interaction is completely abolished above 42°C till date no molecular details of the interactions are available. In the present scenario, an attempt has been made to analyze first the predicted structure of σ^{32} obtained by comparative modeling techniques and then to study the interactions between σ^{32} and DnaK. From this molecular modeling study we could specifically identify the binding sites of the interactions of σ^{32} with DnaK which will enlighten the mechanism of regulation of its activity and stability by DnaK. Our study provides the idea for future mutational experiments in order to find out the possible roles of the amino acids of region2 and region3 of σ^{32} in stability as well as in binding with DnaK.

Keywords: Heat Shock Protein, Protein-Protein Interaction, σ^{32}, DnaK, Docking, Molecular Dynamics Simulations

Background:
The proper conformation of proteins and hence cellular survival is challenged by stress conditions like extreme heat which results in a massive aggregation of proteins inside both eukaryotic and prokaryotic cells. This response called the 'heat shock response' leads to the induction of almost all the universally conserved 'heat shock genes' which encode chaperones, proteases and other stress related proteins. In *E. coli*, this regulation is mediated by *rpoH* gene product, the alternative sigma factor σ^{32}. Its intracellular level is low and increases transiently after temperature up-shift **[1].** The cellular concentration of σ^{32} is tightly controlled at four different levels: transcription and translation of the *rpoH* and activity and stability of σ^{32} protein. Heat induction of σ^{32} mainly occurs at the post transcriptional level. An extended secondary structure in the *rpoH* transcript blocks translation at low temperatures **[2, 3].** Thermal melting of that structure permits ribosome entry followed by translation initiation. Once produced the fate of σ^{32} is determined by its interaction with a number of other proteins including chaperones as DnaK, DnaJ, GrpE and GroEL/ES and proteases as ClpP family, HslUV, Lon and FtsH **[4, 5].**

Under non-stress conditions, σ^{32} is neutralized by an interaction with DnaK and DnaJ proteins. This interaction serves two regulatory functions. It inactivates σ^{32} by preventing it from interaction with the RNA polymerase core enzyme and renders it susceptible to FtsH-mediated degradation **[5, 6]** (half-life of σ^{32} is < 1min) as over expression of σ^{32} is toxic **[7].** Accumulation of unfolded proteins upon heat stress conditions titrates away the DnaK system, leaving behind free σ^{32}, which associates with RNA polymerase and in turn initiates transcription of heat shock genes. Accumulation of σ^{32} only occurs in the initial phase (induction phase) of the heat shock response where the levels and half-life of σ^{32} increase transiently **[1, 8].** Elevated

temperatures introduce a conformational change in σ32 which specifically abolishes interaction with DnaK **[9].** It is assumed that the amino acid residues 190-205 in σ32 are responsible for σ32- specific function and become disordered at higher temperatures **[9].** The structurally altered sigma factor is rapidly turned over by cellular proteases. As a consequence, the cellular level of σ32 decreases and the heat shock response is shut off. Several experiments for studying the dynamical properties as fluorescence resonance energy transfer, fluorescence anisotropy measurements and hydrogen / deuterium exchange have suggested significant structural flexibility in the core of σ32 **[10].** This property is probably the main hindrance for the formation of good crystals and hence high resolution crystallographic 3D structure of the protein is still unavailable. As a result many structural and functional properties of σ32, its interactions with the chaperones as well as with the proteases are still obscure. In the present scenario an attempt has been made to analyze the structural biochemistry of σ32 protein along with its interactions with DnaK. We report a three dimensional model of σ32 built by homology modeling. We have docked the 3D structure of DnaK with the homology model of σ32. This docked model has been used to *illuminate* structural insight of the mechanism by which DnaK interacts with σ32 and regulate its activity and stability.

Methodology:

The amino acid sequence of σ32 protein from *E. coli* K-12 was collected from Uniprot (accession numbers P0AGB3). The amino acid sequence of the σ32 was used to search Brookhaven Protein Data Bank (PDB) **[11]** for suitable template(s) to build homology model using the BLAST software tool **[12].** The BLAST search picked up the crystal structure of *T. thermophilus* RNA polymerase holoenzyme (PDB Code: 2A6H F chain) as the template with 40% sequence identity with σ32 from *E. coli* K-12. Modeler program in the Discovery Studio 2.5 Platform of the Accelrys was used to model the three dimensional structure of the σ32 using the above template. The model of σ32 protein was then subjected to energy minimization using CHARMM force fields **[13]** using steepest descent (SD) algorithm. The stereo-chemical qualities of the three dimensional models were then checked using PROCHECK **[14],** and ERRAT **[15]** which predicts it as a model of good quality and no residues were found to be present in the disallowed regions of the Ramachandran Plot **[16].** In order to build σ32 –DnaK complex, the modeled structure of σ32 was docked with the crystal structure of DnaK protein (PDB Code: 1DKX) using the program GRAMM **[17].** GRAMM produced seven different models of the σ32 –DnaK protein complex. Among them the best structure of the complex was chosen on the basis of the biological relevance as present in literature **[10].** The model of σ32 –DnaK protein complex was then protonated at pH 7.5 using Accelrys Discovery Studio 2.5 and then subjected to 2000 cycles of energy minimization using CHARMM force fields with steepest descent (SD) algorithm until the structure of the σ32 –DnaK protein complex reached the final energy derivative of 0.001 kcal / mole. The stereo-chemical qualities of the docked protein complex were again checked using PROCHECK and ERRAT and results were same as before. The system was solvated with water molecules. Then molecular dynamics (MD) simulations were performed on the docked structure to predict the favorable binding interactions between σ32 and DnaK. Initially the dynamics run was kept constant at 303K (*30°C*). The σ32 –DnaK complex was then heated until *it* reached the temperature 316K (43°C). The same process has been followed for the modeled σ32 protein (i.e., before docking to DnaK protein) at 303K and 316K temperatures. The final structures of σ32 proteins as well as those of σ32 –DnaK complex at 303K and after heating at 316K were then analyzed to find out the possible modes of binding. The two structures were superimposed using 'align and superimpose' module of Discovery Studio 2.5 platform to find out the structural changes of σ32 due to temperature up shift.

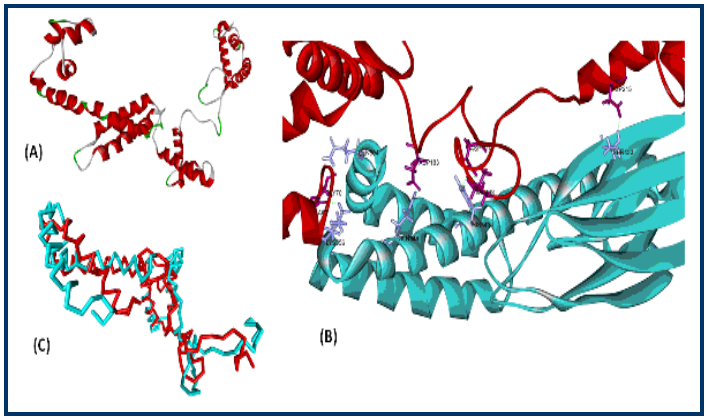

Figure 1: (A) Three dimensional ribbon representation of prokaryotic transcription regulatory protein σ32. Helices are presented in red. The remaining are loops; **(B)** Hydrogen bonding interactions between DnaK and σ32. DnaK is colored in cyan and σ^{32} in red; **(C)** Superimposition of the backbone atoms of the binding interface of DnaK docked σ32 at 32°C (red) and 43°C (cyan).

Discussion:

Structure of sigma32

The σ32 protein from *E.coli* is an all alpha protein with 284 amino acid residues. The protein has a total of thirteen helices **(Figure 1 A).** Structurally the protein can be considered to have four domains. All the domains are made up of helices connected together by loop regions. Overall the protein has similar structural arrangements of its secondary structural elements as in the protein RNA polymerase holoenzyme from *T. thermophilus* (PDB ID: 2A6H_F chain).

Structural changes of sigma32 with temperature

It has been a well established fact that in *E. coli* upon temperature increase from normal to heat shock temperature, cellular level of σ32 protein increases **[8].** Till date no detailed analysis of the conformational changes of σ32 at elevated temperature has been elucidated. In order to account for the loss of σ32 specific function from a structural perspective, the model of the σ32 protein was stepwise heated from 32°C to beyond 32°C. Initially no structural changes were observed but at around 43°C the spatial arrangement of the protein were found to be significantly altered as measured by the r.m.s.d values (10.696 Å) of the backbone atoms of the proteins at these two different temperatures. This clearly indicates a huge change in the conformation of the σ32 protein at elevated temperature.

Interactions between sigma32 and DnaK

The three dimensional coordinates of the σ32 -DnaK protein complex have been generated using molecular docking technique with the help of the software tool GRAMM. When σ32

interacts with DnaK no significant changes occur in the σ32 structure (r.m.s.d. between σ32 in σ32 -DnaK complex and σ32 alone 0.2Å). Analysis of σ32 - DnaK protein complex shows that the interactions between the proteins have been stabilized mainly by H-bonds involving the side chain atoms of the proteins. The residues involved in interactions are shown in **Table 1 (see supplementary material)** & **(Figure 1 B)**. Interestingly, it has been observed that all the amino acid residues from σ32, involved in binding DnaK fall in the region2 and region3 of σ32. Though the aforementioned regions have previously been predicted to be involved in binding with DnaK and responsible for σ32 specific functions **[9, 10],** the molecular details of the binding have not yet been established. *This report* elucidates the structural details of σ32 as well as the interactions between σ32 and DnaK. Heating σ32 -DnaK protein complex beyond 42°C, destroys all six H-bond and results in total loss of interaction between σ32 and DnaK. This is because of the loss of structural arrangements in the binding interface of the σ32 protein. This is exemplified by r.m.s.d value (8.276Å) of the backbone atoms of σ32 protein above 43°C **(Figure 1 C).** This might play a major role in decreasing the degradation rate of σ32. Major structural change has been identified to occur in the binding interface of σ32 -DnaK protein complex. This result is consistent to earlier studies by Chattopadhyay *et al.* 2002, which reports that this abolition of σ32-DnaK interaction is mainly due to the structural changes of σ32 at heat shock temperature.

Conclusion:

In this paper an attempt has been made to analyze the probable molecular details of the interactions of σ32 with DnaK. The three dimensional structure of σ32 has been predicted using the homology modeling technique. The functions of σ32 are dependent on temperature. Therefore, the modeled structure of σ32 has been heated from 32°C to 43°C. It revealed a huge structural change beyond 42°C. The σ32 is known to bind DnaK. In order to elucidate the mode of binding of σ32 with DnaK, the three dimensional coordinates of the σ32 and DnaK have been used to dock the two proteins together by molecular docking. The interaction scheme revealed that the *role* of amino acid residues from the region 2 and region 3 of σ32. This report deals with the detailed molecular biochemistry of temperature dependence of σ32 structure and σ32 –DnaK interactions. Therefore our study *will be* useful for future genetic studies to elucidate the roles of the amino acid residues in the protein-protein interactions for the heat shock response.

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Supplementary material:

Table 1: H-Bond interactions between σ^{32} and DnaK (A: σ^{32}, B: DnaK)

H-Bond	Donor Atom	Acceptor Atom	Distance (Å)
B:SER423:HN - A:ASP215:OD2	HN	OD2	1.93552
B:HIS541:HD1 - A:ASP191:OD2	HD1	OD2	2.31651
B:HIS541:HE2 - A:VAL198:O	HE2	O	1.95011
B:GLN549:HE22 - A:ASP183:OD1	HE22	OD1	1.92394
B:LYS556:HZ1 - A:ALA69:O	HZ1	O	1.95603
B:GLN604:HE21 - A:GLY70:O	HE21	O	2.07719