

Prediction of three-dimensional structure of *Chlamydia trachomatis* heat shock protein 60: An immunodominant antigen.

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Citation

R Jha, H Jha, S Biswas, A Mittal. *Prediction of three-dimensional structure of Chlamydia trachomatis heat shock protein 60: An immunodominant antigen.* The Internet Journal of Genomics and Proteomics. 2008 Volume 4 Number 2.

Abstract

Chlamydia trachomatis infections are the most prevalent sexually transmitted infection in the world and are often associated with various fertility disorders. *C. trachomatis* heat shock protein 60 (cHSP60) or GroEL has been identified as an immunodominant antigen, being strong target of both humoral and cell mediated immune responses. A homology modeling was used for the prediction of three-dimensional (3-D) structure of cHSP60 by using the comparative modeling module of MODELLER. Since the MODELLER-aligned alignments led to asymmetric matching, the necessary sequence alignments were done using ClustalW and mGenTHREADER. The template used was the file with code 1kp8 from the Protein Data Bank (PDB), which gives the crystal structure of GroEL of *Escherichia coli*. The structure was energy minimized by MODELLER's inherent minimization module. From the Ramachandran plot analysis, it was found that 91.8% of residues falls in the most favoured region. Further, this study could shed light on characterizing cHSP60 in wet laboratory.

INTRODUCTION

Chlamydia trachomatis infections are the most prevalent sexually transmitted disease recognized throughout the world. According to World Health Organization (WHO), annually 92 million *C. trachomatis* infections are detected worldwide, of which 43 million cases are reported from South East Asia (WHO, 2001). Up to 80% of women with genital chlamydial infection are asymptomatic and even upper genital tract infection is often clinically silent (Gaydos et al., 1998). Untreated chlamydial infection of lower genital tract can lead to pelvic inflammatory disease (PID) in 10-40% of affected women, which can result in infertility, ectopic pregnancy and chronic pelvic pain (Patric et al., 1997). It is also reported that urogenital infection with *C. trachomatis* may lead to development of an acute inflammatory arthritis (Inman et al., 2000).

Heat shock proteins (HSPs) are highly conserved proteins present in almost all prokaryotic and eukaryotic organisms. The 60-KDa HSPs have been extensively studied for their chaperone function in protein folding and their cooperation with other chaperones in cellular trafficking. More than 150 homologues of HSP60 sequence are currently available with pair-wise similarity at the amino-acid level extending from

40 to 100% (Brocchieri et al., 2000). In Chlamydiae, the 60-KDa (cHSP60) or GroEL is encoded by genes arranged on the bicistronic groESL operon (Morrison et al., 1990). In a panel of 116 recombinant *C. trachomatis* proteins, cHSP60 (Ct110) has recently been identified as an immunodominant antigen, being a strong target of both humoral and cell-mediated immune responses (Follman et al., 2008). In addition, cHSP60 is highly homologous and conserved throughout the evolution. It belongs to a group of chaperone proteins ranging in molecular weight from 15 to 110 KDa which are produced in response to infection, stress, inflammation and other cellular insults (Engel et al., 1990). cHSP60 exhibit greater than 80% homology between *Chlamydia* spp., 60% identity with other bacteria and 50% homology with human HSP60 (hHSP60) and exhibits immuno-regulatory properties primarily by inducing proinflammatory response (Zugel et al., 1999). Recently, Habich et. al. reported distinct epitopes responsible for binding to innate immune cells mediating the pro-inflammatory effects of hHSP60 (Habich et al., 2007).

Persistent chlamydial infection has been reported to cause chronic stimulation of host immune system against immunogenic antigen such as cHSP60 (Hessel et al., 2001).

Numerous studies have shown a consistent immunopathogenic association between antibody response to the cHSP60 and the development of pelvic inflammatory disease (PID), ectopic pregnancy and tubal infertility (Tiitinen et al., 2006; Hartog et al., 2005; Clad et al., 2003). Also, presence of circulating antibodies to a conserved epitope of the cHSP60 is associated with a lower spontaneous conception rate, and increased likelihood of adverse pregnancy outcome in women treated by salpingectomy for the first episode of ectopic pregnancy (Sziller et al., 2008). We previously reported high seroprevalence of anti-cHSP60 in *C. trachomatis* infected women (Dutta et al., 2007) and that detection of anti-cHSP60 antibodies would help in the early prognosis of immunopathological sequelae in *C. trachomatis* infected women (Dutta et al., 2008). In addition, we evaluated cHSP60-specific proliferative responses and our study suggested the probable role of cHSPs in modulation of mucosal immune responses (Agrawal et al., 2007; Srivastava et al., 2008). Recently, we showed higher level of cHSP60 in infertile women than in fertile women in cervical epithelial cells from *C. trachomatis* infected site (Jha et al., 2009).

Till now 3-D structure is not available for cHSP60 which is important since homology modeling has become a useful tool to understand the particular protein. Although several crystal structures of GroEL from other organisms are available and two of them show GroEL in its 14 unit complex, one associated with ATPiS (Boisvert et al., 1996) and a second in an asymmetrical conformation bound to GroES and ADP (Xu et al., 1997). These authors have further reported seven identical GroEL monomers assembled in rings that dimerize in a 14-meric structure with an equatorial interface formed by the E Domains and two non-communicating central cavities. Each Domain E also provides a binding pocket for an ATP/ADP molecule and a Mg⁺ ion. Therefore, the present study was undertaken for predicting 3-D structure model of cHSP60, which is required for identifying major target epitopes for wet lab and also for designing new drugs.

MATERIAL AND METHODS

Retrieval of target sequence: The amino acid sequence of cHSP60 (AAC67701.1), hHSP60 (ABB01006.1) and *E. coli* HSP60 (ABF67773.1) was obtained from sequence database of NCBI (<http://www.ncbi.nlm.nih.gov>). It was ascertained that 3-D structure of cHSP60 is not available in Protein Data Bank (<http://www.rcsb.org/pdb/home.do>), hence the present

exercise of developing the 3-D model of cHSP60 was ascertained. The cHSP60 is 543 amino acid in length and having molecular weight of 58 KDa.

Template searching: A suitable template for modeling of target protein was searched through mGenTHREADER (McGuffin et al., 2003), which is an online tool for searching similar sequences. From the homology searching high resolution of X-ray crystallography structure of the *Escherichia coli* [1kp8] was selected as template protein from protein data bank (Wang et al., 2003). Of the 14 chains, only chain A was used for modeling.

Alignment of Sequence: Amino acid sequence of target and template protein was done using MODELLER. Since the MODELLER-aligned alignments led to asymmetric matching, the necessary sequence alignments were done using ClustalW (<http://www.ebi.ac.uk/Tools/clustalw2/>) (Thompson et al., 1994).

3-D structure modeling: A rough 3-D model of cHSP60 was constructed from the sequence alignment between cHSP60 and template proteins using Modeller 8v0 (Marti-Renom et al., 2000). The structure was energy minimized by MODELLER's inherent minimization module. In the last stage of modeling the structure was evaluated by the inspection of the Psi/Phi Ramachandran plot obtained from PROCHECK (<http://biotech.ebi.ac.uk:8400/cgi-bin/sendquery>) (Laskowsky et al., 1993).

RESULTS AND DISCUSSION

The *Chlamydia* genome database (<http://chlamydia-www.berkeley.edu>) containing both nucleotide and amino acid sequences are available. The retrieved amino acid sequence of cHSP60 from *Chlamydia* genome database and NCBI showed high homology with sequence of *E. coli* GroEL. There are two other ortholog of cHSP60 protein copies in *Chlamydiae* species have diverged functionally after the gene duplication events (McNally et al., 2007) but cHSP60 was found more abundant and immunodominant than the other two. The transcription of cHSP60 gene in *C. trachomatis* has been shown to be immediately induced in infected cells exposed to higher temperature at 45°C (Karunakaran et al., 2003), however, its specific function both under stress and normal conditions remains unclear. We therefore undertook bioinformatics analysis of 3-D structure that will help in understanding chlamydial disease pathogenesis.

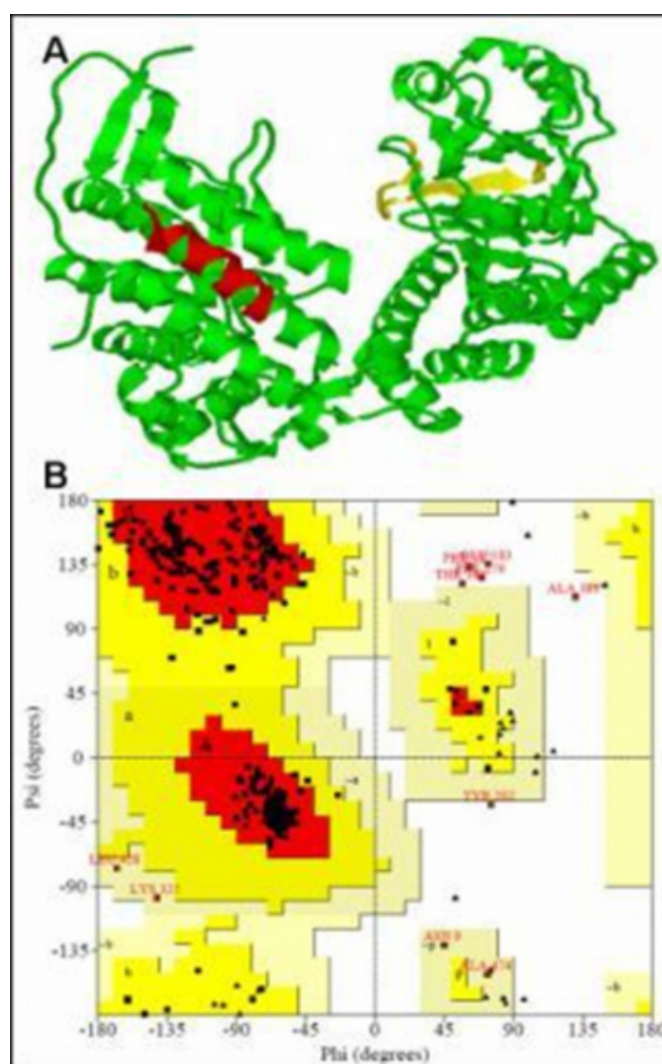
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Homology modeling of proteins has been successful in gaining better understanding of protein function and intermolecular interactions. Manual methods have been successful but, with the advent of genomic sequences database and the significant increase in data, more efficient methods are needed. Therefore, homology modeling was used to produce best model of unknown structure by comparing it with a known template from structurally highly similar molecule. In the multiple alignments of HSP60 proteins, the most conserved residue is glycine (G) (Fig. 3). Brocchieri et. al. earlier reported that sequence positions essential to protein function and structure are conserved over a broad evolutionary range. Freely variable regions of a protein family are generally non functional. It is well recognized that G residues can be a “hinge” for backbone conformations that are generally excluded for other side chains. Conserved G multiplets are also observed in other HSP families, like HSP70 (Karlin et al., 1998) or RecA (Brendel et al., 1997). The Ramachandran plot of all perfectly conserved G residues reveals that many of them belong to backbone conformations or to hinge connections relevant to structural reconfiguration (Fig. 1).

In our study based on the results obtained from mGenTHREADER and ClustalW, the chain-A of X-ray structure of the *E. coli* GroEL were selected as template which was 61% identical with cHSP60. The MODELLER was used for building the model and was further examined and validated using PROCHECK. Further, the total energy values of the predicted 3-D model were calculated as 91.8% of Ramachandran plot (Fig. 1) and the refined model was analyzed by different protein analysis programs including PROCHECK for the evaluation of the Ramachandran plot quality, and WHATIF (Vriend et al., 1990) for the calculation of packing quality. This structure for the corresponding coordinates was found to be satisfactory. The residue properties of cHSP60 indicates that 3-D structure possess 22 α -helix and 18 β -strand (Fig. 2). One of the epitopes (¹⁵¹SANNDAEIGNLI¹⁶²) in the structure falls in the accessible region. Our earlier report suggests that detection of antibodies to this epitope would help in early prognosis of immunopathological sequelae in *C. trachomatis* infected women and thereby instituting appropriate therapy for controlling infection at any stage (Dutta et al., 2008).

Figure 1

Figure 1: (A) Predicted 3-D structure of cHSP60. (B) Ramachandran plot analysis of predicted 3-D structure of HSP60. Based on analysis of 118 structures of resolution with at least 2 Å... and R factor no greater than 20%. A good quality model would be expected to have over 90% in the most favored regions. The Plot statistics are: Residues in most favored regions [A,B,L]- 438 (91.8%); residues in additional allowed regions [a,b,l,p] - 29 (6.1%); residues in generously allowed regions [-a,-b,-l,-p] - 4 (0.8%); residues in disallowed regions \hat{A} - 6 (1.3 %); number of non-glycine and non-proline residues- 477 (100.0%); number of end residues (excl. Gly and Pro) - 2; number of glycine residues (shown as triangles) - 45; number of proline residues - 17; total number of residues- 541.



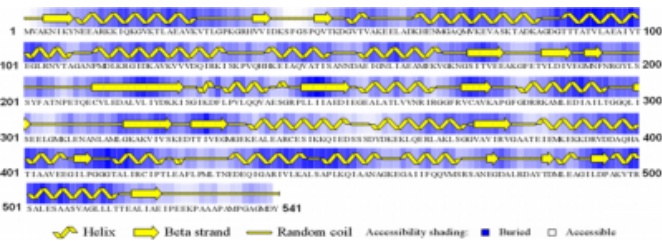
Since, cHSPs are phylogenetically conserved and have high sequence homology, the immune response against epitopes within the region of cHSP60 may elicit an autoimmune response due to cross-reactivity to epitopes of the hHSP60. Also, the homology between bacterial and hHSP60 and the fact that hHSP60 may be increased locally at the site of

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infection may indicate a role for HSP60 autoimmunity in the immunopathogenicity of chlamydial infections (Jones et al., 1993). Further, Yi et. al. reported that seven of 13 B-cell peptide epitopes recognized by human anti-sera exhibited cross-reactive antibody binding to homologous peptide sequences in hHSP60. Such self-reactive B-cell immunity to HSP60 may contribute to chlamydial disease pathogenesis (Yi et al., 1993). Similarly, Domeika et al. examined the association between humoral immunity to unique and conserved epitopes of the cHSP60 and immunity to hHSP60. They have demonstrated that sensitization to hHSP60 is associated with humoral immune response to a conserved epitope of the cHSP60 in patients with PID and also there was serologic evidence of exposure to chlamydiae. This suggests that an autoimmune response to hHSP60 may arise as a consequence of a C.trachomatis upper genital tract infection in those women who develop sensitivity to cHSP60 epitopes that cross-react with epitopes of the hHSP60 (Domeika et al., 1998).

Figure 2

Figure 2: Amino-acid sequence of cHSP60: Secondary structural elements are indicated as α -Helix, β -strand and random coil. Blue (Buried) and white (accessible) regions in the sequence are shown as accessibility shading.



These observations were also supported by co-immunization with mouse HSP60 and cHSP60 inducing strong T and B-cell responses to self HSP60. In particular, T-cell proliferation in response to mouse HSP60 was dramatically increased upon co-immunization. The induction of strong autoimmune responses depended on the homology of amino acid sequences between mouse and cHSP60, since a strong response was not observed when a non-homologous but highly immunogenic protein such as ovalbumin was co-administered with mouse HSP60. The importance of sequence homology implicates cross reaction between the antigen recognition structures on T and/or B cells in the autoimmune response suggesting that molecular mimicry is the basis for autoimmunity induced by HSP60 (Lin et al., 1991). On the other hand, production of cHSP60 antibodies may also dominate over antibodies to another antigenic

component of chlamydiae or high degree of amino acid sequence homology between cHSP60 and other microbial HSP60 that may incite an autoimmune reaction through molecular mimicry (Bavoil et al., 1990). Further, immune responses generated after cross reaction may lead to mediate inflammation in lower and upper genital tract, a key event in the cervicitis, PID, ectopic pregnancy and infertility.

Recently, Kumar et al. implicated HSP90 from Plasmodium falciparum as potential drug target against malaria. Using inhibitor specific to nucleotide binding domain of HSP90, they have shown potent growth inhibitory effect on development of malarial parasite in human erythrocyte (Kumar et al., 2007). Thus, knowledge of 3-D structural details of cHSP60 would also greatly advance the development of drugs targeting this molecule. Further, analysis is required to found the differences between hHSP60 and cHSP60 may make it possible to design inhibitors specific against Chlamydia.

Figure 3

Figure 3: Multiple sequence alignment of HSP60 amino-acid residues of and . The *(star) in the sequence represents identical residues. (C.trachomatis, E.coli, H.sapientiae)



ACKNOWLEDGEMENTS

We acknowledge the Indian Council of Medical Research (ICMR) and University Grants Commission (UGC) for providing financial assistance to Rajneesh Jha, Hem Chandra Jha and Sumit Biswas in the form of a research fellowship. We also thank Mr. Harsh Vardhan, Mr. Rishein Gupta, Ms. Pragya Srivastava and Ms. Apurb Rashmi Bhengraj for his suggestions and critical reading of the manuscript.

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