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In Silico Prediction of the Tertiary Structure of *M. leprae* Hsp65 Protein Shows an Unusual Structure in Carboxy-terminal Region

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Abstract

DNA vaccines have been used with great success in experimental and some clinical therapy. However, the mechanisms of activation of the immune system by these vaccines are not utterly understood yet. Hsp65 is a *Mycobacterium leprae* chaperone whose gene has been efficiently used as experimental DNA vaccine against tuberculosis and clinical trial against tumor. Since little is know about the three-dimensional (3D) structure of hsp65 and modeling of 3D protein structure can increase the information to improve the knowledge about the mechanism action as well as the design of new DNA vaccine formulation, here we used the bioinformatics to get the design *in silico* of hsp65 (heat shock protein) molecule. The determination of hsp65 3D structure was obtained by homology using the software *Modeller* (Eswar et al., 2001). It was used two proteins as models: 1SJP, a 60-kDa chaperonin from *Mycobacterium tuberculosis* in the PDB, and the 1WE3, the crystal structure of the chaperonin complex Cpn60/Cpn10/(ADP)7 from *Thermus thermophilus*). Our results showed an interesting structure in Hsp65 that could be important in development or modulation of immune response.

Introduction

Stories surrounding tuberculosis (Tb) had been told even before Christ birth, but it was only in the XIX century that the *Mycobacterium tuberculosis* was known as the etiological agent of the Tb. After so long, the only prophylaxis against Tb is the BCG vaccine. Although this vaccine is one of the most widely used vaccines in the world, unfortunately it has a dramatically low efficacy in adult pulmonary tuberculosis (Brewer, 2000). Therefore, new strategies to protection are required, such as DNA vaccines.

DNA vaccines are usually used with great success in experimental medicine and some clinical therapies (Guranathan and Kinman, 2000). However, some mechanisms of immune system activation by these vaccines are not completely clear yet. The success of a new vaccine strategy, such as DNA vaccine, is dependent on the interaction between the; immune system and the antigen. The hsp65 DNA vaccine showed efficacy against experimental tuberculosis (Lowrie and Tascon, 1999) and squamous tumor (Michaluart et al., 2008). The hsp65 appears to play an important role in induction of the immune response. The ability of heat shock proteins (Hsps) to participate in innate and adaptive immune responses (Srivastava, 2000) could explain the Hsp65 action. In addition, information obtained by 3D structure may be a key point to improve the actual DNA vaccine, such as dose reduction and maintenance of B e T cells memory.

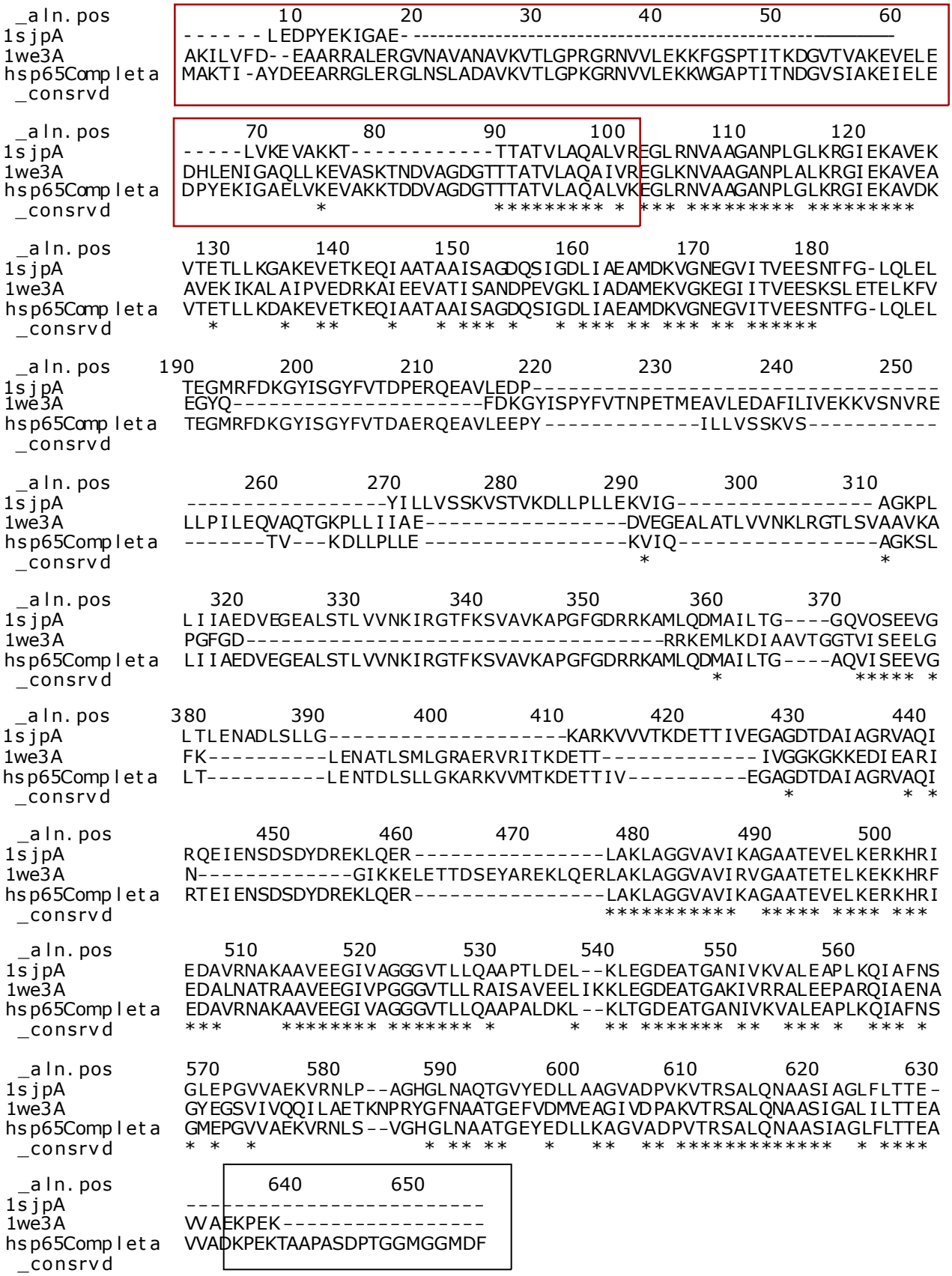

Figure 1: Multiple alignment with 1SJP, 1WE3 and the protein´s subject matter. The alignment was obtained from a Modeller´s script. In red is shown the N-terminal, and in blue the C-terminal.

Material and Methods

The sequence of hsp65 from *M. leprae* used to build the model was retrieved from the public database, Uniprot (www.uniprot.org), having as a corresponding code P09239, while the templates were retrieved from the Protein Data Bank (PDB).

Although the Hsp65 from *M. tuberculosis* (PDB code - 1SPJ) has a high similarity with your counterpart from *M. leprae* (over than 93%), the structure of the latter protein lacks in parameters in the N-terminal. When the *M. leprae* protein model was built the correspondent N-terminal part had a bad fold. To solve this issue, a multiple alignment was used. The other protein used was the chaperonin complex Cpn60/Cpn10/(ADP)7 from *Thermus thermophilus* (PDB code - 1WE3) that share 63% of similarity with Hsp65 from *M. leprae*.

As can be noticed in the multiple alignment, the part of the *M. leprae* Hsp65 that could not find a correspondent in the 1SJP protein, found great parameters in 1WE3 protein, solving the fold problem.

The validation was obtained by Procheck (Lakowski et al., 1993), Verify3D (Vriend and Sander , 1993) and Whatif (Bowie et al., 1991). These three softwares characterize the most important areas to determinate a good 3D model (Silva et al., 2008).

Procheck verifies parameters like Ramachandran plot quality, peptide bond planarity, bad nonbonded interactions, main chain hydrogen bond energy, C-alpha chirality and overall G factor and the side chain parameters.

Verify3D analyzes the compatibility of an atomic model (3D) with its own amino acid sequence (1D). Throughout a validated known score matrix built based in structures from PDB, Verify3d assigns the chemical environment of each residue.

Whatif checks the normality of the local environment of the amino acid. It was used to evaluate residues giving them a quality profile. These measures are calculated using the distribution of each side chain amino acid against validated known structures.

Results and Discussion

The first alignment analysis using ClustalW did not show homology between the Hsp65 from *M. leprae* and the Hsp65 from *M. tuberculosis* in the N-terminal region. As shown in the figure 1, there are many gaps in this region.

The N-teminal problem was solved by the multiple alignment analysis, but as shown in Figure 1, the carboxy-terminal region still had a bad alignment, not able to fold in any secondary structure. Other alignments were accomplished to obtain a better fold, but none of them were successful. Hence, further analysis was made.

Analyzing other proteins structures from the Hsp family, none of them have the carboxy-terminal unfolded as the hsp65 from *M. leprae*. Therefore, this analysis indicated a unique characteristic, revealing different properties of this protein. This interesting result can be a great point of study for future vaccine target. Moreover, the validation of the structure confirmed the carboxy-terminal unfolded structure. The importance of this region in hsp65 will be deter-

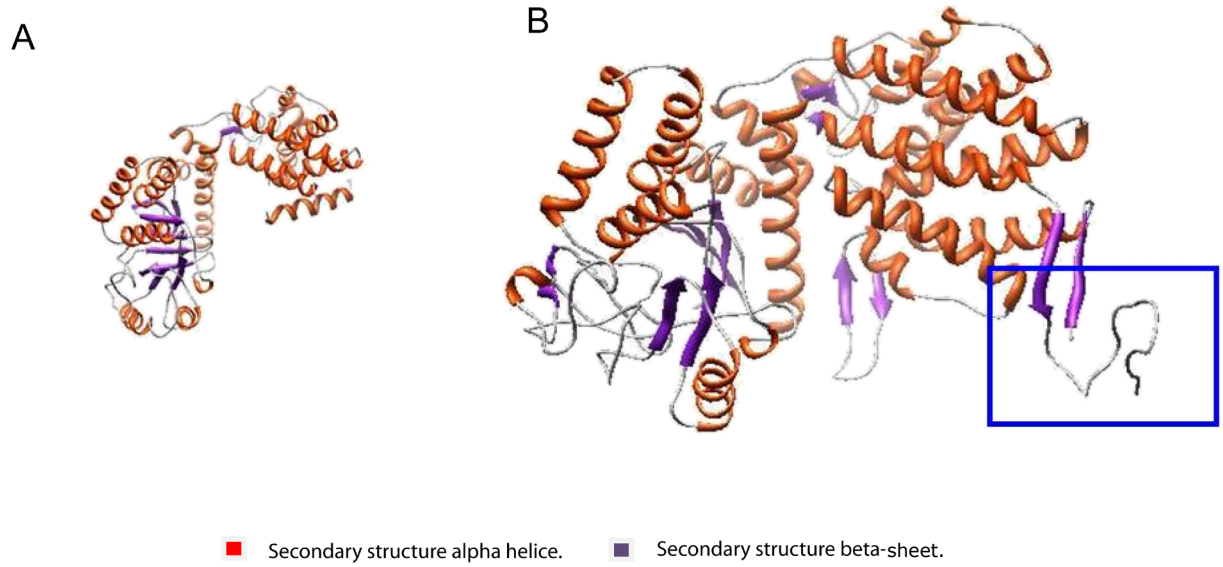

Figure 2: A) Structure of hsp65 from *M.tuberculosis* B) Predicted 3-dimentional struture of hsp65 from *M.leprae*. The blue quadrangle indicates the unfold region in the C-terminal of the protein.

mined by biological assays. The secondary structure of hsp65 from *M. tuberculosis* showed in Figure 2A did not present the unfolded region, as showed in other analyzed protein. Since this region is present in carboxy-terminal, we suggested that it is not involved with sorting or signal peptide. The 3D structure of these proteins is represented by cartoons and colored based on the secondary structure (Fig. 2).

To validation of hypothetical protein generated the accuracy of the protein model obtained, which was judged by PROCHECK analysis. Parameter comparisons of these proteins were made with well-refined structures that have similar resolution. The main output of PROCHECK is the Ramachandran plot (Fig. 3 e 4).

In the Ramachandran plot analysis, the residues were classified according to their regions in the quadrangle. The Ramachandran map for hsp65 is represented in the Figure 3 and the statistics of the residues are showed in Figure 4.

In the plot analysis can be noticed that more than 90% of the residues are in allowed regions, leading to a good validation for the model. The residues that are in bad quadrangles are a reflex from the protein used as template.

Analyses from Verify3D and Whatif have also confirmed a good validation of the model. The plot result from the Verify3D (Fig. 5) shows all residues in the allowed interval. Because the plot is very large, only the first residues of the protein will be showed. So it can be noticed that the part with difficulties to fold was solved. The Whatif output is constituted by indexes that indicated the quality of the contact of the residues. The overall index is -0.88, which corresponds to a good model. When it was analyzed the individual residue it was observed that some residues had a bad

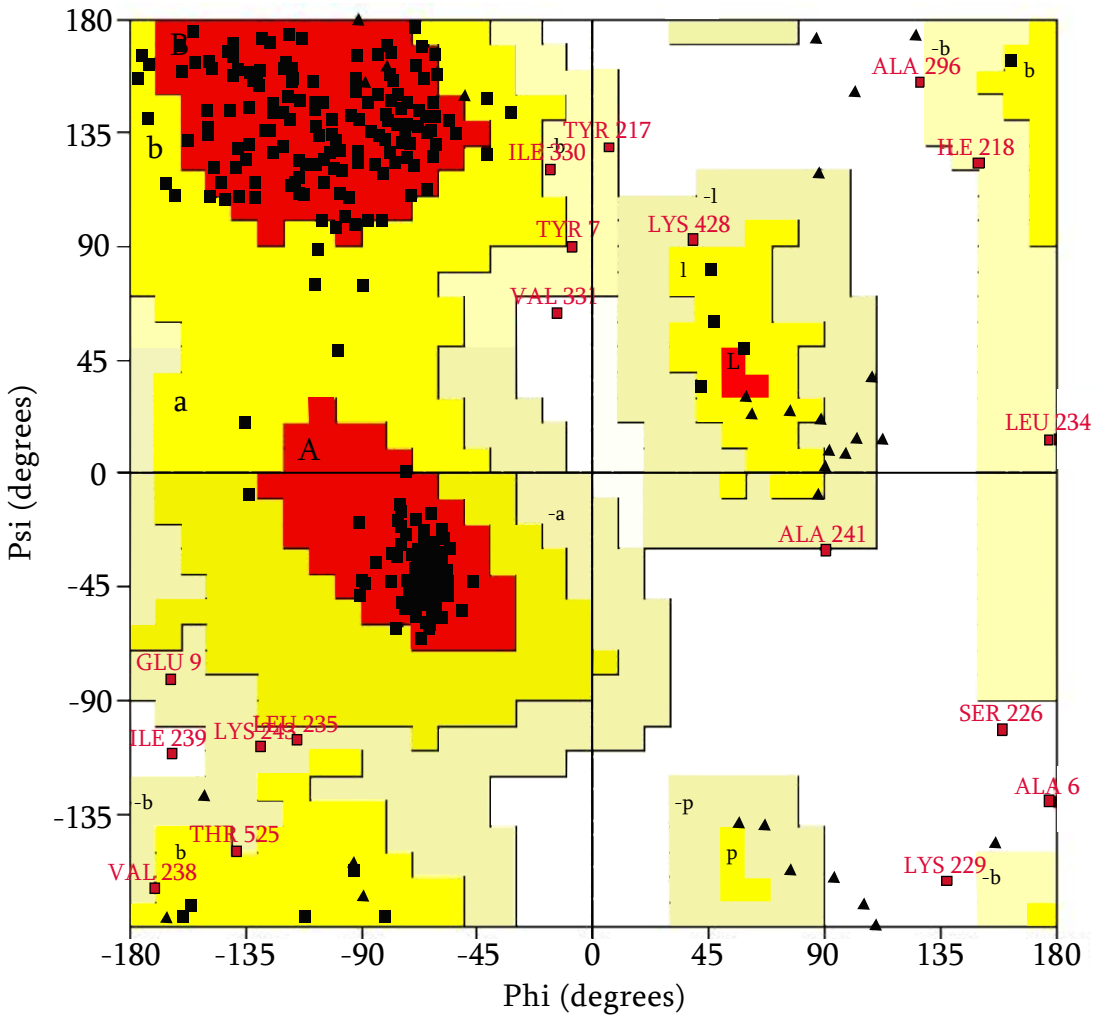

Figure 3: Ramachandran map of *M leprae* hsp65 protein. The Plot calculation was done with PROCHECK program. (Most favored regions - A, B, L; Additional allowed regions - a,b,l,p; Generously allowed region -~a,~b,~l,~p; Disallowed regions – in white).

Plot statistics

Residues in most favoured regions [A,B,L]	429	90.7%
Residues in additional allowed regions [a,b,l,p]	26	5.5%
Residues in generously allowed regions [~a,~b,~l,~p]	11	2.3%
Residues in disallowed regions	7	1.5%
Number of non-glycine and non-proline residues	473	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	52	
Number of proline residues	14	
Total number of residues	541	

Figure 4: Ramachandran map statistics of *M.leprae* hsp65 computed by PROCHECK.

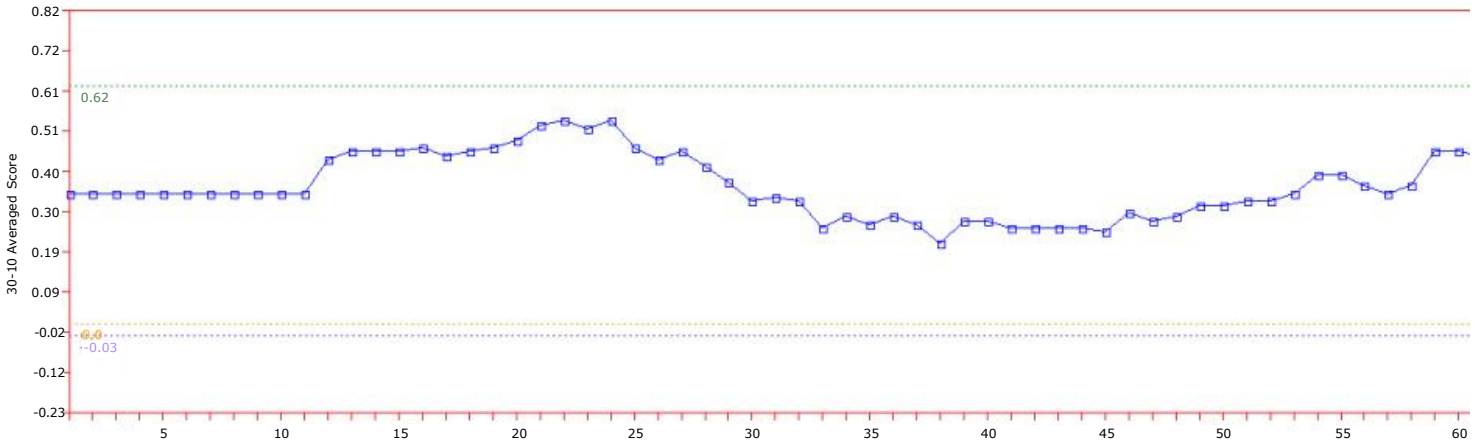

Figure 5: The interval between the scores 0.0 and 0,62 represents the area for a good validation. As shown the structure obtained represents a good model. The first ten residues are disregards as they are in the same score. The vertical axis represents the score and the horizontal axis the protein´s in the same score. The vertical axis represents the score and the horizontal axis the protein´s residues.

index, again a reflex from the template.

Taken together the *in silico* analysis of *M. leprae* Hsp65 protein showed a novel data about the protein structure. The unfold region can be important to development of a protective immune response. So, *in vitro* and *in vivo* analyses are essential to demonstrate the importance of this region during the degradation in eukaryotic cell to induce protective T and B cells. Additionally, this region could be involved with the catalytic activity of Hsp65 from *M. leprae* but not from *M. tuberculosis* (Portaro et al., 2002) or in the interaction with a putative receptor present in cells of the immune system (Srivastava, 2000).

The obtained results open new perspectives to biological or computational assays, emphasizing the importance of bioinformatics analyses to improve biological models.

Future Perspectives

The bioinformatics allowed us to design *in silico* the hsp65 3D structure. The obtained data showed an unusual structure present in carboxy-terminal region only of *M. leprae* hsp65 protein. This novel structure can result in new knowledge about biosynthesis in *M. leprae*, as well as to improve the use of hsp65 as an antigen in DNA vaccine. The future determination of immunogenic regions can reduce the size

of nucleic acid sequence that will be cloned, resulting in safety to this strategy.

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