

Structural Investigation of HSP70-HSP90 and HSP90-TDF Interactions

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Abstract

Tumor Differentiation Factor (TDF) is a pituitary protein, which is secreted into the blood stream and targets breast and prostate. The end effect of TDF on these tissues is differentiation of breast and prostate cells. However, it is not yet clear how TDF induces cell differentiation. Studies in our laboratory determined that the potential TDF receptor candidates are: HSPA8, a member of the 70 kDa heat shock protein family and HSP90 protein. Our previous studies also indicated that TDF may have an inducible receptor, composed of both HSP70 and HSP90, and that TDF signaling depends on the interaction of these proteins. Here we provide additional insights about the proposed interaction between HSP70 and HSP90 and about the HSP90-TDF interaction.

Keywords: Tumor differentiation factor; Protein-protein interactions; Molecular modeling

Introduction

Tumor differentiation factor (TDF) is a recently identified protein, produced in the brain. Work in our lab identified TDF in the brain, specifically in the pituitary, but also in other regions. TDF immunostaining specifically co-localized with markers specific for neurons, but not with markers specific to astrocytes [1].

The TDF protein produced by the pituitary is likely secreted in the blood stream and targets breast and prostate tissue [2]. *In vitro* work using proteomics suggested that the TDF receptor candidates are members of the heat shock family, specifically heat shock protein 70 (HSP70) and heat shock protein 90 (HSP90) [3-5].

Proteomics allows identification and characterization of proteins in large scale [6-10] and TDF receptor candidates (and TDF ligand) were identified by affinity chromatography followed by mass spectrometry [3-5,11]. Our earlier studies also indicated that TDF may have an inducible receptor, composed of HSP70 and HSP90 [3-5]. Furthermore, we also suggested that TDF signaling depends on the interaction of HSP70 and HSP90 proteins with TDF [12]. However, the function of TDF is still not understood and many of these studies still need additional theoretical and experimental confirmation. In addition, the TDF crystal structure has not been established, thus making us rely mostly on structural biology-based work [13]. Therefore, a better understanding of TDF requires additional studies. To further understand the function of TDF, we employed structural biology to investigate the interaction of HSP70, HSP90 and TDF. Here we provide additional insights about the possible interaction between TDF and its potential HSP70 and HSP90 receptors.

Methods

For protein interaction and docking experiments, we have taken the homology model of 3C7NB, a member of the HSP70 as a ligand protein [4] and HSP90-beta as receptor protein. The 3C7NB is the open, weakly ADP bound form [14]. For HSP90, we have selected the homology model based on gi20149594 and template crystal structure 2IOQ Chain B [15]. This model receptor protein is developed using Swiss model [16,17]. 2IOQ is an open form of HSP90.

Protein-protein docking was carried out using GRAMM-X Docking Web Server v.1.2.0 [18,19] and verified by Patch dock and Fire dock servers [20-23]. Descriptions of these docking experiments are described elsewhere [3-5]. For the TDF-HSP90 interaction, we used the same homology model of HSP90 (2IOQ.pdb) as receptor protein [15], but used the model TDF structure as a ligand protein [11,12]. This

model TDF protein was developed using I-Tasser server [24,25]. In our previous experiments, we have used TDF-P1 as a ligand [12].

To further substantiate we have performed another set of experiment using HSP90 model receptor based on template structure 2CG9B.PDB (Chain B) [26]. That part could be found in the supplemental materials (Figures S1-S6).

Results and Discussion

HSP70-HSP90 interaction

HSP70 proteins may interact with the proteins from the HSP90 family. Both proteins were also experimentally determined that interact with TDF. To investigate the details on TDF-HSP70-HSP90 interaction, we conducted docking experiments involving HSP70 and HSP90 proteins, and TDF-HSP90 proteins. HSP90 is composed of N Terminal Domain (NTD), Middle Domain (MD) and C Terminal Domain (CTD). HSP90 forms a dimer at the C-terminal domain [27]. Figure 1A describes the structure of the model receptor protein (HSP90, based on 2IOQ.pdb) colored from N-terminal to C-terminal. Three receptor cavities for potential ligand-binding are also labeled by numbers in this figure. Figure 1B displayed the hydrophobic surface of the model receptor protein. From Figure 1B it is clear that the ligand binding pockets are somewhat hydrophobic in nature. Figure 1C described the structural aspects of model ligand protein. The structure of the model ligand protein is based on HSP70 (HSPA8, 3C7NB.pdb) (Figure 1C). The structure of the model HSP70 is composed of three parts N-terminal nucleotide binding domain (NBD), substrate binding domain (SBD), and a linker that connects the SBD and NBD.

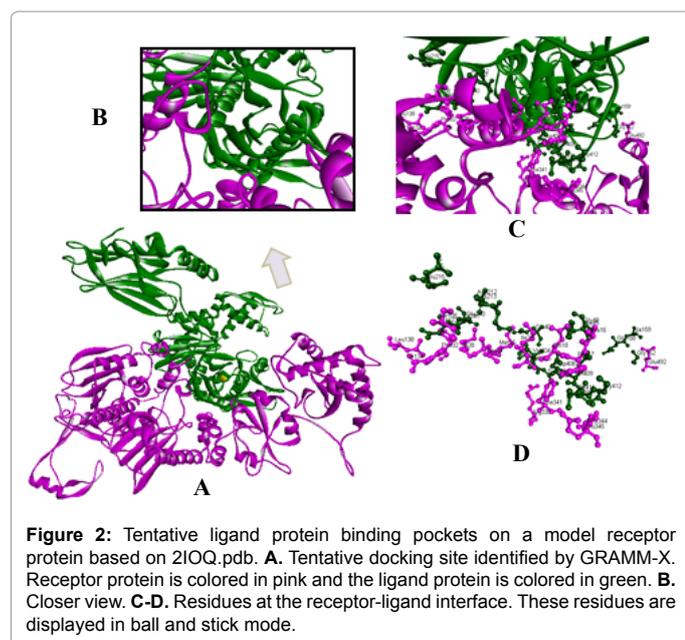
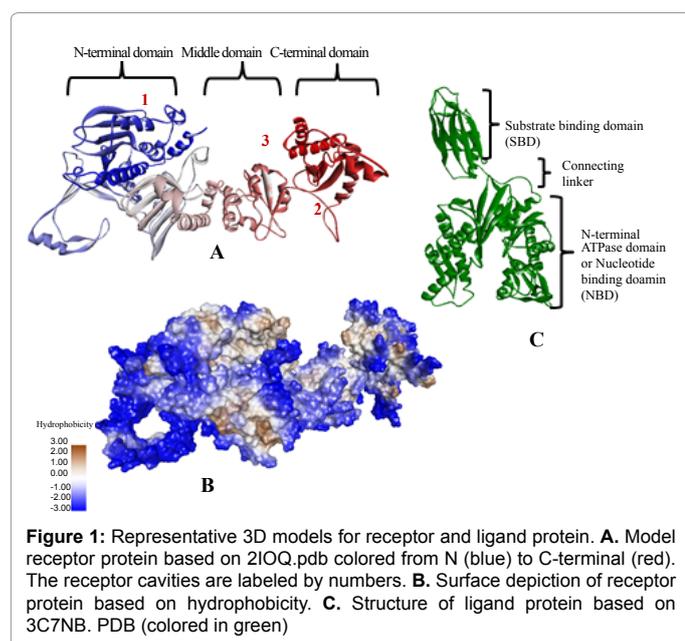
Figures 2 and 3 display the two tentatively identified docking sites (cavity 3 and cavity 1 of Figure 1A) of HSP70 ligand on the HSP90 model receptor model. Interfacial residues are also displayed in Figures 2C-2D and 3C-3D. These docking sites are identified using GRAMM-X docking server. One additional ligand protein binding pocket (cavity

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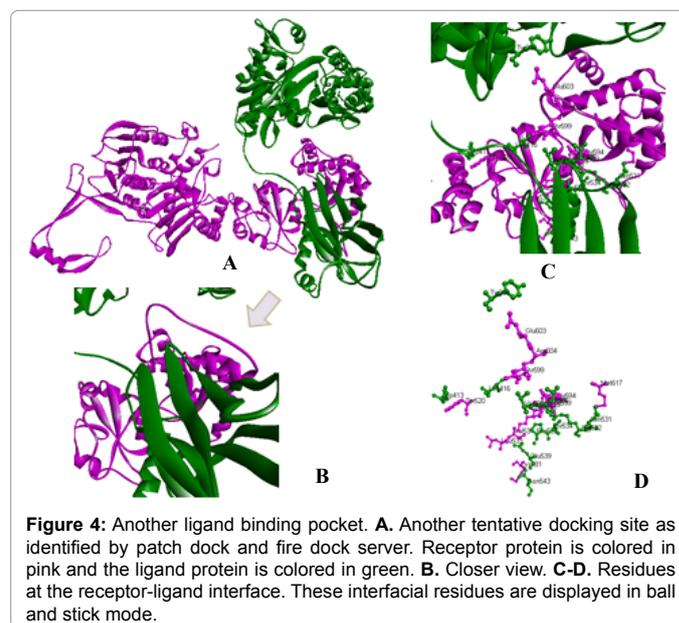
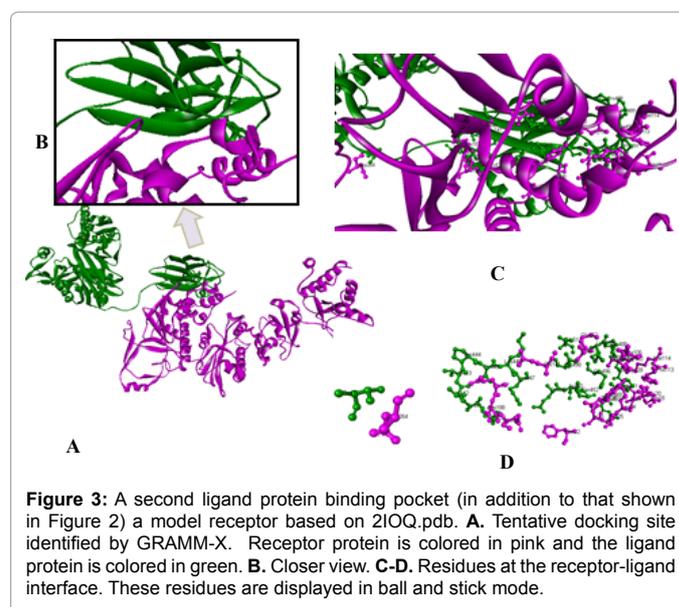
2 of Figure 1A) as displayed in Figure 4 is identified using Patch dock and Fire dock server. Figure 4C and 4D are the residues of the receptor and ligand protein at the interfacial region. In Figure 2, the NBD of the ligand protein is docked onto the receptor cavity 3. In Figure 3, the SBD of the ligand protein is docked onto the receptor cavity 1. In Figure 4, the SBD is docked onto the receptor protein cavity 2. From the above computational study we can see that the SBD of HSP70 is most frequently bound to HSP90. An additional feature is observed while identifying the tentative docking site using Patch dock and Fire dock. In this particular instance, the tentative docking site of the ligand protein is traversed between the receptor protein cavity 2 and 3 of Figure 1A. When docking by GRAMM-X, the ligand protein has a higher tendency to dock in cavity 3 than cavity 1 of model receptor based on 2IQO. In case of patch dock and fire dock web-server however, the ligand protein is mostly docked at cavity 2 and 3 than cavity 1. All these

figures are made using Accelrys Discovery Studio 3.5 [28]. Overall, we have found three tentative docking sites for interaction between HSP70 and HSP90 and that From Figure 1B, we can assume that these pockets are somewhat hydrophobic in nature.

Figure 5 displays the predicted protein-protein interaction network for HSP70 kDa protein 8 (HSPA8). These analyses are performed using the The Biological General Repository for Interaction Datasets (BioGRID) [29,30] and the graphic views are generated using the web based BioGRID graphical network viewer [http://thebiogrid.org/]. The numbers of total interactions are 414 (Figure 5A). If “interactor interactions” are included then the total numbers of interactions for HSPA8 are 7160 (Figure 5B).

HSP90-TDF interaction

We also investigated the interaction between TDF ligand protein [11,12] and HSP90 receptor based on template structure 2IQO.pdb [15]. In this case, when docking by GRAMM-X, the ligand TDF



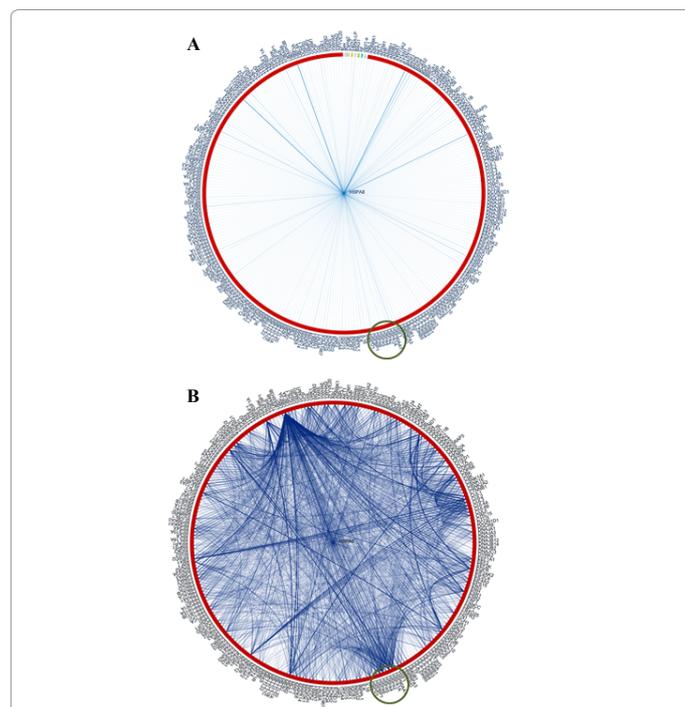


Figure 5: Prediction of interactions between HSP70 (Heat shock 70kDa protein 8; HSPA8) and HSP90. Other heat shock proteins including hsp90 are in close up. B. Graphic view "interactor interactions" of Heat shock 70kDa protein 8 (HSPA8). These figures are generated using the web-based graphical network viewer BioGRID 3.2.102.

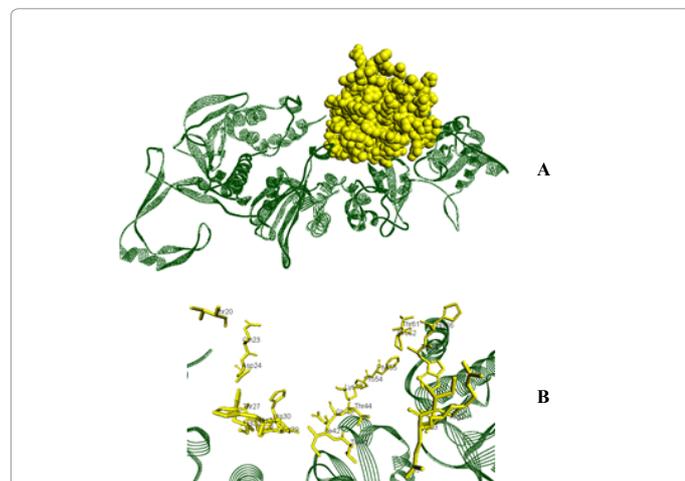


Figure 6: Tentative TDF protein binding pockets on a model HSP90 receptor based on 2IQO.PDB. A. Tentative docking sites identified by GRAMM-X. Receptor protein is colored in green and the ligand TDF protein is colored in yellow space filling mode. B. Residues at the receptor-ligand interface. These interfacial residues of ligand protein are displayed in yellow stick mode.

protein has a higher tendency to dock in cavity 3 than cavity 1 of model receptor. And in this case the TDF protein does not seem to dock strongly at cavity 2 of the model receptor. These docking site using GRAMM-X webserver are displayed in Figure 6A. Interfacial residues of TDF structure are displayed in Figures 6B. In case of patch dock and fire dock web-server the ligand TDF protein however mostly docked at cavity 3 than cavity 1 and 2. Figures 6A and 6B were made using Accelrys Discovery Studio 3.5 [28].

The protein-protein interaction network (physical and genetic) for HSP90 protein is shown in Figure 7. The numbers of total predicted interactions are 906 (Figure 7A). These calculations are based on interspecies interactions and self-interaction. If "interactor interactions" are included (Figure 7B) then the total numbers of interactions are 8456 [29,30].

Conclusions

Overall, the current data suggest that TDF may indeed interact, in addition to HSP70, with HSP90. As we demonstrated previously, TDF may activate a pathway that is specific to breast and prostate cancer cells but not sensitive to other cancer or normal fibroblast or fibroblast-like cells. Our previous report also suggested that TDF-R may be a multi-subunit inducible receptor, composed of HSP70 and HSP90, and that TDF signaling depends on the interaction of these proteins. Thus TDF interacts with its receptors and induces cell differentiation through a unique, non-steroid mechanism [3-5,12].

From the above structural study we can speculate that TDF protein has a higher tendency to bind to the MD of HSP90 receptor protein. The client proteins and co-chaperones are supposed to bind in the MD of HSP90 [31-35]. The NTD of HSP90 contains a lid structure that is closed in ATP bound conformation but remains open in the ADP bound and apo forms [36]. In the case of HSP70, we find that the ligand typically binds to the SBD of HSP70, which is consistent with earlier reported findings [37,38]. Nevertheless, some binding of the ligand protein is found in the linker region of HSP70, and this type of binding has also been reported in the literature [39,40]. For HSP70, the α helical lid in the SBD plays a role in substrate binding. Here, the substrate is encompassed by the lid in a cavity comprised of β sheets. Structural reordering of helical lid is possible before and after substrate binding [41]. Ongoing experimental investigations in our laboratory

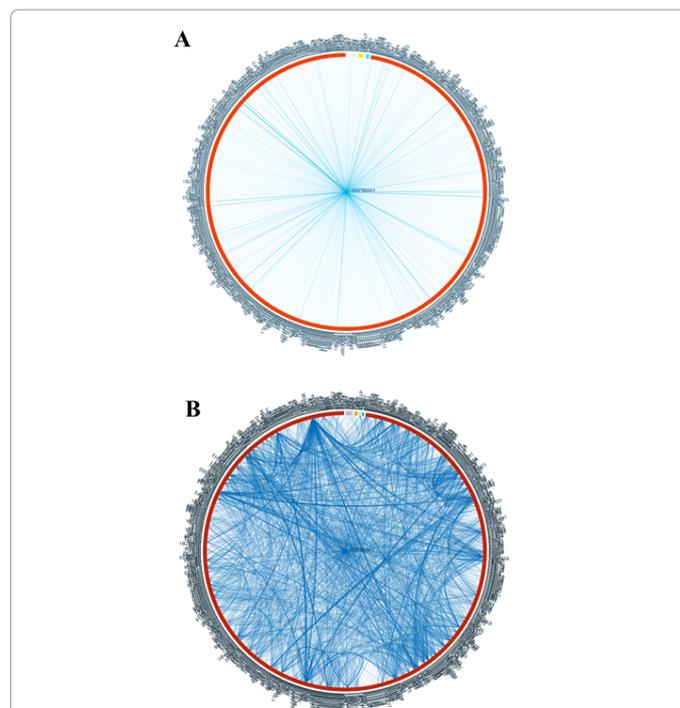


Figure 7: A. Predicted interaction network of HSP90. B. Graphic view of interaction network if "interactor interactions" are included. These figures are generated using the web-based graphical network viewer BioGRID 3.2.102.

will hopefully shed some light in the current interactions identified by structural biology.

Although HSP90 system is largely studied, there are many unanswered questions [42-45]. To our knowledge, complex formation between Hsp90 and Hsp70 are mediated through co-chaperones such as Hop/p60 or Sti1 in yeast [46-48]; no strong experimental evidence or crystal structure is available at this time to verify direct interactions of Hsp70's with hsp90's. The present work proposes the idea of a complex formation between HSP70 and HSP90 (as a TDF receptor), and a recent study on multiple myeloma also suggests the possibility of such direct interaction between Hsp70 and Hsp90 [49]. Nevertheless, since no experimental evidence is available to verify this interaction, this proposal remains an open topic for further investigation.

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