J. Microbiol. Biotechnol. (2017), 27(3), 644–647
https://doi.org/10.4014/jmb.1610.10017

jmb

Interaction of a 22 kDa Peptidyl Prolyl *cis/trans* Isomerase with the Heat Shock Protein DnaK in *Vibrio anguillarum*

Dong Seop Kang, Soo Young Moon, Hwa Jin Cho, Jong Min Lee, and In-Soo Kong*

Department of Biotechnology, Pukyong National University, Busan 48513, Republic of Korea

Received: October 7, 2016
Revised: November 4, 2016
Accepted: November 14, 2016

First published online
November 14, 2016

**Corresponding author*
Phone: +82-51-629-5865;
Fax: +82-51-629-5863;
E-mail: iskong@pknu.ac.kr

pISSN 1017-7825, eISSN 1738-8872

Copyright© 2017 by
The Korean Society for Microbiology
and Biotechnology

Peptidyl prolyl *cis/trans* isomerases (PPIases) catalyze the *cis/trans* isomerization of peptidyl-prolyl peptide bonds preceding prolines. We investigated the protein-protein interaction between a 22 kDa PPIase (VaFKBP22, an FK506-binding protein) and the molecular chaperone DnaK derived from *Vibrio anguillarum* O1 (VaDnaK) using GST pull-down assays and a bacterial two-hybrid system for in vivo and in vitro studies, respectively. Furthermore, we analyzed the three-dimensional structure of the protein-protein interaction. Based on our results, VaFKBP22 appears to act as a cochaperone of VaDnaK, and contributes to protein folding and stabilization via its peptidyl-prolyl *cis/trans* isomerization activity.

Keywords: Peptidyl prolyl *cis/trans* isomerase (PPIase), DnaK, protein-protein interaction, *Vibrio anguillarum*

Protein interactions are critical in determining protein function. It is thus essential to determine how specific proteins react with other proteins in cells [1–3]. Peptidyl prolyl *cis/trans* isomerases (PPIases) catalyze the *cis/trans* isomerization of peptidyl prolyl peptide bonds preceding prolines; they are also able to act as molecular chaperones by preventing the aggregation of denatured substrates [4]. Previously, we reported the isolation and characterization of 22 kDa PPIases from *Vibrio anguillarum* O1. These proteins belong to the FKBP (FK506-binding protein) group. VaFKBP22 has chaperone-like activity; it acts as isomerization factors for protein refolding [5, 6]. VaFKBP22 is predicted to have a V-shaped structure and expression under low temperature (15°C) and alkaline conditions (pH 10) in vivo [6]. During the purification of VaFKBP22, it was always accompanied by a 90 kDa band on SDS-PAGE (Fig. 1A). To identify this 90 kDa protein, we analyzed its amino acid sequence and confirmed that it was DnaK produced by *Escherichia coli*. We therefore assumed that VaFKBP22 and *E. coli* DnaK bind specifically to one another in vivo.

DnaK belongs to the heat shock protein 70 (Hsp70) family. It is one of the most abundant constitutively expressed and stress-inducible molecular chaperones [7, 8]. Typically, Hsp70 family proteins shield hydrophobic surfaces exposed by proteins in their non-native states; they also assist in protein refolding and trafficking to prevent protein aggregation and solubilize insoluble proteins [9–15].

E. coli DnaK and VaDnaK have a homology of 85%. Therefore, VaFKBP22 may be able to bind to VaDnaK in vivo. In the present study, we investigated the protein-protein interaction between VaFKBP22 and VaDnaK using GST pull-down assays and a bacterial hybrid system for in vivo and in vitro studies. In addition, we analyzed the 3D structure of this interaction using automated protein-docking servers. Our data show for the first time the binding of a PPIase to an Hsp70 family chaperone in prokaryotes.

To investigate the VaFKBP22-VaDnaK interaction in vitro, we performed GST pull-down assays. The amplified genes encoding VaFKBP22 and VaDnaK were obtained by PCR and inserted into pET22b+ and pGEX-4T-1, respectively. Next, the plasmids were overexpressed in *E. coli* BL21 (DE3) cells. To investigate the binding of GST-VaDnaK and His6-VaFKBP22, we carried out GST pull-downs using previously reported methods [16]. GST-VaDnaK attached to glutathione agarose beads was used for specific binding with VaFKBP22 in the supernatant of crude *E. coli* extracts. Fig. 1 shows that the recombinant proteins GST-VaDnaK and His6-VaFKBP22 appeared at 90 and 22 kDa, respectively, on SDS-PAGE. Western blotting, performed as described previously [6], detected a band of low molecular weight

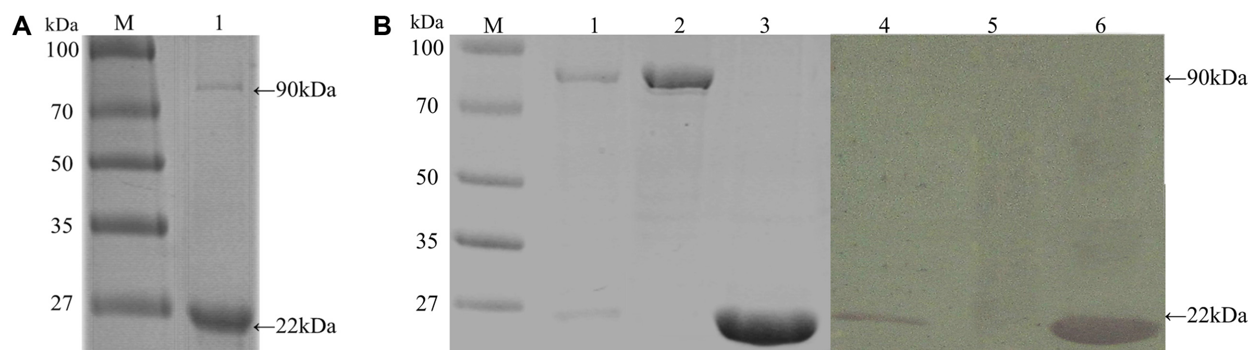

Fig. 1. Purified recombinant VaFKBP22 (22 kDa) from *E. coli* BL21(DE3) on the SDS-PAGE gel and interaction of VaFKBP22 and VaDnaK in vitro.

(**A**) We can see the other band at the range of 90 kDa. It was confirmed as *E. coli* DnaK. (**B**) Lanes 1−3 are results of SDS-PAGE and lanes 4−6 are results of western blotting. Lanes 1, 4: GST pull-down assay result of GST-DnaK and His6-VaFKBP22; lanes 2, 5: GST-DnaK; lanes 3, 6: His6-VaFKBP22. GST pull-down assays were performed using glutathione agarose beads. The molecular binding ratio is 1:4. Proteins bound to glutathione agarose beads were eluted and analyzed by SDS-PAGE and western blotting using recombinant polyclonal His6-VaFKBP22 antibodies.

indicative of His6-VaFKBP22. A 22 kDa band corresponding to VaFKBP22 was confirmed, whereas there was no band in the range of 90 kDa (Fig. 1B). Therefore, we concluded that these two proteins interact physically with each other [17].

The N-terminal (amino acids 1–245; His6-VaFKBP22$_N$) and C-terminal domains (amino acids 270–618; His6-VaFKBP22$_C$) of VaFKBP22 were prepared to determine which VaFKBP22 domain bound to VaDnaK, using GST pull-down assays. The results show that the 13 kDa His6-VaFKBP22$_C$ peptide bound strongly to VaDnaK (Fig. 2A). Conversely, VaFKBP22$_N$ did not bind to VaDnaK (Fig. 2A) [18]. We also investigated the effect of FK506 and ATP on the VaDnaK-VaFKBP22 interaction. A GST pull-down assay in the presence of FK506 or ATP revealed no difference in thickness for any of the VaFKBP22 bands (Fig. 2B). This suggested that FK506 and ATP did not influence the interaction between VaFKBP22 and VaDnaK.

Since VaFKBP22 and VaDnaK interact in vitro, we hypothesized that they interact in vivo. We tested this prediction using a bacterial two-hybrid system. According to the Bacterio-Match II two-hybrid system vector kit, pBT and pTRG were used as bait and target plasmids, respectively. The constructed vectors pBT-VaFKBP22 and pTRG-VaDnaK were transformed to *E. coli* strain XL-1 Blue MRF′ Kan, extracted using a Quick Plasmid Miniprep Kit, and transformed into Bacterio-Match II validation reporter-competent cells. Transformants were screened in M9 His-dropout media containing 3-amino-1,2,4-triazole, a HIS3 inhibitor. Transformed reporter cells expressing pBT-VaFKBP22 and pTRG-VaDnaK were grown in M9 His-dropout media containing 3-amino-1,2,4-triazole (Fig. 3).

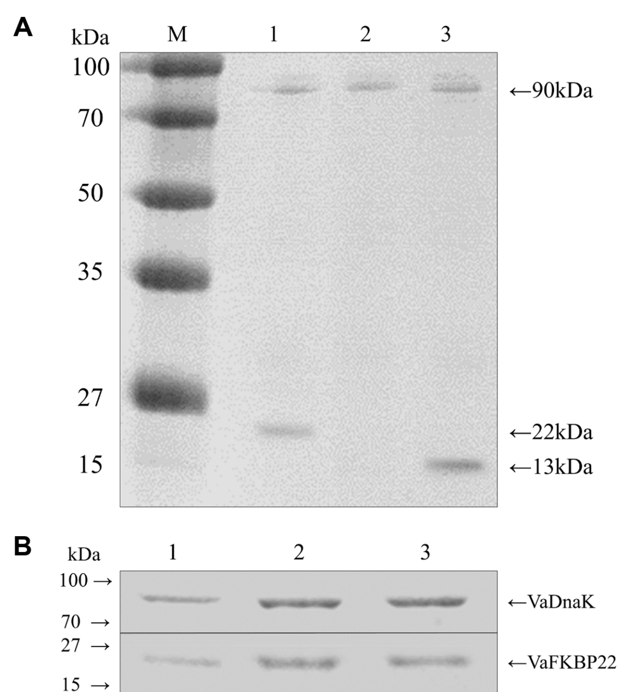

Fig. 2. Interaction of two VaFKBP22 domains (N- and C-terminal domain) and VaDnaK, and effect of ATP and FK506 on protein interaction in vitro.

Each protein interaction was confirmed by GST pull-down assay. (**A**) Lane 1: VaFKBP22$_N$ (full sequence, 22 kDa) and DnaK (90 kDa); lane 2: VaFKBP22$_N$ and DnaK; lane 3: VaFKBP22$_C$ and DnaK. (**B**) VaFKBP22 and VaDnaK were bound at the following conditions. Lane 1: without ATP or FK506; lane 2: addition of ATP; lane 3: addition of FK506.

Reporter cells with the pBT and pTRG vectors failed to grow (Fig. 3A). However, reporter cells expressing pBT-

VaFKBP22 and pTRG-VaDnaK did grow (Fig. 3C). Fig. 3B shows reporter cells expressing pBT-LGF2 and pTRG-Gal11p (which bound to one another) as a positive control. These results suggest that VaFKBP22 interacts with VaDnaK in vivo.

The 3D structures of VaFKBP22 and VaDnaK were predicted using SWISS-MODEL, a protein-modeling web server. Protein-protein interactions were predicted using the PATCHDOCK server, a molecular docking algorithm. Hypothetical 3D structures of the protein complexes were visualized using Chimera 1.11 (Fig. 4). Previously, we found that VaFKBP22 can form a homodimer in vivo because it consists of two domains (N- and C-terminal domains) with different functions. The N-terminal domain contributes to dimerization, whereas the C-terminal domain has PPIase activity [5]. The structure of DnaK is known to differ between its ATP- and ADP-bound forms, and the predicted structures of VaDnaK also have two different forms (Fig. 4) [18, 19]. According to our computer prediction, the VaFKBP22 homodimer can bind to VaDnaK in either of two different states, and we expect that both ATP- and ADP-bound VaDnaK interact with the C-terminal domain of VaFKBP22 (Figs. 4A and 4B).

DnaK is a highly conserved Hsp70 protein that acts as a molecular chaperone. Under stressful conditions, Hsp molecular chaperones also play a role in the refolding of certain denatured proteins. Hsp90 requires accessory proteins or protein cofactors that act as cochaperones, such as immunophilins, which share domains with PPIases [20, 21]. It has been reported that various FKBPs, as well as Hsp70, contain tetratricopeptide repeat (TPR) domains with a PPIase domain at the C-terminus [20–24]. The TPR motif is a common structural feature used by many proteins, and is capable of directing protein-protein interactions. The Hsp70/Hsp90 complex is formed by interactions between the TPR domains, and VaFKBP22 is expected to interact with DnaK using this structural motif [20, 22].

Typically, peptide bonds in a protein exist in the *trans* form, since *cis*-peptide bonds inhibit protein folding through steric hindrance. Because of this steric hindrance, peptide bonds generally have an energetic preference for the *trans* form. The proportion of *cis*-peptide bonds within a protein is about 6%, most of which are found in the Xaa-Pro sequence [25]. Proline has a unique amino acid structure, and preferentially forms the *cis*-peptide bond over the *trans*-peptide bond. This is why converting *cis*-peptide bonds to the *trans* form is helpful for protein folding. Thus, VaFKBP22 binds to VaDnaK and contributes to protein folding and stabilization by PPIase activity. The results of the present study show that VaDnaK and VaFKBP22 interact with each other in vivo and in vitro. Furthermore, we determined that the C-domain of VaFKBP22 binds specifically to VaDnaK, and that the strength of the interaction is not affected by FK506 or ATP. In conclusion, we found that two proteins with chaperone

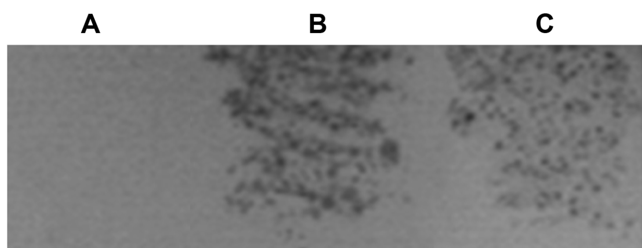

Fig. 3. Protein-protein interaction in vivo.
Protein-protein interaction was confirmed by using a bacterial two-hybrid system. pBT and pTRG vectors (**A**, negative control), pBT-LGF2 and pTRG-Gal11p (**B**, positive control), and pBT-VaFKBP22 and pTRG-VaDnaK (**C**) were transformed to reporter cells, respectively. (**A**), (**B**), and (**C**) were grown in M9 His-dropout media.

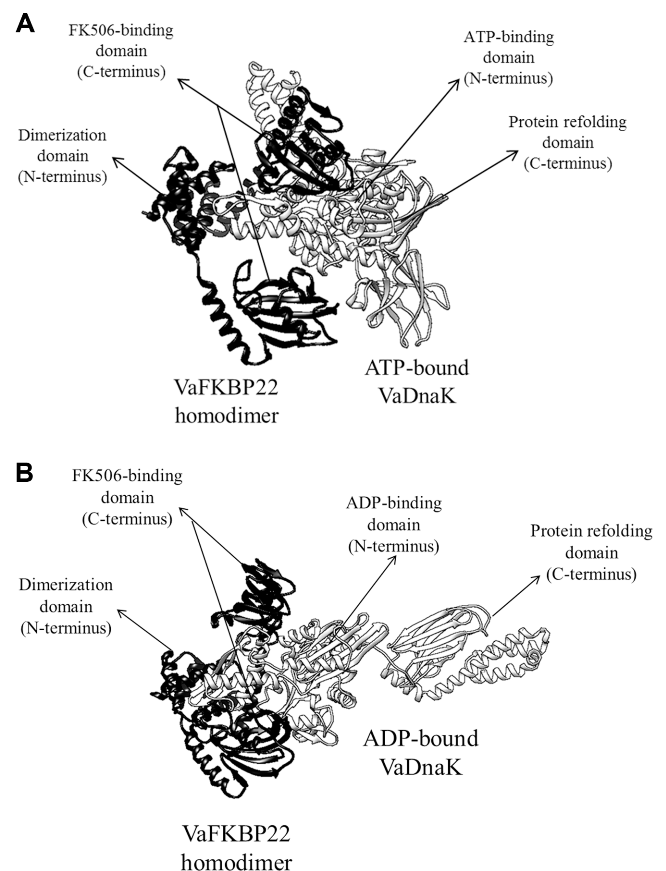

Fig. 4. Hypothetical structure of VaFKBP22-VaDnaK complex.
VaFKBP22 can interact with ATP-bound DnaK (**A**) and ADP-bound DnaK (**B**). The VaFKBP22 homodimer is in black. ADP- and ATP-bound DnaK are in white.

activity are bound in vivo. We therefore predict that VaFKBP22 acts as a cochaperone of VaDnaK. The TPR domains of VaFKBP22 and VaDnaK assist in this interaction via electrostatic and hydrophobic activities. Our future research will investigate the role of VaFKBP22 as a cochaperone of VaDnaK.

Acknowledgments

This work was supported the by Basic Science Research Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Education, Science and Technology (MEST) (NRF-2012R1A1A4A01004957)

References

1. Phizicky EM, Fields S. 1995. Protein-protein interactions: methods for detection and analysis. *Microbiol. Rev*. **59:** 94-123.
2. Klotz IM, Darnall DW, Langerman NR. 1975. Quarternary structure of proteins, pp. 293-411. *In* Neurath H and Hill RL. *The Proteins*, Vol. 1. Academic Press, New York. USA.
3. Rain JC, Selig L, De Reuse H, Battaglia V, Reverdy C, Simon S, Chemama, Y. 2001. The protein–protein interaction map of *Helicobacter pylori*. *Nature* **409:** 211-215.
4. Tremmel D, Tropschug M. 2007. *Neurospora crassa* FKBP22 is a novel ER chaperone and functionally cooperates with BiP. *J. Mol. Biol*. **369:** 55-68
5. Jo GA, Lee JM, No G, Kang DS, Kim SH, Ahn SH, Kong IS. 2015. Isolation and characterization of a 17-kDa FKBP-type peptidyl-prolyl *cis/trans* isomerase from *Vibrio anguillarum*. *Protein Expr. Purif*. **110:** 130-137.
6. Kim SH, Lee JM, Kang DS, Kim DG, Ahn SH, Kong IS. 2014. Expression, purification and characterization of soluble recombinant peptidyl-prolyl *cis/trans* isomerase from *Vibrio anguillarum*. *Protein Expr. Purif.* **101:** 54-60.
7. Calloni G, Chen T, Schermann SM, Chang HC, Genevaux P, Agostini F, Hartl FU. 2012. DnaK functions as a central hub in the *E. coli* chaperone network. *Cell Rep.* **1:** 251-264.
8. Zeiner M, Gebauer M, Gehring U. 1997. Mammalian protein RAP46: an interaction partner and modulator of 70 kDa heat shock proteins. *EMBO J.* **16:** 5483-5490.
9. Bukau B, Deuerling E, Pfund C, Craig EA. 2000. Getting newly synthesized proteins into shape. *Cell* **101:** 119-122.
10. Ha JH, McKay DB. 1994. ATPase kinetics of recombinant bovine 70 kDa heat shock cognate protein and its amino-terminal ATPase domain. *Biochemistry* **33:** 14625-14635.
11. Meiri D, Tazat K, Cohen-Peer R, Farchi-Pisanty O, Aviezer-Hagai K, Avni A, Breiman A. 2010. Involvement of *Arabidopsis* ROF2(FKBP65) in thermotolerance. *Plant Mol. Biol.* **72:** 191-203.
12. Owens-Grillo JK, Hoffmann K, Hutchison KA, Yem AW, Deibel MR, Handschumacher RE, Pratt WB. 1995. The cyclosporin A-binding immunophilin CyP-40 and the FK506-binding immunophilin hsp56 bind to a common site on hsp90 and exist in independent cytosolic heterocomplexes with the untransformed glucocorticoid receptor. *J. Biol. Chem.* **270:** 20479-20484.
13. Pratt WB, Roft DO. 2003. Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp. Biol. Med*. **228:** 111-133.
14. Ryan MT, Pfanner N. 2002. HSP70 proteins in protein translocation. *Adv. Protein Chem*. **59:** 223-242.
15. Young JC, Barral JM, Ulrich Hartl F. 2003. More than folding: localized functions of cytosolic chaperones. *Trends Biochem. Sci*. **28:** 541-547.
16. Zhang X, Wang Y, Li H, Zhang W, Wu D, Mi H. 2004. The mouse FKBP23 binds to BiP in ER and the binding of C-terminal domain is interrelated with Ca^{2+} concentration. *FEBS Lett.* **559:** 57-60.
17. Singh R, Lee MO, Lee JE, Choi J, Park JH, Kim EH, Agrawal GK. 2012. Rice mitogen-activated protein kinase interactome analysis using the yeast two-hybrid system. *Plant Physiol.* **160:** 477-487.
18. Kityk R, Kopp J, Sinning I, Mayer MP. 2012. Structure and dynamics of the ATP-bound open conformation of Hsp70 chaperones. *Mol. Cell* **48:** 863-874.
19. Bertelsen EB, Chang L, Gestwicki JE, Zuiderweg ER. 2009. Solution conformation of wild-type *E. coli* Hsp70 (DnaK) chaperone complexed with ADP and substrate. *Proc. Natl. Acad. Sci. USA* **106:** 8471-8476.
20. Calderwood SK. 2013. Molecular cochaperones: tumor growth and cancer treatment. *Scientifica* (*Cairo*) **2013:** 217513.
21. Young JC, Obermann WM, Hartl FU. 1998. Specific binding of tetratricopeptide repeat proteins to the C-terminal 12-kDa domain of hsp90. *J. Biol. Chem.* **273:** 18007-18010.
22. Assimon VA, Southworth DR, Gestwicki JE. 2015. Specific binding of tetratricopeptide repeat proteins to heat shock protein 70 (Hsp70) and heat shock protein 90 (Hsp90) is regulated by affinity and phosphorylation. *Biochemistry* **54:** 7120-7131.
23. Fellerer C, Schweiger R, Schöngruber K, Soll J, Schwenkert S. 2011. Cytosolic HSP90 cochaperones HOP and FKBP interact with freshly synthesized chloroplast preproteins of *Arabidopsis*. *Mol. Plant* **4:** 1133-1145.
24. Schweiger R, Soll J, Jung K, Heermann R, Schwenkert S. 2013. Quantification of interaction strengths between chaperones and tetratricopeptide repeat domain-containing membrane proteins. *J. Biol. Chem.* **288:** 30614-30625.
25. Kang CB, Hong Y, Dhe-Paganon S, Yoon HS. 2008. FKBP family proteins: immunophilins with versatile biological functions. *Neurosignals* **16:** 318-325.