



Original Research Article

Characterization and identification of polypeptide active partners and the dimer binding sites of HtpG protein applying 12 mer phage library

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ABSTRACT

Keywords

Cloning;
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GST;
GST-HtpG;
Hsp90;
phage display.

In this research work, an attempt has been made to clone the *htpG* gene of *E. coli* AB1157 into pGEX-2T plasmid and used to overexpress the HtpG protein in the form of GST- HtpG fusion protein. The GST- HtpG fusion protein was purified and smeared with 12 mer phage library to ascertain & identify polypeptides which bind with the HtpG protein. Identification of such motifs aided to identify partner proteins that bind with the heat shock protein family *in vivo*. We identified nine of the 12 mer amino acids sequences susceptible to bind tightly with the HtpG protein. Proteins that have homology sequences with any of both the 12 mer sequences were identified from protein data base. Currently, we recognized three binding domains utilized in HtpG protein dimer formation. Moreover, the binding domain of the Hsp90 family of proteins exhibit its function as with client proteins as thyroid hormone receptor, protein kinase, nitric oxide synthase, SMC protein family, DNA polymerase, DNA topoisomerase, 50S ribosomal protein L2, ATP synthase, P53 and a quite number of proteins were determined. These facts clearly designate that the HtpG and Hsp90 family of proteins bind with many proteins *in vivo* to facilitate their protein folding.

Introduction

There have been considerable interest in the identification of most of protein families often complete their role *in vivo* by forming a large protein complexes as Structural Maintenance of Chromosomes (SMC) family which form a protein complex with a partner of a non -SMC family protein (Connelly *et al.*, 1998).

In vivo proteins combine with each other to form high molecular protein structures. Heat shock protein 90 (Hsp90) is a ubiquitously distributed molecular chaperone and is conserved in all organisms ranging from prokaryotes to eukaryotes. In eukaryotes, cytosolic Hsp90 helps to regulate the activities of a number

of transcription factors and kinases involved in signal transduction, cell cycle control, and development (Queitsch et al., 2002). In many eukaryotes, removal of cytoplasmic Hsp90 is known to be lethal, proposing an essential role for this protein in cell growth (Borkovich et al., 1989 and van der Straten et al., 1997). HSP90 is either quickly or firmly associated with specific client proteins that are unstable unless chaperoned with HSP90. Various regions of HSP90 have been proposed to be involved in the interactions with such target proteins. For instance, a highly charged region of chick HSP90 is essential for the binding to estrogen (Binart et al., 1995); this region is also involved in the binding to the subunit of casein kinase CK2 (Miyata and Yahara, 1995). Hsp90 is a dimeric molecular chaperone that contributes to signal transduction by stabilizing a large number of proteins directly involved in signaling pathways. During protein processing, ATP binds to Hsp90 and drives huge conformational changes in the chaperone that ultimately lead to ATP hydrolysis (Sato et al., 2010 and Ali et al., 2006). As in the cases of other molecular chaperones, Hsp90 recognizes non-native proteins and prevents irreversible aggregation during refolding (Buchner, 1999).

HtpG is a prokaryotic homologue of the Hsp90 protein, is present in the cytoplasm of bacteria, yeast and higher organisms. Biochemical studies have revealed that hsp90 is an ATP driven machine that cooperates with a set of co-chaperones (Riggs et al., 2004). The domain building of hsp90 is conserved between *E. coli* and man, therefore, the co-chaperones that support in hsp90 function in the prokaryotes cell may have the same function in eukaryotic. Homologues also exist in the endoplasmic reticulum (Parcell

and Lindquist, 1994). The authoritative expertise of phage display has been broadly applicable in the recombinant libraries displaying small random peptides. Which shall have significant appreciated properties for recognizing peptide sequences contains attraction to several targets. These libraries have produced peptide sequences similar to the epitopes of antigens in order to define the binding specificity of monoclonal antibodies (Devlin et al., 1990, Lane and Stephen, 1993, Huang and Oliff, 2001, Chowdhary et al., 2007). Previously, the phage display used to recognize small peptides which prevent vascular endothelial cancer development, interleukin-6 receptor (Su et al., 2005), targeting neuropilin as an antitumor strategy in lung cancer (Chen et al., 2005a), tumor necrosis factor-alpha (Shibata et al., 2004) and also to elucidate the mechanism of action of anti-HER2 monoclonal antibodies (Baselga and Albanell, 2001).

In our current study, we utilized the phage display diligently to acquire structural functional relationship of the HtpG protein partners. The technique of phage display allows high affinity ligands, presumably related to the HtpG protein to be identified.

Materials and Methods

Chemicals

All the reagents, chemicals, restriction enzymes, DNA polymerase and DNA ligase used were AR or molecular biology grade and obtained from Sigma, BDH chemicals LTD, Bio-Rad and stored as directed by supplier.

Bacterial strains and plasmid DNA

All bacterial strains utilized in the research work were conducted by Dr Picksley, S. M. (Biomedical Science Department, Bradford University, UK). AB1157 (F⁻ *thi-1 thr-1 araC14 leuB6 Δ(gpt proA2) 62 lacY1 tsx-33 galK2 λ⁻Rac⁻ hisG4 rfbD1 rpsL31 str^R kdgK51 xylA5 mlt-1 argE3*) [22], BL21(F⁻ *ompThsdS (r_B⁻m_B⁻)gal dcm*) [23], DH5(*supE44 hsdR17 recA1 endA1 gyrA96thi-1 relA1*) [24], W3110(F⁻ *IN (rrnD-rrnE)1*) [22] pGEX-2T (pGEX-2T GST gene fusion plasmid IPTG inducible Ap^R) Amersham Pharmacia Biotech, pMMY001(pGEX-2T-*htpG* IPTG-inducible Ap^R) This work.

DNA manipulation

Extraction, purification, elucidation and manipulation of both chromosomal and plasmid DNA were carried out as described by Sambrook et al., (1989)

Polymerase Chain Reaction (PCR):

Oligonucleotide DNA forward (5'TGAGGGGATCCTACATGAAAGGA C3') and reverse (5'GAAGGTCATCCGGGATTCCATCA3 ') primers were designed in frame to amplify the *htpG* gene from *E. coli* AB1157 chromosomal DNA. The PCR reaction was carried out referenced to Youssef and Al- Omair (2008).

htpG gene cloning into pGEX-2T DNA

An appropriate DNA fragment containing the *htpG* gene treated with the *Eco* RI and *Bam* HI restriction enzymes. A plasmid DNA vector was purified and linearized with *Eco* RI and *Bam* HI restriction enzymes. The *htpG* gene was cloned into pGEX-2T DNA method reported by Youssef and Al- Omair (2008).

Polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out conferred to Laemmli, (1970).

Overexpression and Purification of GST- HtpG fusion protein

The overexpression and purification of GST- HtpG fusion protein was conceded by Youssef and Al- Omair (2008).

Phage display of affinity ligands and Phage ELISA

The phage display technique was conceded and confirmed to the method described by Bottger et al., (1996).

Preparation of phage single stranded DNA

Phage single stranded DNA was prepared as reported by Sambrook et al., (1989).

DNA sequencing

ssDNA sequences was performed by MacroGen Inc. (MacroGen Inc. 1001, World Meridian Venture Center, #60-24, Gasan dong, Geumchun Seoul, 153-781, Republic of Korea) and determined by the dideoxynucleotide chain termination method using an automated procedure involving differential fluorescent label. Nucleotide sequences were analyzed by using CHROMAS program and DNA was translated into amino acid by ExPASy Proteomics tools (<http://kr.expasy.org/>) web site.

Results and Discussion

The technique of phage display allows high affinity ligands that interact with the

HtpG protein need to be identified. In the adherence of such technique an important step was to express HtpG protein in an easily purified form. One affinity tag commonly used to purify proteins for phage display is the GST (glutathione S transferase) tag. Consequently, the HtpG protein was overexpressed and purified as GST-HtpG fusion protein.

The *htpG* gene contains 1875 nucleotide base pair (bp). The PCR oligonucleotide forward and reverse primers were used to amplify the entire *htpG* gene of bacterial strain AB1157 18 bp upstream of the *htpG* gene to 20 bp downstream of the *htpG* gene. The pGEX- 2T is a suitable vector for the cloning process because its complete sequence is known. The pGEX-2T express GST protein tagged of the target protein of interest HtpG. The pGEX- 2T DNA was digested with restriction enzymes *EcoR* I and *BamH* I to linearize 4.3 kbp DNA of the vector. The 4.3 kb plinear pGEX- 2T and the *htpG* PCR product were ligated using T4 DNA ligase. The ligation mixture was transformed into competent cells of the *E. coli* strain DH5. The recombinant plasmid DNA was designated pMMY001. The pMMY001 plasmid was then introduced into the *E. coli* B protease deficient host strain, BL21. The GST- HtpG fusion protein purified using affinity chromatography. It was noticed that the purification led to the isolation of 96kDa protein corresponding to the GST- HtpG fusion protein (Figure 1 Lane 4).

Before employing the phage display technique on the GST-HtpG fusion protein, the 12 mer phage library was tested using monoclonal antibody DO1 (provided by Dr. Picksley, S. M.). The monoclonal antibody DO1 is known to recognize the N terminal of p53 protein at

SDLxKL motif (Bottgeret *al.*, 1996). This antibody was used as a positive control to confirm the integrity of the 12 mer phage library. The results of the 12 mer phage selected with monoclonal antibody DO1 are shown in Figure 2. The ELISA signal of the 12 mer phage plated onto monoclonal antibody DO1 is about 3 and 4 folds greater than the ELISA signals of the 12 mer phage plated onto BSA, ovalbumin, Marvel (controls) respectively. These ELISA signals of the 12 mer phage selected with monoclonal antibody DO1 indicate that the DO1 antibody is able to select the 12 mer phage. The signals produced from this round for the 12 mer phage are symptomatic and revealed the integrity of the 12 mer phage library.

The integrity of the 12 mer phage library was demonstrated; we then applied the 12 mer phage with the *E. coli*, HtpG protein and in the form of GST-HtpG fusion protein. It is likely to have potential probability that phage specifically selected on the GST-HtpG fusion protein might bind to the GST protein alone. Therefore, the purified GST protein was utilized as a control for GST-HtpG fusion protein work with the phage display technique. The 12 mer phage library (5×10^{11} TU/ml) was used for this resolution and 100 μ l of the 12 mer phage library were plated onto small dishes previously coated with 20 μ g purified GST-HtpG fusion protein. To examine the specificity of the 12 mer first round selected phage with GST-HtpG fusion protein, the 12 mer phage pool selected from these round (1st round) were plated onto an ELISA plate wells previously coated with GST, BSA, ovalbumin, Marvel as a non-specific control proteins and GST-HtpG protein as a specific protein.

The bound phages were perceived by

peroxidase conjugated anti-M13 antibody. The phage display results obtained with the 12 mer phage selected on the GST-HtpG fusion protein is shown in Figure 3. The ELISA signal of the 12 mer plated onto a well coated with GST-HtpG fusion protein in the ELISA plate is approximately 2 fold greater than the ELISA signal detected from plating the phage onto wells coated with GST protein and 4 fold greater than that observed with BSA, ovalbumin and Marvel proteins. Based on the result received from the first round of the 12 mer it had probability that some phage specifically bound to the HtpG protein in the GST-HtpG fusion protein. Subsequently, the 12 mer phage pool produced from the first round were carried forward to a following round of selection by plating 50 μ l of the 12 mer phage pool onto small dishes previously coated with 5 μ g of the purified GST-HtpG fusion protein. This phage were eluted, neutralized and amplified (second round). The specificity of the 12 mer second round selected phage was examined.

As we have displayed in Figure 4, the ELISA signal produced from the second round 12 mer phage plated onto the GST-HtpG protein, which was about 4 fold greater than the ELISA signals obtained from plating the 12 mer phage onto GST and substantially 6 fold greater than the ELISA signals produced with BSA, ovalbumin, Marvel (controls).

We may envisage from these results that the 12 mer phage selected by the GST-HtpG protein should have an affinity ligand displayed on the surface of the phage that binds tightly to the HtpG protein. It was unequivocal to carry forward the 12 mer phage to a further round (third round) of selection with the GST-HtpG protein. The third round of

selection was performed by plating 15 μ l of the 12 mer phage pools from the previous round of selection onto small dishes previously coated with 0.5 μ g of the purified GST-HtpG fusion protein. The phage were eluted and neutralized and the neutral phage was used to transfect *E. coli* strain K-91 and plated onto LB agar plates supplemented with 20 μ g/ml tetracycline. The phage from single colonies were amplified and the specificity of the 12 mer phage amplified from each clone was examined by plating 25 μ l/well onto an ELISA plate wells previously coated with GST, BSA (controls) and GST-HtpG proteins respectively. The bound phage were detected at 450 nm by peroxidase conjugated anti-M13 antibody and TMB substrate. The ELISA signal produced from plating the third round 12 mer phage onto GST-HtpG fusion protein was about 6-8 fold greater than the ELISA signals obtained from plating the 12 mer phage onto GST or BSA controls (Figure 5). The individual 12 mer phage selected after third round each showed strong ELISA signals with GST-HtpG protein rather than the GST or BSA. It was established that the 12 mer phage libraries had a high affinity ligand(s) on their surface that might interact with the HtpG protein after the third round of selection.

To ascertain the sequence of the high affinity ligands displayed on the surface of the 12 mer phage libraries (insert), we have chosen 25 phage clones of the 12 mer phage library that contributed 8 or 7 fold ELISA signals (strong signals) greater than the controls, for sequencing their ssDNA. The following oligonucleotides primer 5'CCCTCATAGTTAGCGTAA3' was deliberate on the basis of the identified sequence of bacteriophage fd, Beck *et al.*, (1978) in order to identify the arrangement of the insert in the 5' end of

the gene III (Parmley and Smith, 1988). The DNA sequence of the region, that encoded for the amino terminal in PIII (contain insert) was conceded by MacroGen Inc. (MacroGen Inc.1001, World Meridian Venture Center, #60-24, Gasan dong, Geumchungu Seoul, 153-781, Republic of Korea) for the 25 phage clones of the 12 mer library to identify the nucleotide sequence of the insert that fused to the gene of coat protein PIII.

The DNA sequences of the insert and their corresponding amino acids in all the positive clones were represented in Table 1 for the 12 mer insert. The DNA sequences were translated into the corresponding amino acids by ExPASy Proteomics tools (<http://kr.expasy.org/>) web site and the results for the DNA sequence of the insert region with its corresponding amino acids are represented in Table 1. The amino acids sequences reveals that these 12 mer amino acids sequences interacts strongly with the HtpG protein and might exist within the sequence of the HtpG protein partner.

The amino acids sequences of inserts in gene III of the 12 mer phage clones were used to search for protein homology using Swiss port protein database (<http://web.expasy.org/blast/>) web site.

Unlike nine 12 mer amino acids sequences have been identified as an insert in the coat protein III of fd phage that bind tightly with the HtpG protein through applying the 12 mer phage library. The amino acids sequences that found to bind firmly to HtpG protein are SMRSWDNDHMKR, DCTWRISDTKVP, CRQHAEPKMDWD, KHPIKFRRVSDR, FSKPDKLFKEDK, KYADPQRTVHSV, ETFQSGAKQLHL, HDEAEHSQTFWL and MERDTLEDSKLF are represented in (Table 1). Proteins that have homology sequence with the above nine 12 mer amino acids

sequences and identified from the protein data base. The family of heat shock proteins 90 is a highly conserved molecular chaperone that is essential in eukaryotes and prokaryotes, where it is involved in many cellular functions, such as protein trafficking, signal transduction and receptor maturation (Taipaleet *al.*, , 2010).

Proteins such as aspartyltRNA synthetase, PspC domain protein, aminotransferase, asparaginyl-tRNA synthetase, histidine kinase and aspartyl-tRNA synthetase identified from protein database to have a sequence homology with the 12 mer amino acids sequence SMRSWDNDHMKR. The *E. coli* protein database also revealed that the carboxyl terminal (amino acids SMRTWDN from 618 to 624) of DNA polymerase V (EC 2. 7. 7. 7) shared sequence homology with the 12 mer amino acid sequence SMRSWDNDHMKR. The DNA polymerase plays a vital role in DNA repair system and from these results , we assume that the Hsp90 proteins may have a role in the DNA repair mechanism. In addition, the transcriptional regulator LuxR family and transcriptional regulator AraC family were identified in protein data base to establish sequence homology with SMRSWDNDHMKR amino acids sequence. Consequently, these proteins may bind to the HtpG or Hsp90 proteins for protein folding. It was testified that, Hsp90 proteins interacts with transcription factors, and plays essential roles in diverse signal transduction pathways (Pratt and Welsh 1994). It has been reported that Hsp90 proteins contribute in the assembly and disassembly of protein complexes. It further plays a significant role in the translocation of specific proteins all the way through intracellular membranes, and in assisting in protein folding (Welch, 1991). This family of protein connects to

cellular kinases, viral, actin, and tubulin. Possessing the critical roles of these stress proteins, considerable sequence and structural conservation (as much as 50%) is evident in species as diverse as bacteria, plants, insects, and mammals (Morimoto *et al.*, 1990).

Some of the proteins such as DNA directed RNA polymerase subunit beta" (EC 2.7.7.6) and transcription-associated protein 1 have an apparent role in protein synthesis. Consequently, the HtpG protein may bind with proteins involved in protein biosynthesis to maintain a nonnative substrate in a soluble folding competent state. Upon addition of HtpG or Hsp90 protein machinery, can be refolded to the native form (Nathan *et al.*, 1997).

The sequence of the 12 amino acids CRQHAEPMKDWD insert was found to have a sequence homology with lysyl-tRNA synthetase (EC 6.1.1.6), tRNA pseudouridine synthase Pus10 and tryptophanyl-tRNA synthetase (EC 6.1.1.2). It was testified that the Hsp90 proteins are implicated as cofactors or chaperones in major cell growth related processes, transcription, translation, DNA synthesis, protein folding and transport, cell division, and membrane function (Gething and Sambrook, 1992). *In vitro*, it was reported that Hsp90 interact with non-native substrate proteins and prevents their stress induced aggregation (Wiechet *et al.*, 1992). The 12 mer amino acids sequence KHPIKFRRVSDR was recognized to have homology sequence with thyroid hormone receptor interactor 12, E3 ubiquitin-protein ligase UPL3, ATPase central domain protein, Beta-galactosidase (EC 3.2.1.23) and STE/STE20/YSK protein kinase. It has been testified that Hsp90 proteins plays vital roles in the maturation of signal transduction proteins, like hormone receptors, various kinases and calcineurin

(Pratt and Toft, 2003). *In vivo*, it has been protruding that there are a growing number of cellular proteins that are found in association with Hsp90. These natural proteins primarily played a role in regulation and signaling pathways, including steroid hormone receptors (Pratt and Toft, 1997) and kinases (Xu and Lindquist, 1993). By supplementary structural changes, Hsp90 is thought to play a regulatory role in these proteins folding. In this process, Hsp90 acts in complex with a set of partner proteins (like immunophilins, Hop/Sti1, p23, p50/cdc37, PP5) (Buchner, 1999) some of which have been shown to possess chaperone like functions themselves (Bose *et al.*, 1996). This binding advocates that the substrate protein is contacted by several proteins in the Hsp90 family heterocomplex.

Hsp90 family facilitates protein folding contained by the cell. This family of proteins interacts with substrate proteins, known as partner proteins in an ATP dependent manner (Young, *et al.*, 2004). ATP binding and hydrolysis facilitates conformational changes in the Hsp90 proteins that are essential for client protein activation (Mayer and Bukau, 2005). Hsp90 proteins appear to interact with partner proteins at late stages along the protein folding pathway, when the client proteins are in near native state conformations (Zhao, *et al.*, 2005). Hsp90 induces slight conformational changes that encourage the requisite and release of ligands or interaction with partner proteins (Pratt and Toft, 2003). Many of Hsp90's partner proteins, counting Cdk4, c-src, and v-src, are oncogenic or otherwise necessary for cell proliferation, making Hsp90 an attractive target for anticancer therapeutics (Neckers, 2007). Hsp90 is believed to induce subtle conformational

changes within its partner proteins, promoting the binding and release of ligands or the interaction with partner proteins. Hsp90 binding also serves to protect some client proteins from ubiquitinylation and subsequent degradation (Caplan, *et al.*, 2007). In eukaryotes and prokaryotic, Hsp90 protein family interacts with a broad array of client proteins that are dominated by signaling and regulatory proteins. These partner proteins comprise serine/threonine and tyrosine kinases, steroid hormone nuclear receptors, transcription factors, and tumor suppressor proteins (Caplan, *et al.*, 2007; Picard, 2006; Pearl and Prodromou, 2003).

All the 12 mer amino acids sequence FSKPDKLFKEDK have a homology sequence with some proteins in data base as phospho-N-acetylmuramoyl-pentapeptide-transferase, ankyrin repeat domain-containing protein 45, glycogen synthase (EC 2.4.1.11), transcriptional regulator SARP family, prenyltransferase UbiA family, UDP-N-acetylglucosamine 1-carboxyvinyltransferase 2 precursor (EC 2.5.1.7). Other proteins identified from protein database include NAD-dependent epimerase/dehydratase family protein, ATP synthase gamma chain, AMP-dependent synthetase and ligase which would share a common function in binding ATP, and it may be a contender for the HtpG protein. Dymock *et al.*, (2005) reported that Hsp90 proteins have facilitated identification of structural elements involved in ATP binding/hydrolysis and others potentially important for client protein binding. Hsp90 family of proteins endures large conformational rearrangements that are modulated by ATP binding and hydrolysis. Several distinguishable conformational states of Hsp90 have been

identified through structural analyses (Hessling *et al.*, 2009). ATP required promotes the alteration from the open to closed conformation (Mayer, 2010). Throughout this transition, a preserved region in the N terminus, known as the active site lid, closes over the nucleotide binding pocket, and the N-terminal domains momentarily interact. Subsequent ATP hydrolysis, dissociation of ADP restores the open conformation. These conformational rearrangements are preserved in bacteria, yeast, and human Hsp90 family members. However, the equilibrium between these conformational states varies across species (Southworth and Agard 2008). Furthermore structures of inhibitor complexes of the eukaryotic Hsp90 proteins have indicated that these compounds block ATPase activity, and hence Hsp90 function, at least in part by occupying the nucleotide binding site contained within the protein and preventing ATP access (Jez *et al.*, 2003). Some of the proteins such as DNA gyrase subunit A, DNA topoisomerase (EC 5.99.1.2), ethanolamine transporter and uridine kinase have a homology sequence with the amino acids sequence KYADPQRTVHSV. Both DNA gyrase subunit A and DNA topoisomerase have a significant role in DNA repair system and this increase our postulation that the Hsp90 proteins plays an exceptional role in cellular proliferation and survival by preferentially stabilizing near native state structures and aiding the dynamic assembly and disassembly of signaling complexes (Whitesell and Lindquist, 2005). In our current study, one of the proteins identified in the protein data base is a putative transporting ATPase that may bind with the HtpG protein. Prokaryotic and eukaryotic Hsp90 proteins have conserved N-terminal domain that binds and hydrolyzes ATP, domains

(Krukenberget *al.*, 2011). It has been reported that the structure/function studies of Hsp90 proteins have established a common mechanistic argument in which ATP binding and hydrolysis trigger conformational changes that alter the hydrophobicity of domains used to employ client proteins (Mayer and Bukau, 2005). Dutta and Inouye, (2000) reported that the Hsp90 is a member of the ATPase superfamily that includes DNA gyrase B/topoisomerase II, DNA repair protein MutL, and histidine kinases.

Proteins that identified in data base and have homology sequence with the 12 mer amino acids sequence ETFQSGAKQLHL, are xanthine dehydrogenase I, lipase/acylhydrolase with GDSL like motif, integrase family protein and malonate decarboxylase alpha subunit. These proteins may bind to the Hsp90 proteins for folding. It further shows the protein database revealed that amino terminal (amino acids FSSAAKQLHL from 20 to 29) of the transcriptional regulator protein shared sequence homology with the 12 amino acids sequence ETFQSGAKQLHL.

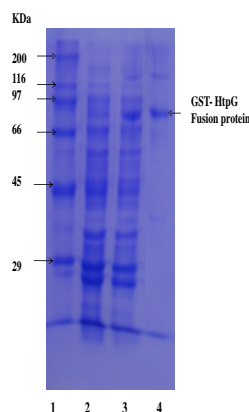
These findings encouraged us to identify more proteins that may bind to the HtpG protein. A search of the prokaryotic protein database also revealed that the amino terminal (amino acids ETRGFQTEAKQLLHL from 8 to 22) of HtpG protein shared sequence homology with the 12 amino acids arrangement ETFQSGAKQLHL. The electron microscopy data revealed that the HtpG protein from a dimer and positioning of the two monomers creates a large central cleft lined by hydrophobic elements (Shiau *et al.*, 2006). Therefore, the amino acids domain ⁸ETRGFQTEAKQLLHL¹² of the HtpG protein is one of the binding

domains that involved in HtpG protein dimer (This work). Also, the amino acids sequence DDAEQYQTFW of HtpG protein from 368 to 377 was recognized to have a homology sequence with the 12 mer HDEAEHSQTFWL amino acids sequence. Therefore, the amino acid domain DDAEQYQTFW of HtpG protein is the second domain participates in HtpG–HtpG protein dimer formation.

Other proteins that have homology sequence with the 12 mer HDEAEHSQTFWL amino acids sequence are transformation competence related protein ComJ, NUDIX hydrolase (protein family of phosphohydrolases), biofilm formation regulatory protein BssS, methyltransferase type 11, propionate-CoA ligase and tetracycline resistance protein. It also contains the 12 mer HDEAEHSQTFWL amino acids sequence was found to homology sequence with some protein that involved in DNA repair machine as putative RNA-dependent RNA polymerase 2, superfamily I DNA and RNA helicase, ATP-dependent DNA helicase PcrA.

The 12 mer amino acids arrangement MERDTLEDSKLF was found to have homology sequence with the following proteins, GTP cyclohydrolase 1 (EC 3.5.4.16), cytidine deaminase, succinate, cytochrome c, oxidoreductase subunit 3 (EC 1.3.5.1), transcriptional regulatory protein (TetR family), methyltransferase type 12, enoyl-CoA hydratase/isomerase, helicase conserved C-domain protein, transcriptional regulator, winged helix family protein (Lyngbya sp.) and protein kinase. The amino acids sequence MERDTLEDSKLF have a homology sequence with Holliday junction ATP dependent DNA helicase RuvB (EC 3.6.4.12) one of the proteins

Figure.1 A figure showing the purification profile of GST- HtpG fusion protein



Lane 1: protein marker Sigma SDS6H2-1VL (M.Wt 30,000- 200,000 KDa).Lane 2: Crude extract of *E. coli* strain overexpressing GST- HtpG protein. Lane 3: GST- HtpG protein eluted from DEAE-sepharose column by using 100 ml gradient of 50-500 mM of NaCl in buffer A.Lane 4: GST- HtpG protein eluted from glutathione sepharose 4B affinity column by using 25 ml 10 mM reduced glutathione in buffer C.

Figure.2 Illustrate the binding affinity of 12 mer phage library selected with DO1 antibody to bind with DO1, BSA, Ovalbumin and Marvel

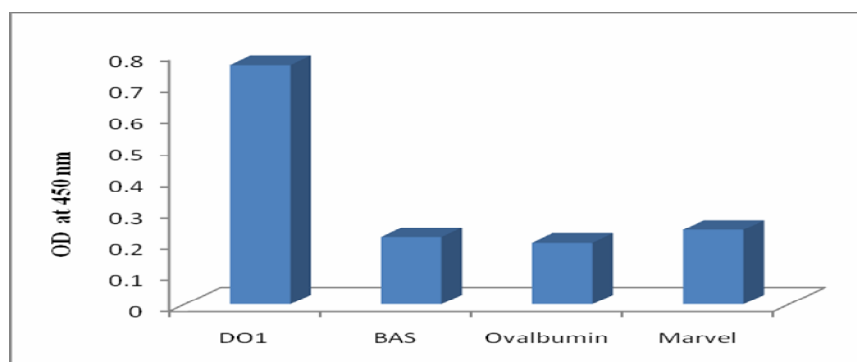


Figure.3 Illustrate the binding affinity of the 1st round 12 mer phage library selected with GST-HtpG fusion protein to bind with GST-HtpG, GST, BSA, Ovalbumin and Marvel proteins.

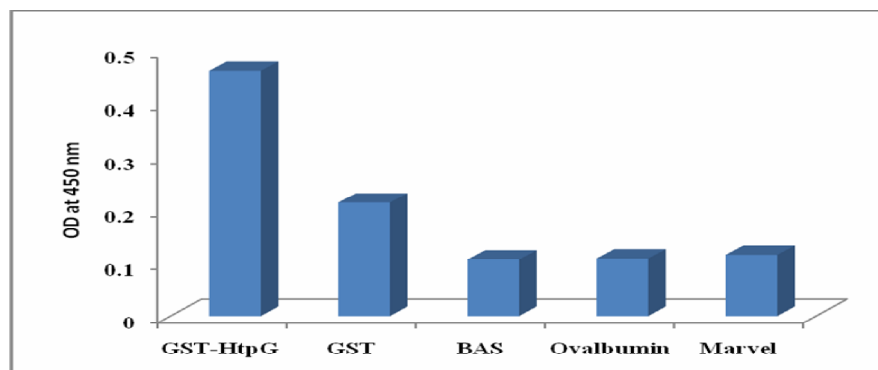


Figure.4 Illustrate the binding affinity of the 2nd round 12 mer phage library selected with GST-HtpG fusion protein to bind with GST-HtpG, GST, BSA, Ovalbumin and Marvel proteins.

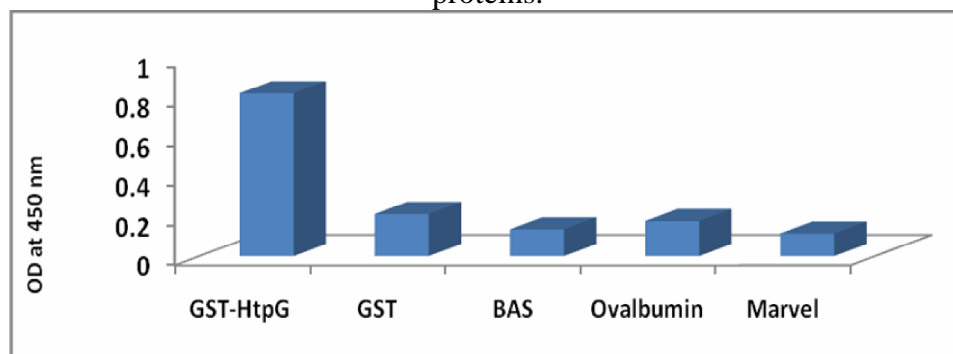


Figure.5 Illustrate the binding affinity of the amplified phage single colonies of the 3rd round 12 mer phage library selected with GST-HtpG fusion protein to bind with GST-HtpG (1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 52, 55, 58, 61, 64, 67, 70, 73, 76, 79, 82, 85, 88, 91, 94, 96), GST (2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, 68, 71, 74, 77, 80, 83, 86, 89, 92, 95) and BSA (3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 66, 69, 72, 75, 78, 81, 84, 87, 90, 93, 94) proteins.

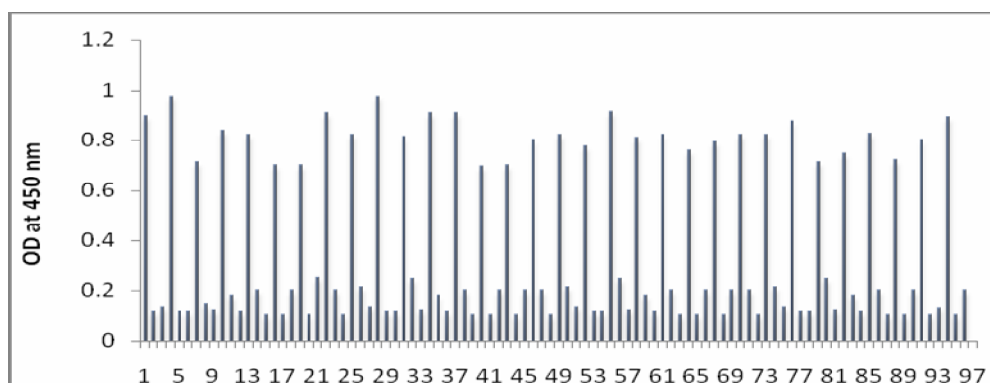


Table.1 Nucleotide sequence of ssDNA of inserts in gene III of the 12 mer phage library clones and their corresponding encoded amino acid sequences that bind tightly with HtpG protein.

No	Nucleotide sequence of ssDNA of inserts in gene III of the 12 mer phage library clones	amino acids sequences of inserts in gene III of the 12 mer phage clones	Number of isolated phage clones
1-	5' AGCATGCGCAGCTGGGATAACGATCATATGAAACGC3'	SMRSWDNDHMKR	2
2-	5' GATTGCACCTGGCGCATTAGCGATACCAAAGTGCCG3'	DCTWRISDTKVP	4
3-	5' TGCCGCCAGCATGCGGAACCGATGAAAGATTGGGAT3'	CRQHAEPMKDWD	1
4-	5' AAACATCCGATTAAATTTCCGCCGCGTGAGCGATCGC3'	KHPIKFRRVSDR	4
5-	5' TTTAGCAAACCGGATAAACTGTTTAAAGAAGATAAA3'	FSKPDKLFKEDK	3
6-	5' AAATATGCGGATCCGCGAGCGCACCGTGCATAGCGTG3'	KYADPQRTVHSV	4
7-	5' GAAACCTTTTCAGAGCGGCGCGAAACAGCTGCATCTG3'	ETFQSGAKQLHL	3
8-	5' CATGATGAAGCGGAACATAGCCAGACCTTTTGGCTG3'	HDEAEHSQTFWL	2
9-	5' ATGGAACGCGATACCCTGGAAGATAGCAAACCTGTTT3'	MERDTLEDKSLF	2

repair to solve Holliday junction (Zhang *et al.*, 2010). Moreover, the MERDTLEDSKLF have sequence homology with C-terminal domain protein of the highly conserved SMC family, which have asignificant responsibility in chromosomal condensation and dosage compensation. The SMC protein proteins usually complete its entire role *in vivo* by binding with non SMC family proteins (Connelly *et al.*, 1998). Consequently, the Hsp90 proteins may bind with the SMC protein family to achieve its entire function *in vivo*. Hsp90 interacts directly or through its cochaperones, with yeast proteins involved in chromatin silencing as well as transcriptional activation (Grad and Picard, 2007). It also involved with DNA helicases involved in chromatin remodeling (Zhao *et al.*, 2005).

Protein compulsory sites are modules that interact with proteins, other macromolecules, and small ligands. These interactions are accountable for protein complex formation as well as principal diverse biologic pathways. Predicted obligatory sites are a hopeful starting point for pharmacologic target identification, drug design studies and protein engineering. In addition, these sites can be receptor in providing sites in identifying protein function, guide docking, and establish networks of protein- protein interactions. Phage display enables the appearance of a large number of peptides on the exterior part of phage particles. Such libraries are able to be tested for binding to goal molecules of interest by means of affinity selection. The powerful technology of phage display has been extensively used for identifying peptide sequences that have affinity to various targets as clearly stated in previous studies that recombinant libraries displaying short random peptides can be of valuable

resources. These libraries have yielded peptide sequences that bear a resemblance to the epitopes of antigens to identify the binding specificity of monoclonal antibodies (Corey, *et al.*, 1993, 1995, Bottgeret *et al.*, 1996).

HSP90 proteins, named according to the 90 kDa average molecular mass of their members are highly conserved molecular chaperones that account for 1–2% of all cellular proteins in most cells under non-stress conditions (Csermelyet *et al.*, 1998). They participate and contribute in the regulation of the stress response (Chen *et al.*, 2005^b) when it is associated with other cochaperones, function in correctly folding newly synthesized proteins, stabilizing and refolding denatured proteins after stress, preventing misfolding and aggregation of unfolded or partially folded proteins, and assisting in protein transport across the endoplasmic reticulum and organellar membranes (Schatz and Dobberstein, 1996; Rutherford and Lindquist 1998; and Young *et al.*, 2001). The 12 mer phage library was utilized effectively with the HtpG protein in the form of GST-HtpG fusion proteins. Furthermore, in our current study, we have identified the HtpG protein dimer domains. The HtpG partner proteins binding domains as aspartyl-tRNA synthetase, transcriptional regulator, asparaginyl-tRNA synthetase, histidine kinase, aspartyl-tRNA synthetase, DNA polymerase, thyroid hormone receptor interactor 12, ATPase central domain protein, DNA gyrase subunit A, DNA topoisomerase, Uridine kinase, RuvB helicase, nitrate reductase, nitric-oxide synthase, glucocorticoid receptor and cellular tumor antigen p53 were identified. All of these provided data need more work to recognize the exact mechanism of Hsp90 proteins with all the

partner proteins. In our current study, we conclude that the Hsp90 chaperone protein family fulfills dual roles: (i) Supplementary collaborator proteins, most of them inherently metastable, in dissimilar subcellular locations, to maintain accurate conformation; (ii) On condition that the ‘unfolded protein response’ that attempts to refold mutated or stress-denatured proteins to a useful state, or, if that proves impossible, then chaperoning their passage to the proteasome for destruction (Shiauet *al.*, 2006).

This issue is particularly pronounced for the bacterial Hsp90, HtpG, for which there are no known cochaperones to improve the efficiency of client protein recruitment. Hsp90 communication with glucocorticoid receptors is not inadequate to the cytoplasm but continues during translocation into the nucleus where the chaperone assists in the receptor’s DNA binding, transcription control, DNA release and nuclear recycling (Grad and Picard, 2007).

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