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Isolation and Characterization of Heat Shock Protein 70-Ipek 1 from *Toxoplasma gondii*

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ABSTRACT

Toxoplasma gondii is a common intracellular parasite that causes the toxoplasmosis. Heat shock proteins (Hsps) have a critical role in pathogenesis of toxoplasmosis. Hsps are highly conserved proteins in evolution among living organisms. This protein family responsible for a wide range of biological processes such as protein folding, protein translocation, protein aggregation. In the present study, Hsp70, a member of the Hsp family, was isolated from *T. gondii* and its sequence and motifs were determined by PCR, cloning, sequencing and homology modelling analysis. ATP hydrolysis, luciferase folding, and luciferase aggregation experiments were performed for determination of its chaperone activity while the stability and secondary structure of the Hsp70 were discovered by using biophysical experiments (FTIR, florescence and quenching experiment). In addition, *in silico* analysis were used to determine the physicochemical characteristics of Hsp70. The results revealed that Hsp70 protein obtained from *T. gondii* (Hsp70-IPEK1) is similar to Hsp70s from other organisms. Also, the chaperone activity, stability and secondary structure of Hsp70-IPEK1 were determined. Hsp70-IPEK1 together with other chaperones in the presence of nucleotide were dramatically increased protein folding and aggregation. According to these results, it is thought that Hsp70 has a potential to contribute many research areas such as pharmaceutical analysis.

Keywords: *Toxoplasma gondii*, Heat Shock Protein 70, Stress.

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Introduction

T. gondii is a highly prevalent intracellular parasite that causes toxoplasmosis. This obligate intracellular parasite leads to infection of one out of three people in the world. Toxoplasmosis is a significant health problem in humans and animals and especially it poses a potential risk for pregnant women and immuno-compromised patients in human [1,2]. Moreover, Toxoplasmosis can lead to severe diseases in high-risk group individuals (e.g., still birth, hydrocephalus, cerebral calcification retinochoroiditis and pneumonia) [3,4].

T. gondii has two distinct forms: definitive host cat and intermediate warm-blooded host. It is generally transmitted to humans through contaminated water and food, especially by ingestion of undercooked meat. Also, humans may be infected by blood transfusion and organ transplantation [3]. The life cycle of this parasite consists of three forms: oocysts, tachyzoites and bradyzoites. The rapidly replicating tachyzoites and the slow-replicating bradyzoites have asexual replication stages within intermediate hosts while the sporozoite-containing oocysts has sexual replication stages in the definitive hosts such as cat and other felids. The life cycle between the tachyzoite and bradyzoite forms is important for pathogenesis and survival of *T. gondii* within intermediate hosts [2]. *In vitro* studies indicate that Heat Shock Proteins (Hsps) can induce differentiation between tachyzoite and bradyzoite forms [3]. For example, Hsp100 participate pathogen intracellular survival and pathogen infectivity in pathogenic bacteria. Also, inhibition of Hsps lead to suppression of bradyzoite development in host cell. Therefore, Hsps can play a significant role in pathogenesis of toxoplasmosis [1,5].

Hsps are evolutionary conserved proteins among living organisms and structurally related but functionally distinct in many organisms. Hsps are localized at different parts of the cell compartments such as endoplasmic reticulum, mitochondria and cytosol. Hsps as molecular chaperones are divided into six main groups based on their molecular weight (Hsp40, Hsp60, Hsp70, Hsp90, Hsp100 and small Hsps (<40 kDa)). Hsps function to fold nascent chains properly, translocate proteins across membranes, disaggregate proteins and target damaged proteins for degradation. Hsp expressions are affected by many factors such as heat, infections, inflammation, oxidative stresses and growth factors. Therefore, many metabolic diseases are associated with expression levels of Hsps [6].

Hsp70s are a highly conserved Hsp family found in all organisms. Hsp70s are responsible for many biological processes including protein assembly, protein folding and protein denaturation [7,8]. Hsp70s have an N-terminal nucleotide binding domain (NBD) and a C-terminal substrate binding domain (SBD). N-terminal nucleotide binding domain, also known as ATPase domain,

hydrolyses the nucleotide and sends a signal to the SBD [6]. While the NBD domain is a highly conserved region, the SBD domain is a variable region [7]. The NBD contains a lid-like structure and peptide-binding groove. Substrate binds a hydrophobic region in the SBD. Unlike ADP bound state, Hsp70s have lower binding affinity but faster exchange ratio for substrate at ATP bound state. Hsp70 functions usually occur via repeated cycles including substrate binding, ATP binding and ATP hydrolysis [9].

The aim of this study is to isolate and characterize Hsp70 obtained from *T. gondii* RH strain (Hsp70-IPEK1). Putative Hsp70-IPEK1 sequence was estimated by in silico analysis. Cloning, sequencing and modeling experiments were performed to determine Hsp70-IPEK1 sequence and motif. After purification and expression processes of recombination protein, chaperone activity of Hsp70-IPEK1 was investigated by ATP hydrolysis, luciferase folding and luciferase aggregation experiments. The stability and secondary structure content of the Hsp70-IPEK1 were determined by using biophysical experiments including FTIR, fluorescence and quenching methods.

Materials and Methods

In Silico Analysis

Putative Hsp70-IPEK1 sequence was predicted from *T. gondii* database (www.toxodb.org) and NCBI Conserved Domain Search (http://www.ncbi.nlm.nih.gov). *T. gondii* ME49 strain was used as template (AAC72002.1) due to this strain shows high sequence similarity to RH strain.

Expasy's ProtParam prediction server and SOSUI server were used to predict Hsp70-IPEK1 physicochemical properties such as molecular weight, amino acid composition and theoretical isoelectric point (pI) and whether it is a soluble or a transmembrane protein. Homology modelling of Hsp70-IPEK1 was performed by using I-TASSER and SWISS-MODEL tools. The model of Hsp70-IPEK1 was shown in (Figure 1).

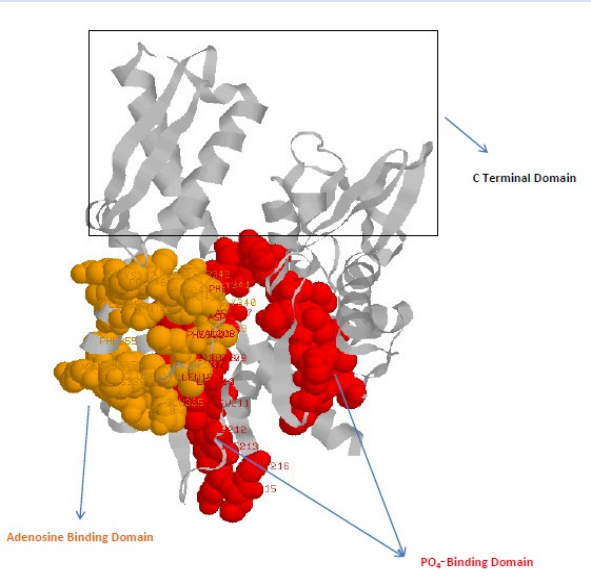

Figure 1. The tertiary structure of the Hsp70-IPEK1 and ATP and peptide binding sites

PCR Amplification, Sequencing and Sequence Analysis

T. gondii RH strain was obtained from the Saydam Public Health Centre, Ankara, Turkey. Total RNA of *T. gondii* was isolated and cDNA synthesis was performed with a commercial kit (Roche Diagnostic) according to the manufacturer's instruction. Hsp70 gene fragment of *T. gondii* was amplified by designed primers (forward primer: 5'-CACCATGGCGGACTCTCCTGCTGTG-3' and reverse primer: 5'- ATCAACTTCCTCCACGGT-3'). PCR reaction was carried out in 50 µL volume containing 1 µg cDNA, 4 µL forward and reverse primer (10 mM of each primer), 5 µL dNTP mix (2 mM), 5 µL 10×Pfu buffer with Mg_2SO_4 and 2 µL Pfu DNA polymerase (2.5 u/µL, Fermentas).

The PCR amplifications were performed in a gradient thermal cycler instrument (Thermo Scientific) with the following thermal cycling program: initial cycle, 30 s at 95 °C; next 35 cycles, denaturation for 30 s at 95 °C; 30 s at 64.2 °C temperature; and polymerization at 72 °C for 1 min; and a final cycle of 7 min at 72 °C. Amplified PCR products were run on a 1% agarose gel stained with ethidium bromide. The PCR product was sequenced and nucleotide sequence of Hsp70-IPEK1 was given in the Figure 2. Comparison of *T. gondii* Hsp70 with other organism was shown and same domains marked in (Figure 3a, Figure 3b).

ATGGCGGACTCTCCTGCTGTGGGTATTGACCTTGGCACCACCTATTCTTGCGTA
GGTGTGTGGAAGAACGATGCTGTGGAAATCATCGCGAACGACCAGGTCCACA
GGACGACCCCGTCCTACGTCGCGTTCACCGACACGGAGAGACTTGTCGGTGAT
GCTGCGAAGAACCAAGTCGCACGCAACCCGGAAAACACCATTTTCGATGCCAA
GCGCCTAATCGGTCGCAAGTTTGATGATCCCTCGGTCCAGTCGGACATGAAGC
ATTGGCCATTCAAGGTCATTGCTGGTCCGGGAGACAAGCCCCTCATTGAAGTC
ACGTACCAGGGAGAGAAGAAGACGTTCCACCCTGAAGAGGTTTCCGCCATGG
TTTTGGGCAAAATGAAGGAAATCGCGGAGGCTTACCTCGGCAAGGAAGTGAA
GGAGGCCGTCATTACCGTTCCTGCGTACTTCAACGATTCGCAGCGTCAGGCTA
CCAAGGATGCTGGTACTATTGCCGGCCTCAGCGTCCTCCGCATTATCAACGAG
CCCACAGCGGCTGCCATTGCTTATGGTCTGGACAAGAAGGGCTGCGGTGAGA
TGAACGTCCTCATCTTCGACATGGGTGGCGGTACGTTCGATGTGTCGCTGCTT
ACAATCGAAGACGGTATCTTTGAAGTCAAGGCCACCGCTGGTGACACCCATCT
TGGTGGTGAAGATTTCGACAACCGTTTGGTGGACTTCTGCGTCCAGGACTTCA
AGCGCAAGAACCGCGGAAAGGACATCAGCACCAACAGCCGTGCCCTTCGTCG
CCTGCGTACTCAGTGCTGGCGCACCAAGAGAACTCTCTCTAGCAGCACTCAGG
CAACCATCGAAATTGACTCTCTTTTTGAGGGCATTGACTACTCTGTGTCTATCTC
TCGTGCGCGCTTTGAGGAGCTTTGCATGGACTACTTCCGCAACTCCCTGTTGCC
CGTCGAGAAGGTCCTCAAGGACTCTGGTATTGACAAGCGCTCGGTCAGCGAA
GTTGTGTTGGTTGGTGGATCTACCCGTATCCCCAAGATTCAGCAGCTCATCACT
GACTTCTTCAACGGAAAGGAGCCGTGCAGGTCGATCAACCCCGATGAGGCCG
TTGCGTACGGTGCTGCTGTCCAGGCACCGATCTTGAAGGGAGTTACCAGCTCT
CAGGTGCAGGATTTGCTTCTTCTGGATGTTGCGCCTCTCTCTCTCGGTCTGGAG
ACAGCTGGTGGTGTCATGACCAAGCTGATTGAAAGAAACACAACGATCCCGA
CCAAGAAGTCTCAGACCTTCACCACGTACGCGGACAACCAGCCAGGAGTGCT
GATTCAGGTGTACGAAGGTGAGCGTGCGATGACCAAAGACAACAACCTCCTG
GGCAAATTCCACCTGGATGGTATCCCCCCCGCCCCCCGTGGTGTCCCCCAAATC
GAAGTCACTTTCGATATCGACGCTAACGGTATCATGAACGTCACAGCGCAAGA
CAAGTCCACCGGAAAGAGCAACCAAATCACCATCACGTACGACAAGGGCCGC
CTCAGTGCGTCCGAAATCGACCGCATGGTGCAAGAGGCAGAGAAGTACAAAG
CCGAAGACGAACAGAACAAGCACCGTGTGGAGGCGAAGAATGGCCTGCTAA
ACTACTGCTACCACATGAGACAGACCTTGGATGACGAGAAGCTTAAGGACAA
GATCTCCTCTGAGGACAGAGACACTGCCAACAAGGCCATCCAGGAGGCCCTT
GACTGGCTGGACAAGAACCAACTAGCAGAGAAGGAGGAATTCGAGGCGAAG
CAGAAGGAAGTTGAGTCCGTCTGCACACCAATCATCACCAAGCTGTACCAGGC
AGGTGCGGCTGCAGGTGGCATGCCTGCCGGTATGGGCGGTATGCCTGGTGGT
ATGGGCGGTATGCCTGGTGGTATGGGCGGTATGCCCGGCGGGATGGGCGGT
ATGCTCGGTGCAGGCATGGGAGGCTCTGGCGGCCCCACCGTGGAGGAAGTTG
ATTAA

Figure 2. Nucleotide sequence of Hsp70

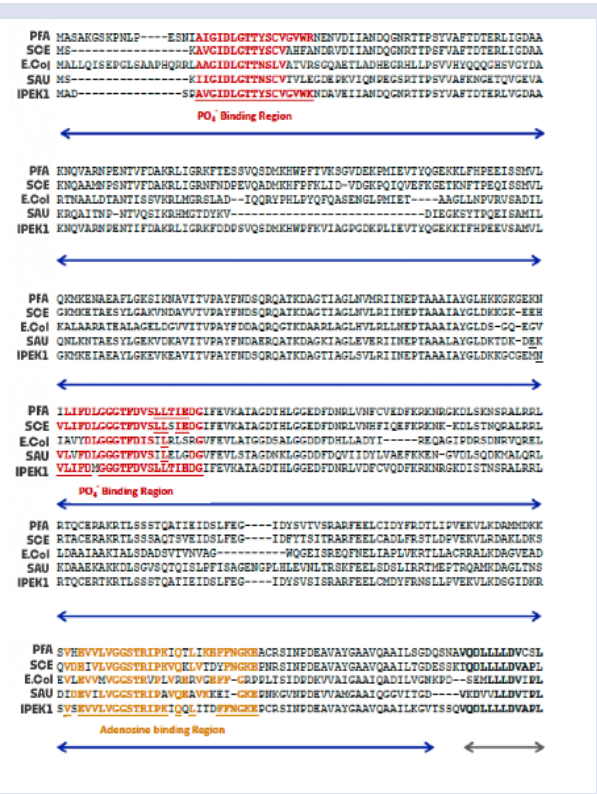

Figure 3a. Comparison of Hsp70-IPEK1 sequence with Hsp70s in different organisms and common motifs. N terminal domain

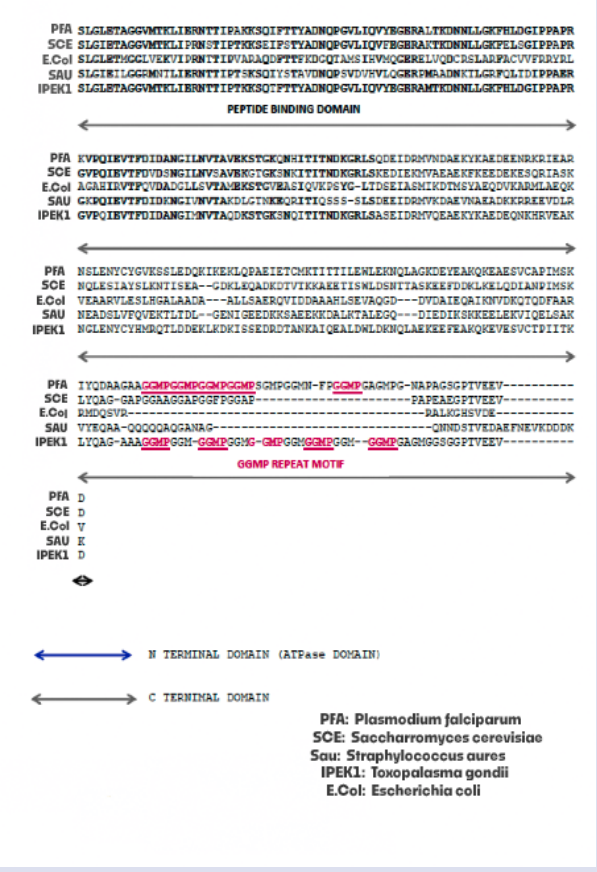

Figure 3b. Comparison of Hsp70-IPEK1 sequence with Hsp70s in different organisms and common motifs. C terminal domain.

Cloning and Purification of Recombinant Hsp70-IPEK1 Protein

The PCR product was cloned into the Champion pET Gateway Expression Kit Pet-161 (Invitrogen). Recombinant Hsp70-IPEK1 protein was produced by using topoisomerase experiments without a sub-cloning process. Firstly, topoisomerase recognition site (underlined sequence in materials and methods 2.2) was added before the forward primer. The PCR product was cloned into Pet-161 vector and then isolated plasmid DNA was transformed into BL21 Star™ (DE3) cells. Transformed cells were grown into 10 mL of LB agar plates containing 100 µg/mL ampicillin. A single colony was inoculated into 10 mL LB media (containing 100 g/mL ampicillin) and incubated overnight at 37 °C with shaking at 200 rpm. The growing cells were inoculated into 1 L of LB (containing 100 g/mL ampicillin) until OD550 was reached 0.4 and then these cells were induced with 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) for 3-4 hours. At the end of the induction process, the growing cells were centrifuged and the pellet was lysed by bead beater using glass beats in the binding buffer (pH 7.4, 20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole).

After centrifugation process, purification of recombination Hsp70-IPEK1 protein was carried out by 5 mL HisTrap™ FF column (GE Healthcare) and was eluted with imidazole gradient (20 mM–500 mM) with using Fast Protein Liquid Chromatography (FPLC, AKTA-Purifier). The collected fragments were analyzed by SDS–polyacrylamide gel electrophoresis on a 10% gel and transferred to nitrocellulose membrane via semi-dry blotter. The target protein was detected with western blot analysis by using primary and secondary antibody (anti-mouse IgG). Bands were determined with NBT (Nitro blue tetrazolium chloride) and BCIP (5-Bromo-4-chloro-3-indolyl phosphate, toluidine salt) (data not shown).

Fluorescence Experiments

Fluorescence experiments of Hsp70-IPEK1 were performed according to the method given in the reference [1]. Briefly, emission spectrums of Hsp70-IPEK1 were measured at 290 nm with 5 nm excitation and between 300 and 500 nm (Shimadzu RF 5301). The experiments were carried out in different protein concentrations at 25 °C with 20 mM Hepes, pH: 7.4, 100 mM NaCl titrations.

Quenching experiments were performed in the presence or absence nucleotides and different acrylamide. Data were analyzed according to the Stern–Volmer equation.

ATP Hydrolysis Experiments

ATPase activity screened with previously established method in our lab [8]. The enzymes used for ATP hydrolysis experiments are obtained from Roche and the reaction mixture contains 2 mM phosphoenolpyruvate, 0.2 mM NADH, 2 units/ml pyruvate kinase and 10 units/mL L-lactate dehydrogenase (both from rabbit

muscle). Each analysis was performed with two replicates and the averages of them were used in the data analysis.

FTIR Experiments

FTIR experiments were carried out by Perkin–Elmer FTIR spectroscopy device at 1 cm−1 resolution. FTIR spectrum of protein solution was detected at amid-1 region and secondary structure of Hsp70-IPEK1 was determined in the absence and presence of nucleotides. Proteins were dialyzed in D2O to eliminate any interference caused by water band spectrum. Hemoglobin (76 % α-helix, 13 % ß-sheet, 11 % random, 0 % ß-turn), conconavalin A (3 % α-helix, 44 % ß-sheet, 28 % random, 25 % ß-turn), lysozyme (36 % α-helix, 10 % ß-sheet, 19 % random, 36 % ß-turn), chymotrypsin (11 % α-helix, 35 % ß-sheet, 30 % random, 25 % ß-turn) were used as standard to determine in secondary structure of Hsp-IPEK1. In the experiments, ATP and ADP concentrations were adjusted to 2 mM and Hsp70-IPEK1 protein was dialyzed against 30 mM sodium phosphate buffer solution, pH 7.4.

Luciferase Folding Experiments

The luciferase folding experiments were measured with Biofix lumi-10 Macherey–Nagel luminometer. Luciferase was used as substrate protein and denatured with urea. Denatured luciferase was diluted in prepared solution (pH 7.4, 25 mM Hepes, 50 mM KCl, 5 mM MgCl2, 2 mM ATP, and 5 mM dithiothreitol). This mixture was incubated with different amounts of IPEK1. In addition, IPEK1 protein was treated with different combinations of Hsps (Hsp40 and Hsp100) in the presence of ATP. The level of luciferase refolding with IPEK1 was measured at 320 nm as described previously [10].

Luciferase Aggregation Experiments

The luciferase aggregation experiments were measured with Shimadzu RF5301 at excitation and emission wavelengths of 341 nm. Luciferase was diluted 10-fold with the reaction mixture (pH 7.4, 25 mM Hepes, 50 mM KCl, 5 mM MgCl2, 2 mM ATP, and 5 mM dithiothreitol). The degree of aggregation was determined by light scattering by substituting different Hsps (Hsp40 and Hsp100).

Results

In Silico Analysis of Hsp70-IPEK1

A 2004 bp PCR product was obtained for Hsp70-IPEK1. Nucleotide sequence of IPEK1 was shown in Figure 2. Theoretically, molecular mass of IPEK1 is approximately 70 kDa. Nucleotide sequence of Hsp70-IPEK1 was analyzed by Expasy's ProtParam prediction tool to determinate physicochemical characteristics. In the amino acid composition of Hsp70-IPEK1, alanine (24.2%), cysteine (26.9 %), glycine (28.4 %) and threonine (20.5 %) were found as the most abundant amino acids.

Length (2004 amino acids), molecular weight (164131.83 Da), theoretical pI : 4.89, extinction coefficient, instability index, aliphatic index and grand average hydropathy (GRAVY) of the Hsp70-IPEK1 protein were analyzed using this tool. According to the computed isoelectric point (pI), Hsp70-IPEK1 is an acidic protein (pI lower than 7).

The computed extinction coefficient of IPEK1 protein was 33750 $M^{-1}cm^{-1}$ at 280 nm. Additionally, the experience showed that Hsp70-IPEK1 does not contain any Trp residues. The instability index value (II) was found to be 49.11 and hence this protein is predicted as unstable (Instability index >40) [10]. The aliphatic index (AI) for IPEK1 was 24.15. The low AI of a protein indicates the it is thermally unstable and more flexible [11]. The grand average hydropathicity (GRAVY) of Hsp70-IPEK1 was found to be 0.851. This protein was identified as a transmembrane protein when analyzed by SOSUI tool.

Toxoplasma Hsp70-IPEK1 sequence was also compared to homologs (*Plasmodium falciparum*, *Saccharomyces cerevisiae*, *Escherichia coli* and *Staphylococcus aureus*). It was showed that motifs and domains of Hsp70-IPEK1 were conserved in both sequence and modelling platform. The Hsp70-IPEK1 sequence contains PO^{-4} binding region, adenosine binding region and GGMP repeat motif (Figure 3).

Fluorescence Experiments of Hsp70-IPEK1

Conformational changes of Hsp70-IPEK1 protein by using fluorescence spectroscopy were investigated in the presence and absence of nucleotides. Fluorescence spectrum was blue shifted 10–15 nm when ATP and ADP are added. Experimental results are indicated that ATP binding causes conformational change in Hsp70-IPEK1 (Figure 4).

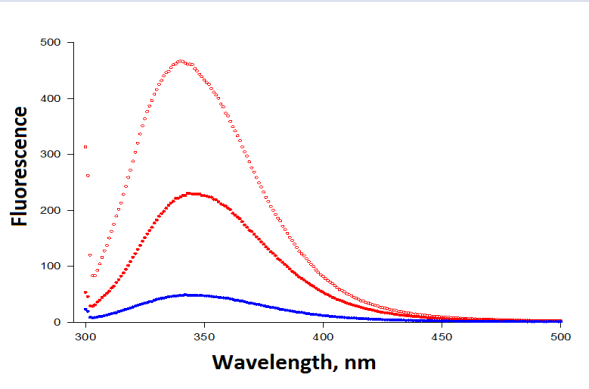

Figure 4. Intrinsic fluorescence spectra of the Hsp70-IPEK1 in the presence of ADP and ATP

ATP Hydrolysis Experiments of Hsp70-IPEK1

Hsp40 and peptide increase the activity of Hsp70. Therefore, ATP hydrolysis is an indirect indicator measure in substrate protein folding of Hsp70 and was measured by ATP regenerative system. The experiment results are shown in Figure 4. Hsp40 provides substrate proteins by binding to J domain of Hsp70 and thus, the activity of Hsp70 increases with presence of Hsp40. According to our results, the increase in the amount of Hsp40 and peptide increased the Hsp70 function. When Hsp40 was 5 µM ATP hydrolysis of Hsp70 was the highest (Figure 5).

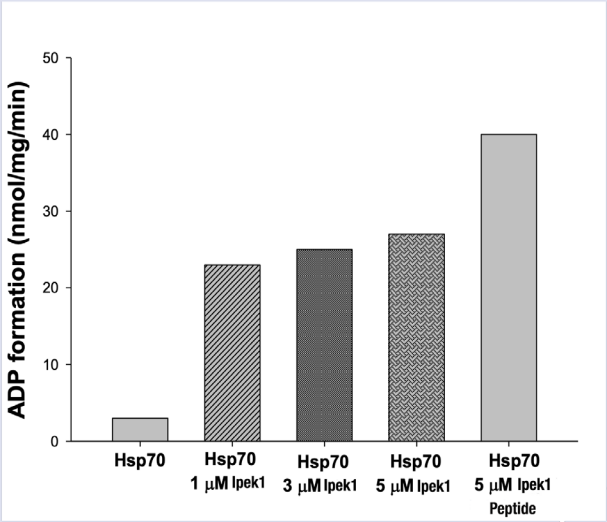

Figure 5. ATP hydrolysis under different conditions

FTIR Experiments of Hsp70-IPEK1

FTIR experiments were used to estimate the secondary structure of Hsp70-IPEK1 at Amid I region. FTIR spectra were determined by measuring different protein concentrations in the presence and absence of nucleotide. Similar to results of fluorescence experiments, FTIR results showed that conformational changes were observed in secondary structures of Hsp70-IPEK1 when it binds to nucleotides (Figure 6).

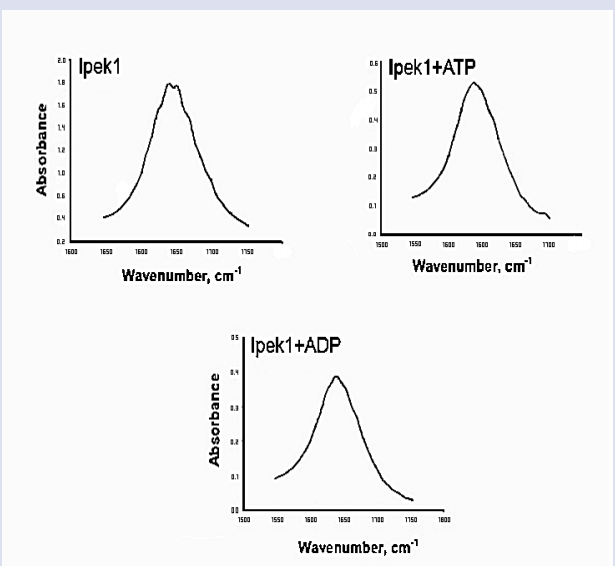

Figure 6. FTIR spectra of the Hsp70-IPEK1 in the presence of ADP and ATP

Quenching Spectra of Hsp70-IPEK1

Quenching method is an alternative method to determine the conformational changes of Hsp70-IPEK1 based on presence and/or absence of ADP. Changes affecting temperature-ligand in the protein surface chromophore were monitored with fluorescence quenching spectrum (Figure 7). In the presence and the absence of ADP form, K_{sv} values were measured 0.250 and 0.375 M^{-1} respectively.

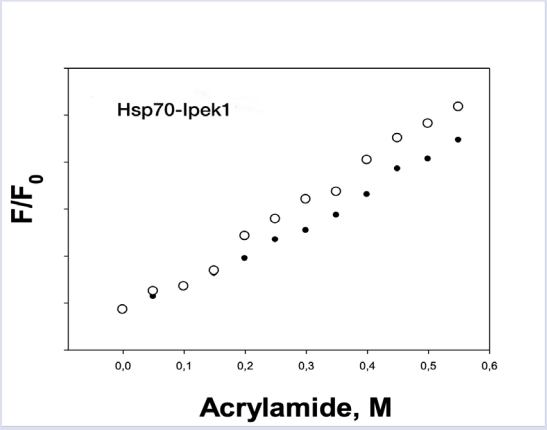

Figure 7. Fluorescent quenching results of Hsp70-IPEK1 in the presence and absence ADP

Luciferase Folding Experiments

In this experiment, the luminescence of the denatured luciferase and luciferase was accepted as '0' and '100', respectively. Addition of Hsp-IPEK1 to experiment helped luciferase folding. Moreover, the light scattering degree increased dramatically in the presence of Hsp40-Hsp70-IPEK1 complex with ATP. Thus, it is thought that Hsp70-IPEK1 can help to find the correct conformation of substrate proteins, especially when coordinated with Hsp40 and ATP.

Luciferase Aggregation Experiments

Luciferase aggregation experiment was performed in the absence and presence of different Hsps (Hsp40 and Hsp100) and ATP presence/absence. The luciferase aggregation was measured with light scattering. The aggregation degree is proportional to intensity of the scattered light. Denatured luciferase formed aggregates however these aggregates dissolved in different combinations of Hsps and ATP (Figure 8). According to the results of luciferase aggregation experiments, Hsp70-IPEK1 protein with ATP and without ATP dissolved aggregate to a certain degree. When Hsp40 and Hsp100 were added to reaction mixture in the presence of ATP, it was observed that dissolution of the aggregates was suddenly increased.

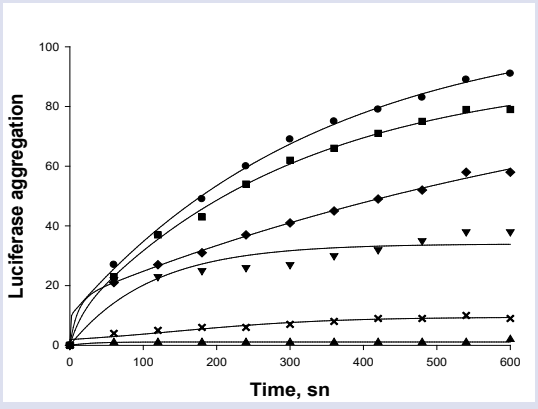

Figure 8. Luciferase aggregation prevention by Hsp complexes in the presence of ATP. Hsp70 alone (●), Hsp70IPEK1 + Hsp40 (■), Hsp70 + Hsp40 + Hsp100 (◊). Addition of Hsp40 and Hsp100 help Hsp70 to solubilize more aggregates and increase the stability by 10 °C

Discussion

Hsp70s are an important family of chaperones and are expressed in response to stress conditions. Hsp70s are involved in major cellular processes. They bind to their protein substrates to prevent non-productive interactions caused by aggregation and protein folding [9]. Also, Hsp70s help the folding of newly synthesized proteins, the subcellular transport of proteins, the degradation of misfolded proteins, assembly and disassembly of macromolecular complexes and in gene induction and apoptosis [9,13]. In folding mechanism of the proteins, Hsp70s interact with other chaperones such as Hsp40s, Hsp100s and nucleotide exchange factors. Many studies have been carried out on structural, biochemical and molecular functions of Hsp70s. However, knowledge about chaperone activity of Hsp70s is still unclear, especially the interaction between Hsp70s and their cofactors [8]. Recent studies revealed that misfolded and aggregate proteins lead to a range of diseases including cancer and neurodegeneration diseases [4,13,14]. Understanding of molecular mechanism in Hsp70s may contributed to the prevention and treatment of various diseases.

Hsps have been characterized in protozoan parasites such as Leishmania, Toxoplasma and Plasmodium [2,14]. These parasites can infect humans and cause serious diseases. Hsps have a critical role in these pathogenic protozoa. Also, *T. gondii* has improved a stress response mechanism by using Hsps to protect itself when exposed to stress. For example, *T. gondii* Hsp90 gene has been described in invasion and growth of the parasite in vitro. Moreover, it has demonstrated that this gene associates with virulence in BALB/c mice *in vivo* [2].

T. gondii leads to toxoplasmosis that is a parasitic disease. During toxoplasmosis, Hsp70s are an important control mechanism in the host immune system. Hsp70s obtained from infected cells by *T. gondii* cause a serious decrease of parasite burden [16]. Nucleotide sequences of Hsp70-IPEK1 isolated from *T. gondii* were transformed to three-dimensional protein structures by simulation. Both these structures and nucleotide sequences exhibited similar with homologous Hsps. Characteristic Hsp70 motifs indicate that this protein family are conserved among species as mentioned in the literature [3,12,17].

In this study, some physicochemical properties of Hsp70-IPEK1 were investigated by using *in silico* analysis and thus some structural characteristics of IPEK1 protein was identified such as solubility and stability. On the other hand, the roles of IPEK1 in folding and aggregation of protein were determined. Hsp70s help the proper folding of protein by ATP regulated cycles of substrate binding and release in cells [18]. This protein functions together with other co-chaperones to prevent the protein aggregation and to provide optimum protein folding [9,18]. Similarly, Hsp70-IPEK1 protein isolated from *T. gondii* assisted to protein folding and increased the solubility of the aggregates with Hsp40 and Hsp100 in the presence of ATP.

FTIR experiments, fluorescence experiments and quenching analysis were performed in the presence and absence of nucleotide to determine the conformational changes in secondary structures of *T. gondii* Hsp70. According to results of these experiments, Hsp70-IPEK1 protein exhibited conformational changes in the presence of nucleotide. Understanding of biochemical properties of Hsps is crucial to use it as a potential therapeutic target. It is thought that Hsp70 upregulated in most cancers is interacted with constituents of the apoptotic and the pro-survival pathway and thus conferring a survival advantage [18]. Hsp70s have some superior biochemical properties such as increased ATP hydrolysis [12]. However, most biochemical properties of Hsp70s are not fully known due to the crystal structure of Hsp70s is yet unavailable.

Conclusions

Hsp70-IPEK1 protein obtained from *T. gondii* were isolated and characterized for better understanding of Hsp70 mechanism. Hsp70-IPEK1 exhibited similar function with homologous Hsp70 proteins found in other organisms. Hsp70 proteins assist cellular proteostasis such as protein folding and protein aggregation. Therefore, characterization of Hsp70s, major players in cells, is essential for future studies (e.g. biotechnology and pharmaceutical areas) and results of the present work have the potential to contribute to the current literature.

Conflicts of interest

There is no conflict of interest in this study.

Acknowledgements

I would like to thank Professor Yusuf Tutar for his expert advice and for the laboratory equipment he provided for our experiments.

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