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Molecular Chaperones: Present Scenario and Future Perspectives

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ABSTRACT

Molecular chaperones (or Chaperones) are very well known for their protein stabilizing functions. After leaving ribosome nascent peptide molecules are very prone to degradation when they expose their hydrophobic region to endoplasmic reticulum (ER). Chaperones present in ER are believed to protect these proteins. Chaperones which belong to heat shock protein (Hsp) are also responsible for protection of protein in stress conditions (e.g. heat, chemical, aging etc.). Recent studies showed that Hsp have the ability to resolubilize the proteins which are already aggregated. For this reason they are now being investigated in treatment of diseases where proteins are deposited as insoluble materials like Alzheimer's disease, Parkinson's disease etc. Although research is in nursery stage it was found some interesting results that chemical and pharmacological chaperones which are newly added compounds in this class have the potential to act like molecular chaperones. Pharmacological chaperones showed better result than Chemical chaperones. Some synthetic derivatives like 4,4'-Dianilino-1,1'-binaphthyl-5,5'-sulfonate (bis-ANS) and 1-anilinonaphthalene-8-sulfonate (ANS) are used in stabilization of proteins in animal model.

Keywords: Molecular chaperone, Chaperone, Heat shock protein, Application of chaperone, Chemical chaperone Pharmacological chaperone, Osmolyte

Abbreviations: Hsp- Heat shock protein; sHsp- small Heat shock protein; ClpB- Caseinolytic peptidase B homolog; ClpA- caseinolytic peptidase A homolog; TCP-1 complex-T-complex protein 1 subunit alpha; CCT- Chaperonin containing TCP-1 complex; Grp94-ER isoform of heat shock protein 90; HtpG – homolog of heat shock protein 90; IbpA-immunoglobulin-binding protein A IbpB-immunoglobulin-binding protein B

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INTRODUCTION

Molecular chaperones are class of proteins which help newly formed proteins into biologically active form by proper folding. These also convert the proteins which are already misfolded into properly folded protein. It seems that chaperone channels newly formed polypeptide chains from ribosome into folding process by inhibiting alternative assembly pathways that may produce biologically inactive proteins. Chaperone literally means "older woman who are in charge of young unmarried woman on certain social occasions". These belong to protein class known as heat shock protein (Hsp) or stress protein. Stress may be in the form of radiation, heavy metals, free radicals, toxins etc. There are wide varieties of chaperones are found in nature ranging from plants to animals and also from prokaryotes to eukaryotes differing in structures and functions. Faulty formation of protein which could be due to malfunction of specific chaperone may lead to disease formation. For example malformed "Tau" protein is found in Alzeimer's disease.

History of Chaperone discovery

In literature the word "Molecular Chaperone" is traced a long back in 1978 described by Ron Laskey to demonstrate the function of a nuclear protein called nucleoplasmin which facilitates chromati assembly by preventing improper interactions between histones and DNA during the assembly of nucleosomes (Laskey et al., 1978). In 1987, this term is generalized by R. John Ellis to include such proteins which are responsible for post-translational modifications in protein (Ellis, 1987). In 1988, it was quite clearly understood that there are similar types of proteins present in both prokaryotic and eukaryotic cells. Later in 1989 details of mechanism of action are described (Hemmingsen et al., 1988 and Goloubinoff et al., 1989). For first instance, it looks like chaperones are only associated with protein folding but it is not totally correct. The first protein which was discovered in nucleus and later known as chaperone was found in stabilization of nucleosomes from folded histones and DNA.

Mechanism of Chaperone Activity

After leaving ribosome the nascent polypeptides expose their hydrophobic segments to cytosol. Being packed with other macromolecules these peptide chains are very prone to aggregation. Although is not clear about how this aggregation occurs but studies shows that it may be due to association of hydrophobic region of C-terminals with either completely folded or partially folded proteins or adhesion with endoplasmic reticulum (Schlieker et al., 2002). It is observed that chaperone-ADP complexes generally have high binding affinity to non-native or faulty folded proteins but surprisingly this affinity is vanished towards native proteins when they are performing their biological functions. Chaperones are found in both prokaryotic and eukaryotic cells and even in plants. They are differ in their structural components like number of subunits, composition of subunits present (Table: 1) but central dogma of action is same that they bind to faulty folded and unfolded protein to prevent aggregation. Folding occurs inside chaperone-ATP complex which is known as folding chamber. Some chaperone do not required ATP for action. Group-I type is found in bacteria, mitochondria and plastids and the Group-II

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type is observed in the eukaryotic cytoplasm and in archaea. Smaller segment is known as co-chaperones which select target proteins and regulates the association and dissociation of chaperone with target protein. Group-II possesses an additional helical structure at top (Pratt and Toft et al., 2003). Stress stimulates heat shock factor-1 (HSF-1) which in turn increases the synthesis of heat shock protein after being transcribed and translated in cell (Soti et al., 2005).

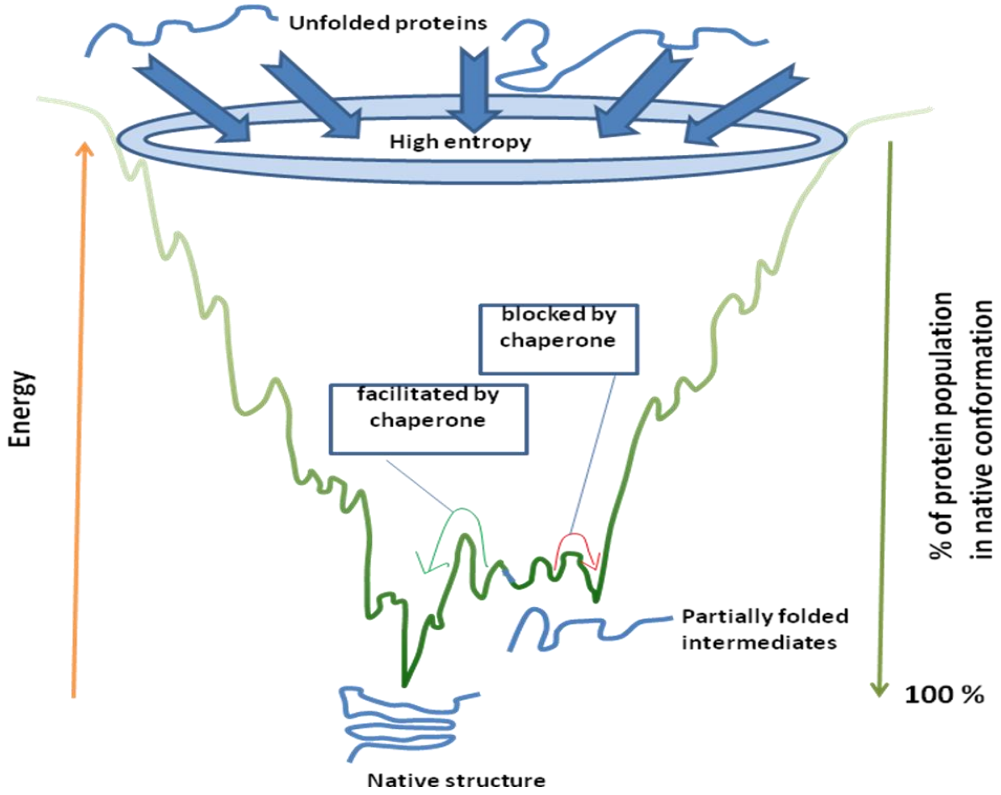

Figure 1: Mechanism of protein folding and role of chaperone

At the top, large number of conformations is present that increases the entropy. At this stage only a small fraction of native structure is present. As folding progresses down the funnel number of states decreases (entropy decreases). The folding mechanism of various proteins is not clear but it is observed that larger proteins require chaperone for proper folding than smaller peptides. Here chaperone does not actually assist in folding but it prevents aggregation of protein during folding process (Grantcharova et al., 2011). Chaperone generally reduces the unfolded proportion of protein of total protein population as folding progresses. At the starting of folding process, different unfolded proteins have different energy levels which depend on differences in energy between its present structure and native structure. Chaperone prevents aggregation and channels them towards native structure (figure 1).

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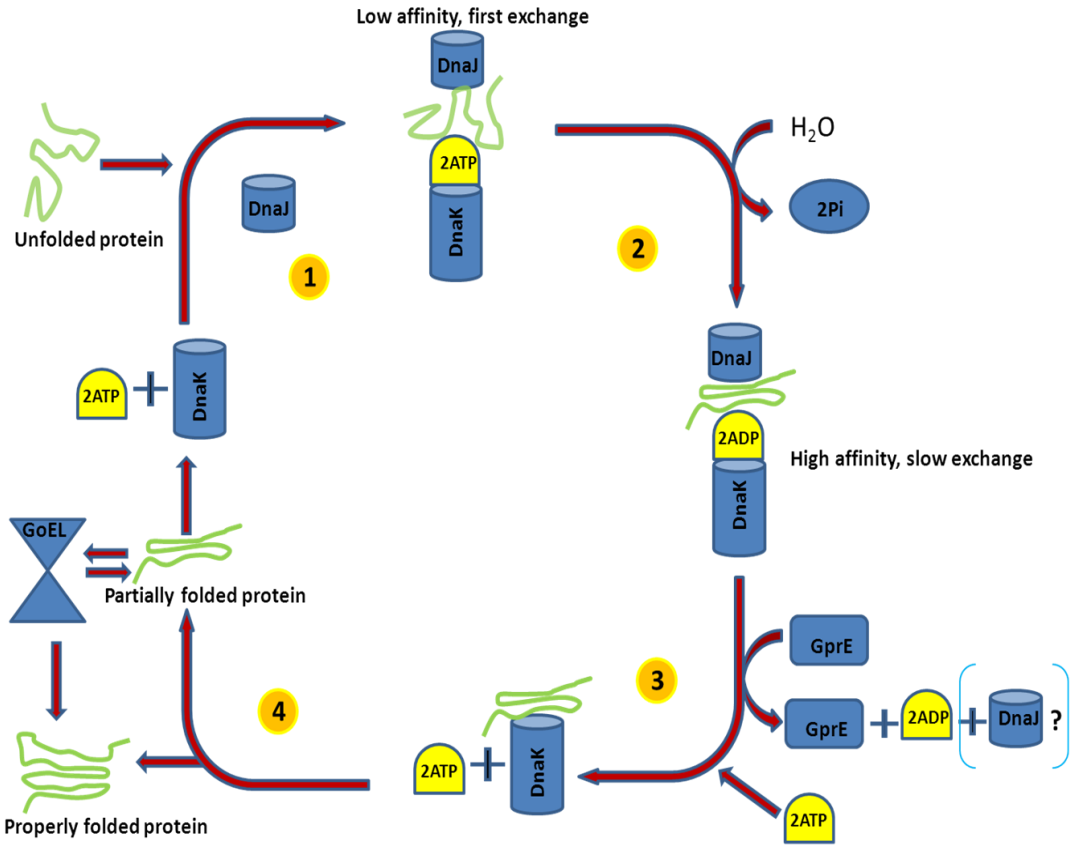

Figure 2: Schematic diagram for mechanism of action of chaperone in *E. Coli*

(1) Unfolded or partially folded proteins attach to DnaK–ATP complex which then attaches to DanJ subunit. Here, two molecules of ATP are required. DnaK and DanJ are homologs of Hsp70 and Hsp40 present in eukaryotic cells. (2) ATP is hydrolysed by addition of water. (3) Another subunit, GprE (present in prokaryotic cell) reacts transiently that result in release of ADP and probably DnaJ. Here, two molecules of ADP are obtained. (4) At this stage properly folded native proteins are released. If some partially folded proteins are produced that is folded by GoEL system. A small percentage of protein requires transfer between GprE and GoEL chaperonin system through free folding intermediate to reach native biologically active structure.

Although mechanism of action of chaperone is complex that is yet to be discovered, we can summarize the generalized action of chaperone inside the cell as below-

a) Most chaperones exhibit ATPase activity and bind to both ADP and ATP. This is very essential step in their folding effects
b) ADP-chaperone complex shows high affinity for unfolded protein which when bound stimulates release of ADP with consequent attachment of ATP.
c) ATP-chaperone complex in turn releases segments of the protein that have folded properly and the cycle involving ADP and ATP binding is repeated until the folding of

protein is completed totally (figure 2).

Table 1: (a) Chaperone family

Chaperone present in Eukaryotic cells (e.g. Mammal)	Structure	Chaperone present in Prokaryotic cells (e.g. *E. Coli*)	Functions
Hsp100	Hexamer (Lee et al., 2003)	ClpA,	Help in thermo-tolerance activity
		ClpB	Protein aggregate Solubilization with DnaK
Hsp90	Mono or Dimer (Owen et al., 1997)	HtpG	ATPase activity
Hsp70	Monomer (Artigues et al., 1997)	DnaK, DnaJ/GrpE (co-chaperone)	Assist in protein folding and resist aggregation of Protein in high temperature
Hsp60	Monomer and oligomer (Chandra et al., 1997)	GroEL, GroES(co-chaperone)	Mitochondrial protein folding
sHsp	8-24-mer	IbpA, IbpS	Prevent protein from heat shock

(b) Some useful chaperones with their major functions

Class of chaperone	Major Functions
Bip (Binding immunoglobulin heavy chain binding protein)	Present in ER and keep abnormally folded immunoglobulin far from ER that prevent degradation
Calnexin (a Ca^{+2} binding protein)	It is also present in ER and binds with monoglycosylated species of glycopretein to prevent their degradation
Calreticulin (a Ca^{+2} binding protein)	Similer as previous
PDI (Protein disulfide isomerase)	This enzyme allows rapid alternation of disulfide bond until correct structure is produced
PPI (Peptidyl prolyl cis-trans isomerase)	This enzyme is responsible for rapid cis-trans isomerization of bond between proline with other amino acids that ultimately stabilize the protein

Appications of Chaperones

In last ten decades a huge research has been carried out in the investigation of chaperone activity. The outcome of this research has appeared as many publications in various reputed journals. It is observed that with these investigations many new roles of molecular chaperone inside the cell are coming in reality. It can be assumed that more roles of it will be traced in future. Actually, all the functions, showed by molecular chaperone are more or less similar to one with another and it is not very difficult to understand why they are similar to each other. Basically, chaperones are specialized proteins that help other protein to get proper folding. All the disorders due to defective chaperones are result in non-native, faulty proteins.

Non-functional or defective chaperones are generally responsible for aggregation and inactivation of proteins. In therapeutic approach targeting chaperone family, our aim is to solubilize aggregated proteins by modulating chaperone system.

Chaperones in Biotechnology

E. Coli is generally preferred for both fermentation and production of various eukaryotic proteins but major drawback which is faced in biotechnology is these heterologous proteins get deposited due to misfolding and aggregation. The exact reason is not known but it can assume that as eukaryotic proteins have larger average size that *E. Coli* proteins will expose more hydrophobic surface patches than smaller proteins. In addition, deposition could be due to insufficiency of protein stabilizing system. Cell could not handle huge amount of proteins which are additive result of *E. Coli* protein and heterogeneous protein. Folding kinetic could be slow. It is therefore advisable to use molecular chaperone like GroEL/ES, or DnaK to increase solubility and stabilize structure. Although various chaperones are found to show different action in heat shock but DnaK is most efficiently in prevention of aggregation of a wide variety of heat-denatured proteins both in *E. Coli* cell extracts and in vivo that other chaperone systems like GroEL/GroES, HtpG, ClpB and IbpB[9]. DnaK is 8-fold more abundant than any other major cytosolic chaperone in yeast. Bi-chaperone system of ClpB and DnaK which are prokaryotic representatives of the Hsp100 and Hsp70 families could be applied for such resolubilization but none of the individual chaperone is found that could be used as general to resist aggregation for any protein[10]. This process depends on both aggregation kinetics and relative affinities of the chaperone system to the folding intermediates. As these parameters are difficult to determine, the application of molecular chaperone is very trivial.

Mechanism of resolubilization done by DnaK/ClpB system is not clear and two models are used to describe its action. In first model ClpB makes complex with protein aggregate which then attaches to DnaK. This model is supported by the fact that DnaK shows less affinity to bind with small protein aggregates (Diamant et al., 2000). Second model tells that ClpB/DnaK complex attaches with protein and it is determined by specificity of component chaperones (Krzewska et al., 2001).

Various processes are used for chaperone application to increase protein production. The first method depends on interaction among TF, DnaK and GroEL. These chaperones are found very important in *E. Coli* for protein synthesis. It is observed that simultaneous overproduction of at least two (e.g. TF and GroEL/ES) rather than one chaperone system together with the protein of interest would significantly increase the amount of desired proteins (Nishihara et al., 2000). In second method, ClpB/DnaK combination is used to resolubilize the protein which is already aggregated. This is mediated by co-overproduction of this combination with the desired protein that is independent of refolding and aggregation kinetics (Tomoyasu et al., 2001). This is contrary to previous method which is largely dependent on assisting in de novo protein folding process. A recent study showed that protein aggregates can be reversibly solubilized in *E. Coli* by chaperone and stopping protein synthesis simultaneously at low temperature. But the amount of native protein obtained is very limited

due proteolysis. This indicates that a competition is present between chaperones and proteases for substrate binding (Carrio et al., 2001). ATP dependent protease activity of Lon, ClpAP, ClpXP and HslUV is very important in *E. Coli*. It is supported by the fact that *E. Coli* cell which is devoid in protease cannot grow at elevated temperature (45°C) (Kanemori et al., 1997). This is an indication of synergistic action of proteases and the DnaK system in preventing protein aggregation (Tomayasu and Mogk et al., 2001).

However till now there is no promising application of the ClpB/DnaK bi-chaperone system to solubilize aggregated proteins in vivo that are produced in recombinant technology. In spite of that this system could be a great promise in future to increase the solubility of a wide variety of heterologous proteins in *E. Coli*. The reasons are their huge disaggregation capacity and broad substrate specificity.

Chaperones and cancer

With the recent developments in technology in the form of high throughput screening, combinatorial chemistry, proteomics and genomics, drug discovery process has been changed very abruptly. There is no doubt that these technologies has delivered pace to anticancer drug discovery. Recent researches show that cancer is occurred due to appearance of oncogenic genes which result further in the formation of tumour. There are several examples of drugs like Glivec, Herceptin and Iressa which are emerged from postgenomic mechanism-based approach. Several molecules are still to come in preclinical and clinical trial. It would be a better approach if we block Hsp90 because Hsp90 is essential for the correct folding, stability and function of oncoproteins and it evidenced that cancer is due to mutation or over expression of these oncoproteins.

Studies in animal model treated with 17-allyl-17-dimethoxygeldanamycin (17AAG), a derivative of geldanamycin showed very promising result in phase-I trial. An interesting point to note about it is that this derivative has the ability to block several oncogens simultaneously so it could be used as broad spectrum anticancer agent (Stebbins et al., 1997). This derivative showed belter result when it is given in combination with other anticancer agents. Combination of 17AAG and taxol gave better result in model with breast cancer in comparison of using 17AAG alone (Paul, 1997). Currently 17AAG is in to enter phase –II trial (Neckers, 2002). The concern about this compound is that it produces a dose related liver toxicity in animal (Supko et al., 1997). More potent Hsp90 inhibitors like radicicol and purine scaffold inhibitors have also been currently investigated for clinical applications (Soga et al., 1998 and Chiosis et al., 2002). Calreticulin which is a 46-kDa Ca^{2+} binding chaperone has also found to cause some cancers. For example calreticulin might be an excellent marker for prostate cancer (Zhu and Wang, 1997).

Cardioprotective activity of chaperone

Expression of cardiac chaperones such as Hsp70, alphaB-crystallin and Hsp22 (alphaC-crystallin) are found to increase in during the development of cardiac hypertrophy. Studies showed that alphaB-crystallin is more important in decreasing cardiac hypertrophy whereas

Hsp70 is responsible for induction of that (Kumarapeli et al., 2008). This is possibly due to that former deactivates nuclear factor of activated T cells (NFAT) pathway but Hsp70 stimulates histone deacetylase-2 (HDAC-2) (Kee et al., 2008). It is observed that synthesis of Hsp70 is dramatically reduced in the blood vessels of older animals than younger in acute hypertension. Exact reason for the declined response is not known; it probably alters Hsp-gene regulation which ultimately transcribed to heat-shock factors (HSF) (Udelsman et al., 1993).

Investigating among the level of Hsp90, Hsp72, Hsp70, Hsp27 and Hsp60 in failing human heart showed that only the expression of Hsp60 was high. Possible explanation could be that Hsp60 acts by NF-κB which is not present in others (Wang et al., 2010. There is no direct evidence but research showed that high circulating level of Hsp60 is observed in severe congestive cardiac failure (CHF) (Niizeki et al., 2008). Different compounds like Geranylgeranylacetone (GGA), Arimoclomol, Celastrol and Statins are being investigated for this purpose (Monte and Cam, 2010). GGA is a cyclic polyisoprenoid whereas Celastrol is a triterpenoid obtained from *Tripterygium wilfordii*. Arimoclomol is a synthetic derivative. Statins are considered because it is identified that patient taking statin produce less Alzheimer's disease (Li et al., 2004). Calreticulin found in various species and it is basically a 46-kDa Ca^{2+} binding chaperone. Normally it regulates intracellular Ca^{2+} homeostasis and endoplasmic reticulum (ER) Ca^{2+} storage capacity. It is observed in clinical studies that calreticulin knockout mice showed cardiac irregularities. This indicates that calreticulin is very essential in development of protein in early stage of cardiac development and recent studies showed that it is actually true in case of human heart also. In embryonic stage of human secretion of calreticulin is high and then decreases abruptly after birth. Chaperones are generally non-specific in nature. In addition modulation of Ca^{2+} level in cell calreticulin is found for responsible for other functions like lectin-like chaperone activity (Trombetta, 2003), modulation of gene expression (Michalak et al., 1999) and modulation of cell adhesion (johnson et al., 2001).

Chaperones and ageing

Study of stress in completely different cells ranging from bacteria and archaea to human cell have show that it is due protein denaturation. In almost all proteinopathies (disorders due to irregular proteins) it is found that aggregated proteins form precipitates (deposits) both inside as well as around the cell, which are a cause of cell damage. Senescence is primarily manifested by a progressive decline of vigor function in body resulting in inactivation of many molecules due development of mutations, wear and tear. Probably other factors are also accelerated the condition. Body has its innate anti stress mechanism and chaperoning systems are key anti-stress equipment. In case of senescence which is an age-associated disease is due partly progressive failure of the chaperoning systems and it would proceed faster if a defective chaperone appears early in life due to a genetic defect. Although in some patients affected by these type of diseases chaperone system are found normal (Alberto and Macario, 2002). If proteins denatured misfolded due to mutation to a degree beyond that which can be reverted by chaperones are degraded before they aggregate and precipitate. Chaperone systems are also work in collaboration with other cellular protein degrading systems like proteasome and other proteases. Alpha B-crystalline belonging to small heat-shock proteins (sHsp) is associated

with a myopathy in which the accumulation of desmin aggregates in muscle cells; both skeletal and myocardial are found (Vicart et al., 1998).

Chaperones in Neurodegenerative disorders

A recently study on abnormal expression of Hsp investigated in the adult brains of Alzheimer patients by using two-dimensional polyacrylamide gel electrophoresis and matrix-associated laser desorption ionization mass spectroscopy (MALDI-MS) showed that Hsp60, Hsp70RY, Hsc71, Grp75, Grp94, and alpha-crystallin B chain were abnormal with varied expression pattern depending on the brain region examined but Hsp70.1, Grp78 and the epsilon subunit of the TCP-1 (CCT) chaperonin exhibited normal expression patterns (Yoo et al., 1998).

The neurodegenerative diseases may be either abnormal depositions of protein associated with abnormal chaperone or due to defective regulation of chaperone-encoding genes. Mainly Hsp90 are involved in both the folding of nascent proteins and re-folding of proteins which was denatured by stress. In addition to this they are also helping in prevention of aggregation and assisting in degradation of protein by the proteasome and lysosomes. It is found that the level of heat shock proteins in body is apparent to decline with age. Oxidative damage may be the cause that chaperones get when they expose to protein surface. In age-related diseases (e.g. Alzheimer's disease, Parkinson's disease, and cataract), the less-soluble/damaged proteins accumulate in neuron especially in post synaptic part. In Alzheimer's disease, chaperones (e.g. Hsp70) present in these positions are probably involved in to sequester the β-amyloid and other damaged proteins (Soti and Csermely, 2003) but small heat shock protein like alphaB-crystallin is identified to increase neurotoxicity of the amyolid-β 1–40 peptide probably by keeping it in a nonfibrillar and highly toxic form (Stege et al., 1999). Parkinson's disease also shows expression of alphaB-crystallin similar to Alzheimer's disease that high amount of neurofibrillary tangles are present in neuron with higher concentration of various heat shock protein than present in normal (Jellinger, 2000).

In case of some long lasting chaperones like AlphaB-crystallin, the level of is not decreased with age but the induction of heat shock protein after stress is decreased in aged animals, were observed. It is also evident that the level of HSF1 (heat shock factor-1) does not change but a decline in the activation and binding of its DNA binding site has been observed with age. Carole J. Proctor and coworker showed with a mathematical model on effect of stress on Hsp90 activity that level of Hsp90 is increased in stress and this can prevent the aggregation of protein if the stress is not severe or long-lasting. If the oxidative damage of chaperone goes to such a limit which is beyond the capacity of chaperone then damaged protein starts to precipitate. Low level of ATP which is generally associated with aging can also worsen the situation because it is required for both refolding and degradation of protein (Carole et al., 2005).

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CONCLUSIONS AND FUTURE DIRECTION

Most of functions of chaperone are found nonspecific in nature and with the help of recent advances in science especially development in molecular biology, new roles of molecular chaperone are constantly coming in front of us. It is like frequently finding a new star while moving in space, but cannot know when our finding will end. The major headache is characterization of activity of chaperones which are already identified. Chaperones are nonspecific so proper characterization and application in specific disease is still a great challenge for scientists. Another factor which increases burden in this case is mechanisms of action of most of chaperone are not still clear. So characterization of chaperone is equally laborious and challenging to finding new classes of chaperone.

Application of suitable chaperone in biotechnology would be very useful in future. Cultures of organism like yeast, *E. Coli* are generally used in recombinant technology to produce desired protein but many times we cannot make them as reliable and sustained source of protein supply because amount of protein obtained is substandard and less. Additional advantage of using chaperone in biotechnology is that most of chaperones are naturally present in bacterial cell so they would be more efficient that other processes. It will possibly be agreed by most of us that investigation of chaperone as therapeutic agent in treatment of neurodegerative disorders would be advisable when there is very less promising drugs available. Some recent studies showed very encouraging result about application of heat shock proteins against cancer. As the chaperones are very nascent in their therapeutic development we need more information to come in any conclusion.

Scientists are able to find some chemicals which are claimed to act like chaperone. Compounds like 4,4'-Dianilino-1,1'-binaphthyl-5,5'-sulfonate (bis-ANS) and 1-anilinonaphthalene-8-sulfonate (ANS) are used as probe in protein folding study (Xinmiao et al., 2005). It is identified that bis-ANS can effectively block aggregation of alcohol dehydrogenase, insulin or the whole cell extract of Escherichia coli in elevated temperature (45ºC) or in chemical stress. But result with ANS is not promising like bis-ANS. The bis-ANS is able to prevent heat induced aggregation of citrate synthase. According to a recent publication it is found that bis-ANS can suppress amyloid formation in neuron. This indicates that it could very useful in neurodegerative disorders (Cordeiro et al., 2004). Osmolytes present in organisms which live in an environment of high or variable salt concentration (Yancey et al., 1982). The Examples of natural osmolytes are trimethylamine *N*-oxide (TMAO), glycerophosphorylcholine, myo-inositol, taurine, dimethylsulfoniopropionate, trimethylglycine, sarcosine, betaine. These types of molecule are low molecular weight, uncharged or zwitter molecules and are also known as chemical chaperone. Osmolyles are compatible with macromolucles and major role is to correct the folding defects in mutant proteins (Arakawa and Timasheff, 1985). However, chemical chaperones are toxic for in-vivo applications as they are required in high concentrations for effective (Bai et al., 1998). This limitation can be overcome by replacing chemical chaperone with pharmacological chaperones (Morello et al., 2000). These are small molecular weight compounds which produce stronger bonds with proteins and are more specific (Welch et al., 1996). For these reasons pharmacological chaperones act at much lower concentrations, i.e.,

physiologically acceptable concentrations (Arakawa et al., 2006). They can selectively attack mutant proteins and cross blood brain barrier. Pharmacological chaperones (or pharmacoperone) hold great prospect in future drug development era. Pyrimethamine is now investigated in the treatment of Late Onset and Juvenile Tay-Sachs disease. A recent publication is cited about multivalent pharmacological chaperone in Treatment of Lysosomal Storage Disorders (Decroocq et al., 2012).

Contributions

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