International Journal of Biological Macromolecules 102 (2017) 367–375

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Review

An overview on molecular chaperones enhancing solubility of expressed recombinant proteins with correct folding

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ARTICLE INFO

Article history:
Received 7 February 2017
Received in revised form 14 March 2017
Accepted 6 April 2017
Available online 12 April 2017

Keywords:
Molecular chaperone
Inclusion body
Heat shock protein
Peptidyl-prolyl isomerase
Disulfide bond formation

ABSTRACT

The majority of research topics declared that most of the recombinant proteins have been expressed by *Escherichia coli* in basic investigations. But the majority of high expressed proteins formed as inactive recombinant proteins that are called inclusion body. To overcome this problem, several methods have been used including suitable promoter, environmental factors, ladder tag to secretion of proteins into the periplasm, gene protein optimization, chemical chaperones and molecular chaperones sets. Co-expression of the interest protein with molecular chaperones is one of the common methods The chaperones are a group of proteins, which are involved in making correct folding of recombinant proteins. Chaperones are divided two groups including; cytoplasmic and periplasmic chaperones. Moreover, periplasmic chaperones and proteases can be manipulated to increase the yields of secreted proteins. In this article, we attempted to review cytoplasmic chaperones such as Hsp families and periplasmic chaperones including; generic chaperones, specialized chaperones, PPIases, and proteins involved in disulfide bond formation.

Contents

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http://dx.doi.org/10.1016/j.ijbiomac.2017.04.025
0141-8130/

1. Introduction

Stanley Cohen and Herbert Boyer were applied cloning and expression methods to produce recombinant proteins in different organisms [1]. At the beginning of the recombinant protein expression systems, *Escherichia coli* (*E. coli*) is a remarkable hosts due to its rapid growth rate, requirement for inexpensive carbon sources, low costs and well-characterized genetic structure [2,3]. However, *E. coli* has some defects such as inability for posttranslational modifications, ineffective cleavage of the amino terminal methionine, inability to produce proteins containing complex disulphide bonds, and expression of proteins as insoluble inclusion bodies (IBs) (IBs) [4,5].

IBs, are inactive form of proteins, which are formed in both prokaryotic and eukaryotic cells that have been miss folded and are not in proper intra-molecular interaction [6]. The natural IBs in some bacteria is like β–lactamase which is a secretion protein [7]. The reasons of IBs formation in protein expression are amount and level of their expression that high level expression of recombinant protein led to IBs formation [8]. The microscopic analysis showed that the size and shape of IBs were different in cytoplasmic and periplasmic spaces [8]. IBs have been cylindrical or ovoid forms [9] and diameter of the shapes changed from 0.5 to 1.3 μm [10]. Moreover, IBs are highly hydrated and have spongy structures that can be simply separated by high speed centrifugation [11,12]. The studies showed that IBs have dynamic, reversible, and kinetic structure that is consequent of unbalance between soluble and insoluble proteins [9,13]. Also, shifting of IB proteins in to active forms in in vitro is really hard and need to several approaches containing isolation, solubilisation, and refolding of IBs, which has low efficiency [12,14]. The solubilisation of IBs are mostly dependent on denaturant agent such as urea, HCL, and guanidine hydrochloride which stimulate disruption of intramolecular interactions [13]. In production of recombinant proteins, several procedures have been used for soluble expression and preventing of IB formation such as using molecular chaperones, low temperature, suitable promoter, ladder tag to secretion of proteins into the periplasm, gene protein optimization and chemical chaperones [5,15]. Cells normally have molecular chaperones and other factors to correct folding of proteins [16]. Numerous studies demonstrate positive effects of molecular chaperons on correct folding formation of recombinant protein and prevention of IBs formation in the cytoplasm and periplasm [17,18]. Also molecular chaperons have clinical and experimental application such as immunomodulatory [19], treating neurodegenerative diseases [20], and morbidity of chronic illnesses [20,21]. In this review we focused on cytoplasmic and periplasmic chaperones category and their applications in recombinant protein expression with correct folding.

2. Molecular chaperones

Molecular chaperones are proteins existing in bacteria and eukaryotic cells which have an important roles to assist cellular homeostasis under normal and detrimental growth situation and cell defences to inhibit aggregation of mis-folded proteins [22,23]. Various genetic sources have molecular chaperones because these agents have critical roles in cell such as prevention of accumulation and mis-folding protein, and also, help to preparing correct folding of protein [24,25]. Chaperones do not have steric information of target protein structure to determine exact folding. So, they prevent improper interactions within and between other polypeptides by enhancing the yield of correct folded proteins. However, they don't increase the amount of folding reactions. Unfolded proteins have hydrophobic residues, which are abnormally exposed to the cell solution that own to form stable inactive accumulations of these proteins. Molecular chaperones are able to bind to hydrophobic residues of unfolded proteins and assist to create the correct folding of protein [22,26]. Molecular chaperones exist in the cytoplasm and organelles of eukaryotic cells like nucleus, mitochondria, endoplasmic reticulum, chloroplast, and periplasmic space of prokaryotic cells [27]. Molecular chaperones are divided in cytoplasmic and periplasmic categories that have been reviewed (Table 1).

2.1. Cytoplasmic chaperones

Most of cytoplasmic chaperones are mainly expressed under stress conditions such as high temperature [28]. Significantly, the chaperones bind to misfolded proteins to unfold them and in turn substrates are released from the chaperones to provide a new chance for unfolded protein to gain correct folding [16]. These chaperones can be categorized into five families based on their molecular weight including heat shock protein100 (Hsp100), Hsp90, Hsp70, Hsp60, and small Hsps (sHsps) that have molecular weight between 12 and 43 KD [19]. Some type of cytoplasmic chaperones have an ATP-dependant mode of substrates interactions (Hsp100, Hsp90, Hsp70, Hsp60) to have functional system, but in contrast, majority of sHsps don't need ATP consumption [29]. Among them, Hsp90 and Hsp70 are the main chaperones responsible for protein holding to stop association of target protein with another protein [30]. Cytoplasmic chaperones based on their function and mechanisms are divided to three sub-classes including disaggregate chaperones that enhanced the solubilisation of stress-induced accumulated proteins [31], holding chaperones that keep and hold the folded proteins on their surface [32,33], and folding chaperones that interfere the refolding of protein and enhance the proteolysis of mis-folded proteins [34,35]. In this section, we focused on the cytoplasmic chaperones based on their molecular weights.

2.1.1. Hsp100

Hsp100 or (Clp) family are Hsps with molecular weight of 100–104 KDa, which give the organisms tolerate excessive stress and have variety of proteolysis functions [36]. Also, Hsp100 is responsible for protein disaggregation and degradation that lead to omitting non-functional and harmful polypeptides that is vital for the maintenance of cellular homeostasis [37]. Unlike the other Hsps, Hsp100 family solubilize accumulated proteins, thermally [38]. At first the members of this family were recognized as components of the two subunit bacterial Clp protease system, which contains regulatory ATPase/chaperones such as (ClpA and ClpX) and proteolytic (ClpP) subunits [39]. Then this family separated to two classes and eight individual subfamilies within this classes [40]. Furthermore, Hsp100 family cooperate with Hsp70, which is another ATP-dependant chaperone system for saving proteins from aggregation. The Hsp70 system gets the solubilized proteins from Hsp100 and refolds them [41]. The Hsp 100 is a member of AAA^+ superfamily (ATPases associated with various cellular activities) proteins, which get their energy from hydrolysis of ATP to stimulate creation of correct folding in target proteins [42]. All AAA^+ superfamily members have AAA domain which have two motifs for nucleotide binding and hydrolysis. Moreover, the AAA domain is responsible for protein oligomerization that lead to made of hexameric structures with a central pore [43]. Hsp100 chaperones are differ from each other based on the number of AAA domains which are one or two in each protomer and the existence of extra domains [43].

2.1.2. Hsp90

The Hsp90 family are highly conserved and proteins, which exist in all organisms from bacteria to humans and their expressions rises in response to the stress conditions in prokaryotic and eukaryotic