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Mini Review

The Role of Small Heat Shock Proteins on Folding Processes of PfAdoMetDC/ODC Protein as a Malarial Drug Target

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Abstract

Malaria remains one of the leading causes of death on the African continent due to the lack of effective vaccines and treatment against the disease. In order to design drugs, large, pure, and active quantities of proteins are considered as main objective to study their structure-functional features. *Plasmodium falciparum* S-adenosylemethionine decarboxylase ornithine decarboxylase (PfAdoMetDC/ODC) is considered a malarial drug target protein due to its role in polyamine synthesis. Polyamines are involved in cell proliferation, differentiation, and cell growth. Therefore, blocking functional activities of PfAdoMetDC/ODC by effective compounds has been regarded as an alternative method to combat malaria. However, contaminant proteins that co-purify with PfAdoMetDC/ODC as a drug protein have delayed the design of active compounds. Though molecular chaperones have been proposed as major tools that may assist in improving the production of some target proteins, they have not been effective in obtaining a pure protein because the final product produced is always contaminated with what seems to be small heat shock proteins. This review looks at some of the methods that maybe explored in regulating the activities of small heat shock proteins during the synthesis of PfAdoMetDC/ODC in order to produce not only a pure but also an active protein in large quantity.

Keywords: Small heat shock proteins; E. coli; PfAdoMetDC/ODC; Malaria; Molecular chaperones

Introduction

Molecular chaperones play a key role in protein quality control both as single or co-operative partners depending on the structure of the substrate. They function as folding catalysts and proteases, which are crucial for cells to produce functional proteins as well as to eliminate harmful misfolded/aggregated proteins. Molecular chaperones are classified according to their sizes, which are the Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and small Heat Shock Proteins (sHSPs) [1]. Each member of Hsps from these families acts upon a specific spectrum (broad or narrow) of substrate proteins in helping their folding, refolding, oligomeric assembly, translocation, and/or degradation [1]. Small heat shock proteins operate as molecular chaperones, preventing stress induced aggregation of partially denatured proteins and promoting their return to native state when favorable conditions pertain [2]. Small heat shock proteins, ubiquitously existing in all forms of life [3], were found to suppress protein aggregation in an ATP-independent manner and stabilize stress-damaged cell membranes [4]. Under in vitro conditions, sHSPs can effectively interact with unfolded model substrate proteins and keep them in a folded-competent state for subsequent refolding that is facilitated by ATP-dependent chaperones such as Hsp70s and Hsp100s [5]. The sHSPs are known to non-selectively interact with almost all forms of aggregated-prone unfolded model substrate proteins under in vitro conditions [6]. Table 1 depicts the various forms of sHSPs from different origins and highlights their role in substrate folding. Trigger factor is one of the essential molecular chaperones that interact with almost all substrates upon their exit from ribosomes. However, Nakamoto [6] suggested that it holds on to the substrate for a long time such that it co-purifies with the final product as an unwanted protein. Williams and colleagues [7] observed that the malarial drug target PfAdoMetDC/ODC which was meant to be isolated as a pure protein, tends to co-purify with contaminant proteins that are believed to be molecular chaperones. Briefly, PfAdoMetDC/ODC is a key during the synthesis of polyamines and they are involved in cell proliferation, differentiation, and cell growth. Polyamines are found in almost all forms of life including *Plasmodium falciparum* (*P. falciparum*) a causative of malaria [8]. Therefore, PfAdoMetDC/ODC protein is regarded as malarial drug target since it is the driving engine for the production of the polyamines [8]. Escherichia coli host system has been used for various expressions of recombinant proteins for structural studies and therapeutic purposes. Some of the advantages of using this system include the affordability, easy to use and manipulative. However, a number of challenges have been encountered when using this system for some recombinant protein production that includes aggregation, low production or no production at all and the formation of inclusion bodies [9]. As a result, most of the proteins have been very challenging to study their structure-functional activities towards the development of effective drugs, particularly for malaria. This review covers both advantages and disadvantages of having sHSPs in E. coli host system during the folding stages of PfAdoMetDC/ODC protein. This may

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Table 1: Different sizes of Small heat shock proteins.

Name	Protein function	References
Hsp16.6	Prevent protein aggregation	Mu et al.[18]
CeHsp17	Exhibit chaperones activity by preventing exposed cells at 58°C	Ezamaduka et al., Zhang et al.[13,14]
Hsp18.1	It Cooperate with Hsp70 to reactivate a heat-denatured protein	Lee GJ, Vierling.[15]
Hsp20.2	Possesses chaperone activity	Bukach et al.[19]
Hsp27	Prevent heat denatured protein aggregation via ATP-independent formation	Vidyasagar et al.[20]
Trigger factor	Function as a protein folding	Lakshmipathy et al.[21]

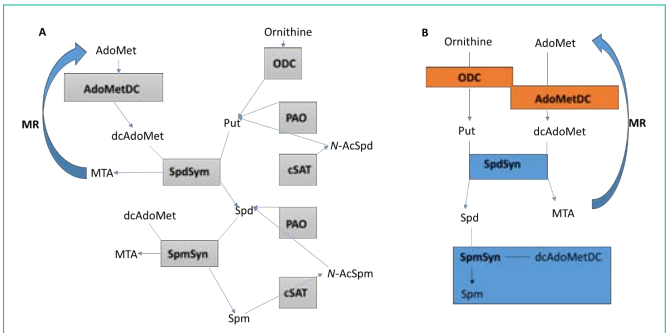

Figure 1: Synthesis and regulation of polyamines in Human and Plasmodial systems. (A) In human cells, Ornithe Decarboxylase (ODC) and S-Adenosylmethionine decarboxylase (AdoMetDC) are highly regulated, and have very short half-lives (<1h), whereas the synthases of SpdS and SpmS are constitutively expressed. Spermidine/Spermine-N1-acetyltransferase and Polyamine oxidase, provide a pathway for back conversion from spermine to spermidine to putrescine. (B) *Plasmodium falciparum* has a bifunctional AdoMetDC-ODC and lacks Spermine synthase. Abbreviations: MTA, 5'methylthioadinosine; SAT, spermine-N1-acetyltranferase; PAO, polyamine oxidase [18].

give a broader idea on why there is a prolonged binding of sHSPs to PfAdoMetDC/ODC, though produced in E. coli. Could it mean that PfAdoMetDC/ODC is not completely folded, as a result, the sHSPs end up co-purifying with it as a target protein? If this is the case, what means could be employed to regulate the sHSPs activities during the folding of the former? Again, what influence they may possess or have if they were to be eliminated or supplemented during the synthesis of PfAdoMetDC/ODC protein?

Polyamine metabolic pathways in humans and *Plasmodium falciparum*

Polyamine metabolism in mammalian cells is regulated by two separate proteins, the S-Adenosylmethionine Decarboxylase (AdoMetDC) and Ornithine Decarboxylase (ODC). Mammalian cells are regulated by feedback mechanisms and involve multiple routes of synthesis and interconversion (Figure 1A). Studies suggest that these complicated regulatory mechanisms enable cells to adapt to considerable changes in extra-and intracellular polyamine concentrations [10]. The ways in which polyamine synthesis is regulated by ODC and AdoMetDC between Plasmodial and human systems differs (Figure 1). For example, both ODC and AdoMetDC are highly regulated, and have short half-lives (<1 h), while in plasmodial systems they have longer half-lives and the pathway also lacks spermine [7]. This could be the reason why this pathway has been proposed as a drug target in plasmodium systems.

Polyamine synthesis in *P. falciparum* is regulated by a single open reading frame that encodes both rate limiting enzymes in

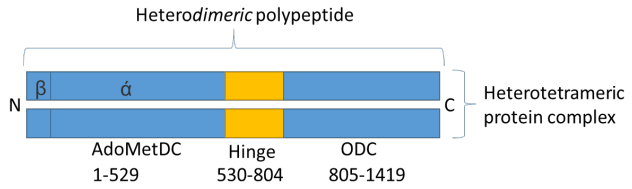

Figure 2: Schematic organization of the bifunctional AdoMetDC/ODC from *P. falciparum* [6,11].

the polyamine pathway such as ODC and AdoMetDC [11] (Figure 1B). AdoMetDC is an enzyme that catalyses the formation of polyamines such as putrescine and spermidine which are needed by parasites for proliferation, differentiation, and growth [7]. Ornithine is decarboxylated to putrescine and subsequent attachment of aminopropyl groups to its terminal amino substituent's to form spermidine. The aminopropyl groups are donors of decarboxylated S-Adenosylmethionine (dcAdoMet) in the presence of AdoMetDC (Figure 1B). Both ODC and AdoMetDC are rate limiting in this step. Interference with polyamine biosynthesis by inhibition of the rate limiting enzymes (ODC) and (AdoMetDC) has been proposed as potential chemotherapy of cancer and malaria parasite infections [11]. Birkholtz [12] suggested that this pathway is unique to *P. falciparum*.

Structural organization of the bifunctional AdoMetDC/ODC from *P. falciparum*

A bifunctional enzyme from *Plasmodium falciparum* (PfAdoMetDC/ODC) is in the form of a heterotetrameric of approximately 330 kDa, formed by a 150 kDa heterotetrameric AdoMetDC (α and β subunits) and the 180 kDa homodimeric ODC [13] (Figure 2). The functional heterotetrameric complex therefore consists of two subunits each of the 160 kDa α-AdoMetDC/ODC and the 9 kDa β-AdoMetDC [7,11,12]. The PfAdoMetDC domain is found at N-terminal domain of the bifunctional peptide (residues 1-529). Like that of the human enzyme, it consists of an (α β) 2 dimer but unlike other AdoMetDC enzymes, the plasmodial enzyme is not stimulated by putrescine. The ODC sub-domain of the protein is found at the C-terminal of the bifunctional peptide from residues 805-1419 (Figure 2). Polyamines such as putrescine and spermidine play an important role in differentiation and proliferation of cancerous cells and malaria parasite (*P. falciparum*). S-Adenosylmethionine decarboxylase/ornithine decarboxylase (PfAdoMetDC/ODC) has been described as an ideal antimalarial drug target due to the important role it plays in biosynthesis of the polyamines and uniqueness of its structure [11]. However, the production of this large bifunctional protein to facilitate its biochemical characterization

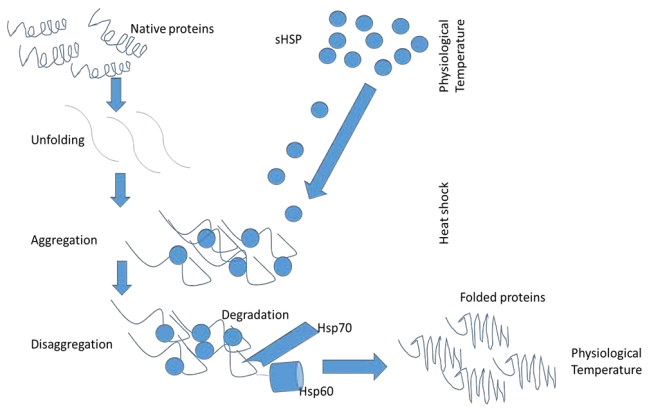

Figure 3: Cooperation between small heat shock proteins as ATP-Independent and ATP-dependent molecular chaperones during the folding of polypeptides under stress conditions [23].

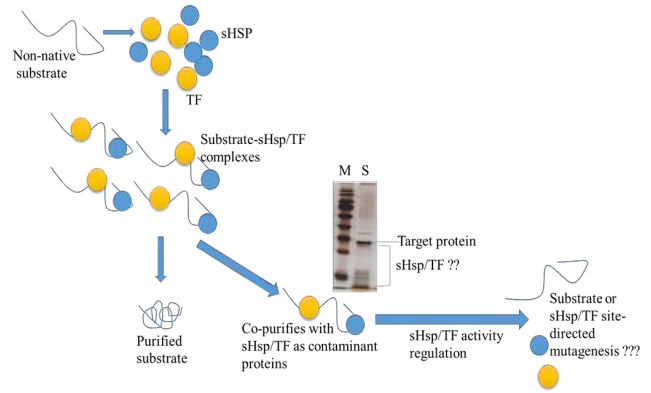

Figure 4: Proposed method of small heat shock regulation on substrate binding. Non-native proteins interact with small heat shock proteins including Trigger factor. Most protein co-pufiries with small heat shock proteins. In order to regulate sHsp in the system its either binding is controlled by performing site-directed mutagenesis on the substrate or heat shock proteins.

is hampered by the poor yields of recombinant protein. It has previously been proposed that co-expression of target recombinant malaria proteins with molecular chaperones of malarial origin in E. coli could improve the yield of the target recombinant proteins [13]. It was also proposed that PfHsp70-1 presumably protected E. coli cells against thermal stress by preventing protein aggregation [14]. Small heat shock protein from Caenorhabditis elegans (CeHsp17) was reported to prevent the cells exposed at 58oC for half an hour [15]. It has been suggested that molecular chaperones are cooperative in terms of helping substrate during folding process. Lee and Vierling [16], reported that small heat shock protein cooperates with Heat shock protein 70 system to reactive a heat-denatured protein. Thus, it may be suggested that co-expressing small heat shock proteins along with Hsp70produced in E. coli may improve the yield and quality of PfAdoMetDC/ODC proteins. Figure 3 shows a simplified model of small heat shock proteins as ATP-independent and heat shock protein 70 as ATP-dependent molecular chaperones interacting with aggregate prone substrates under stressful conditions.

The role of small heat shock proteins on the folding of PfAdoMetDC/ODC substrate

Previously conducted studies have shown that small heat shock proteins play a significant role under stressful conditions [16,15]. It has been widely reported that heterologous over-expression of certain exogenous molecular chaperones or an endogenous transcriptional regulator is able to significantly increase the viability of E. coli cells against heat shock treatment at lethal temperatures around 50°C [15]. This suggests the significant role played by molecular chaperones, in particular the small heat shock proteins, as protective tools inside the cellular system. However, as important as they may be, some molecular chaperones jeopardize the objective of obtaining pure proteins (Figure 4). As a result, the work aimed at developing a method or technique that may be utilized to regulate the activity of molecular chaperones is underway [17]. Admittedly so, these groups of proteins are needed for the folding steps of newly synthesized proteins but are not supposed to be in the final product of the target proteins. Williams and colleagues [6] reported that PfAdoMetDC co-purifies with endogenous molecular chaperones. The same scenario was also observed when PfAdoMetDC/ODC was expressed without the supplementation of molecular chaperones (Figure 4). As one of the malarial target proteins, it is very difficult to isolate this protein without the contaminant proteins, particularly small heat shock proteins. Thus, hampers the progress of developing alternative drugs against malaria.

Regulations of molecular chaperones as interactive partners of non-native proteins

Escherichia coli are the most popular system for recombinant protein production. Other researchers have developed an E. coli system that does not produce certain sizes of molecular chaperones in order to avoid contaminant proteins on the final product [18]. For example, the residence time for DnaK on peptides varies from 30 s to 25 minutes; only proteins that interact only with DnaK upon synthesis are released fast [18]. On the other hand, proteins that exhibit low cellular abundance, a higher number of DnaK-binding sites, and those that tend to assume dynamic structural intermediates (slow folding proteins which do not easily bury their hydrophobic patches) exhibit higher DnaK residence time. Typically, such proteins require DnaK for their maintenance. Consequently, the extended binding of DnaK to peptides may slow their folding, resulting in detrimental consequences. For this reason, it has been proposed that the expression of recombinant proteins in DnaK minus strains of E. coli be used to circumvent DnaK contamination [18]. This method however is not a solution for other recombinant proteins because it does not fulfill all the requirements of the protein that can be considered for structural characterization [18]. The inclusion of ATP-Mg during the washing steps of protein purification has also been used for removal of DnaK/Hap70molecular chaperone as a causative of contamination. This method has not been successful to all proteins produced in E. coli as a host system. However, the development of a method based on site-directed mutagenesis aimed at looking at the role or effects of small heat shock proteins on PfAdoMetDC/ODC protein produced both in their presence and absence in an E. coli system is essential. The development of this method may help in the production of pure proteins, particularly those that are known to be difficult to be produced both in pure and active form.

Conclusion

Escherichia coli is the most studied bacterium and is a widely

utilized host cell for producing pharmaceutically important recombinant proteins. The production of some recombinant protein has been difficult in this system owing to the extent of the quality of the final product and activity. Unfortunately, contaminated proteins cannot be considered for structure-functional studies towards drug design purposes. Endogenous molecular chaperones have been the cause of contamination to some recombinant proteins such as PfAdoMetDC/ODC, a malarial drug target protein [6]. As a result, investigating a universal method for the regulation of molecular chaperone activity is crucial. The main focus should be on small heat shock protein which seems to be the major cause of contamination on the final product of the purified proteins. One method to be explored is using site-directed mutagenesis to mutate those sites of molecular chaperones that seems to bind longer on the substrate. This can be either conducted to small heat shock proteins themselves or to the substrate and test their role or influence on the folding pathways of PfAdoMetDC/ODC produced in E. coli. This will be conducted both in vivo and in vitro system in order to get a clear picture of their role, thus regulate their activities in protein folding steps [22-25].

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