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FUNCTIONAL CHARACTERIZATIONS OF GROUP II CHAPERONIN AND PREFOLDIN FROM HYPERTHERMOPHILIC *THERMOCOCCUS* sp. KS-1 IN ORGANIC CO-SOLVENT

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ABSTRACT

Molecular chaperones have function in maintaining the quality of protein within a cell. Chaperones are indicating that are designed to function under stress. Chaperones functions under thermal stress have been intensively studied. Unfortunately, study about chaperone function under other stresses such as at extreme pressure or in chemical/solvent denaturants were limited. In this study we characterized the function of two kind chaperones from hyperthermophilic archaea *Thermococcus* sp. strain KS-1, prefoldin and chaperonin in organic co-solvent, by using polar protics and aprotic co-solvents. The results showed that the prefoldin and chaperonin only reduced aggregation of citrate synthase in 30% v/v acetonitrile which is polar aprotic. The chaperonin also showed ATPase activity in the 20% v/v acetonitrile. Based on those results we suggest that they could apply for maintaining protein substrate in the polar aprotic co-solvent for advance industrial application.

KEYWORDS

Chaperonin, prefoldin, organic co-solvent, stability and acetonitrile

INTRODUCTION

Molecular chaperones play central role in maintaining protein structure within a cell[1, 2]. There are consisting several kinds of molecular chaperones in the cell[1]. Prefoldin (PFD) and group II chaperonin (CPN) are two kinds of molecular chaperone which found exclusively in archaea and eukaryotic and have been studied that they have cooperative interaction each other,[3, 4, 5, 6]. PFD is heterohexameric protein composed of two or six kind of subunits[7, 8]. Biochemical studies have shown that PFDs capture and stabilize unfolded target polypeptides and subsequently deliver them to CPN for completion folding[4, 6, 9]. CPN consists of 60 kDa proteins assembled into double-ring toroidal with two cavities where folding occurs[10, 11].

Many chaperones are up regulated upon heat shock, indicating that are designed to function under stress. Chaperone-protein interactions upon thermal stress and over the temperature ranges at which the chaperones function have been extensively studied[12, 13, 14]. Unfortunately, characterization of chaperone functions under other stresses such as at extreme pressure or in chemical/solvent denaturants are limited. Regard to solvent stability, it was suggested that thermostable proteins are also more stable to solvent. The single subunit CPN from *Methanocaldococcus jannaschii* (rTHS) has been shown to preserve enzymes activities in water miscible organic co-solvent mixtures, the CPN stable in 30% v/v acetonitrile (ACN), 30% v/v ethanol (EtOH), 50% v/v methanol (MeOH) and 30% v/v tetrahidrofuran (THF) at 25°C[15].

We have published the results of various investigations into the protein-folding mechanism of PFD and CPN using hyperthermophilic PFD-CPN systems of *Thermococcus* sp. KS-1 (*T*. KS-1)[6, 13, 16, 17, 18]. *T.* KS-1 has two homologous chaperonin subunits, α and β[19, 20]. The natural composition of subunits in the hexadecameric double ring changes with temperature[20]. The β subunit is significantly more abundant at the higher temperature than the α subunit[20]. The homo-oligomer of the β subunit is also more thermostable than the homooligomer of the α subunit[19]. It has been suggested that the stability can be attributed to differences in 20 amino acids at the C-terminal end[21].

The *T.* KS-1 also expresses two pairs of prefoldin subunits genes; two α subunit genes, pfdα1 and pfdα2, and two β subunit genes, pfdβ1 and pfdβ2. We have functionally characterized four recombinant *T.* KS-1 PFD complexes (PFDα1-β1, PFDα1-β2, PFDα2-β1, and PFDα2-β2). All four complexes make similar hetero-hexameric structures and exhibited chaperone activity to suppress thermal aggregation. Moreover, the transfer of a denatured GFP to CPNα for folding was demonstrated for PFDα1-β1. The affinities of PFDα1-β1 and PFDα2-β1 for CPNα were almost identical whereas CPNβ exhibited

relatively strong affinity for PFD complexes[13]. A previous study on the cognate *Thermococcus* species, *T. kodakaraensis*, showed that PFDα1-β1 and α2-β2 complexes are dominantly expressed in cells. Transcriptional analysis and translational analyses showed that the β1 subunit of *T. kodakaraensis* is constitutively expressed under normal and heat-stressed conditions and β2 expression is highly induced at elevated temperatures[22].

In this study we characterize the stability of *T.* KS-1 PFDs and CPNs in organic co-solvent. Because *T.* KS-1 PFDα2-β2 and T.KS-1 CPNβ more termostable than *T.* KS-1 PFDα1-β1 and *T.*KS-1 CPNα, respectively, we suggested that they also more stable in organic co-solvent.

MATERIALS AND METHODS

(i) Bacterial strains, plasmids, reagents and proteins:

Escherichia coli strains BL21 Star (DE) (Invitrogen, Carlsbad, CA) was utilized for protein expression. *T.* KS-1 CPN wild-type homo-oligomers, Cpnα and Cpnβ, were expressed and purified as described[23, 24]. *T.* KS-1 PFDα1β1 and PFDα2β2 were also prepared as described previously[13]. The concentrations of CPN and PFD were determined employing the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA) using bovine serum albumin as the standard. The concentrations are expressed as molar concentrations of hexadecamer for CPN and heterohexamer for PFD.

(ii) Protection of CS from thermal aggregation

Thermal aggregation of CS was monitored with light scattering at 360 nm with a spectrofluorophotometer at 25 °C. CS (100 nM as monomer) was incubated in the assay buffer (100 mM Tris-Cl pH 8.0 containing 100 mM KCl, 5 mM DTT and 30% v/v organic co-solvent (methanol, ethanol or acetonitrile) in the presence or absence of chaperonins (50 nM) or prefoldin (100 nM).

(iii)ATP hydrolysis activity

ATP hydrolysis activities were measured at 25 and 60°C in 20% v/v acetonitrile and the buffer (50 mM Tris-HCl, pH 8.0, 300 mM KCl, 1 mM $MgCl_2$), respectively. In a 300 μl reaction mixture containing 1 mM ATP with or without 15 μg chaperonins. The reaction was initiated by the addition of ATP and terminated by mixing with ice-cold 2% perchloric acid. The amount of Pi that was produced by spontaneous hydrolysis of ATP was the substrate used to calculate the ATP hydrolysis activity.

RESULTS AND DISCUSSIONS

1. Chaperonin function in organic co-solvent

Chaperonin has two main functions, first, holding the protein substrate in preventing from aggregation or denaturation and second, assisting protein substrate for correct folding in ATP-dependent manner[16, 25]. To observe the function of *T.* KS-1 CPN in organic co-solvent, we used the activity of CPN in preventing citrate synthase (CS) from aggregation and ATP hydrolysis activity. Refer to the results of Bergeron *et al.* 2008, we used three different co-solvents, 30% v/v

EtOH and MeOH represent to polar protic solvent and 30% v/v ACN that represent to polar aprotic solvent[15], the result showed that the CS denatured and aggregates in the three conditions solvent, and this process can be observed with light scattering. When chaperonins are included in the 30% v/v EtOH and MeOH the aggregation of CS is not inhibited (Fig. 1A and 1B). It showed that the chaperonin lack their function in polar protic solvent. Interestingly in the case of 30% v/v ACN, the aggregation of CS in inhibited. The efficiency of preventing CS aggregation of CPNβ higher than CPNα, we suggest it because CPNβ more thermostable than CPNα.

The ATPase activities of CPNα and CPNβ in the aqueous buffer the ATPase activities of *T*. KS-1 CPNs depend on temperature in temperature below 70 °C, The ATPase activity of CPNα was higher than CPNβ and upper that the ATPase activity of CPNβ was higher than CPNα[19, 23]. Based on those results, we then examined the ATPase activities of the chaperonin in the 20% v/v ACN compared with in the aqueous buffer at 60 °C (Table 1), because the *T.* KS-1 CPNs almost not have activity below 40°C. Interestingly, that the ATPase activity of CPNβ in the 20% v/v ACN was higher than CPNα, this condition represent to the activity of CPNβ at the higher temperature. We suggest that thermostable of *T.* KS-1 PFD and CPN related to stability of them in polar aprotic co-solvent.

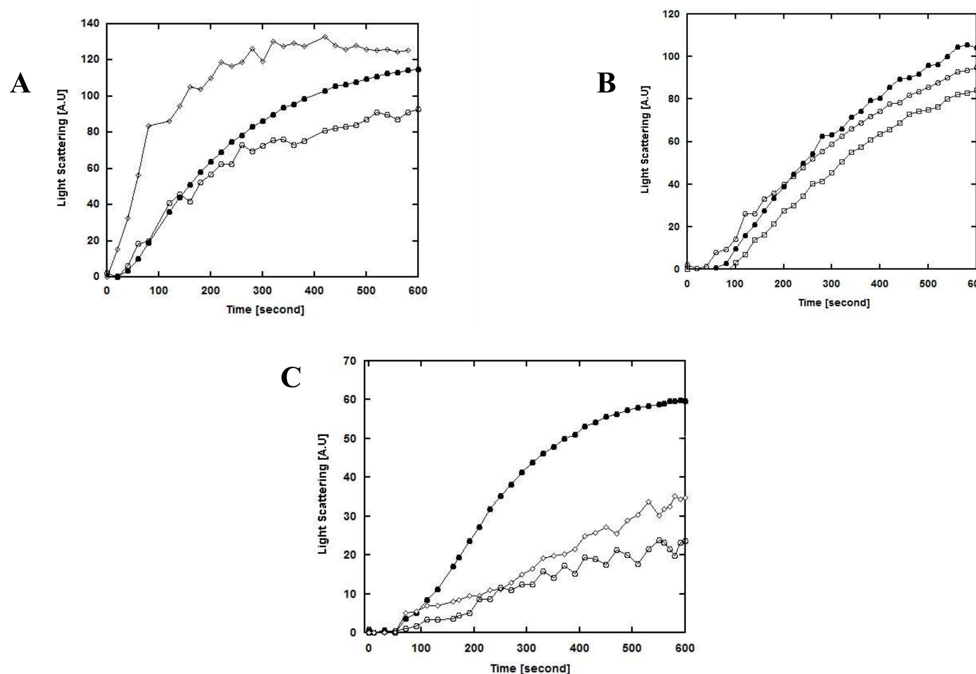

Fig. 1

Prevention of Citrate synthase (CS) aggregation by Chaperonin in organic co-solvents. Turbidity of 100 nM measured in TKM buffer at 360 nm and 25°C. CS aggregation in the absence of chaperone (filled circle), and in the presence 50 nM T. KS-1 CPNα (open diamond) and T. KS-1 CPNβ (open circle). A for 30% v/v ethanol (EtOH), B for 30% v/v methanol (MeOH) and C for 30% v/v acetonitrile (ACN).

Table 1
ATPase activities of T. KS-1 CPNs

	ATPase activity (nmol/min/mg)	
	CPNα	**CPNβ**
20% v/v acetonitrile at 25 ^{0}C	18.1	38.5
TKM buffer at 60 ^{0}C	32.7	20.3

2. *Prefoldin stability in acetonitrile 30% co-solvent*

Prefoldin is co-chaperone that captures protein substrate and transfers it to the group II chaperonin[9]. Recently, it was found that prefoldin also showed the ability to assist protein folding at low temperature in ATP independent manner[26].

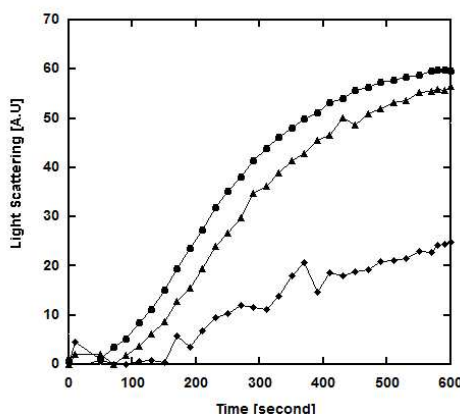

Fig. 2

Prevention of Citrate synthase (CS) aggregation by prefoldin in 30% acetonitrile. Turbidity of 100 nM measured in TKM buffer at 360 nm and 25°C. CS aggregation in the absence of chaperone (filled circle), in the presence 100 nM T. KS-1 PFDα1-β1 (filled triangle) and 100 nM T. KS-1 PFDα2-β2 (filled square).

The *T.* KS-1 expresses two pairs of prefoldin subunits genes; two α subunit genes, pfdα1 and pfdα2, and two β subunit genes, pfdβ1 and pfdβ2. We have functionally characterized four recombinant *T.* KS-1 PFD complexes (PFDα1-β1, PFDα1-β2, PFDα2-β1, and PFDα2-β2). All four complexes make similar hetero-hexameric structures and exhibited chaperone activity to suppress thermal aggregation[13]. A previous study on the cognate *Thermococcus* species, *T. kodakaraensis*, showed that PFDα1-β1 and α2-β2 complexes are dominantly expressed in cells. Transcriptional analysis and translational analyses showed that the β1 subunit of *T. kodakaraensis* is constitutively expressed under normal and heat-stressed conditions and β2 expression is highly induced at elevated temperatures[22].

To confirm the correlation between thermal stability and organic co-solvent stability, we analyzed the activity of *T.* KS-1 PFDs in 30% v/v ACN. In this study, we

selected PFDα1-β1 and PFDα2-β2, and analyzed their ability to prevent CS aggregation caused by solvent stressing. Figure 2 showed the result of analysis, the CS denatured and aggregates in the 30% v/v ACN and this process can be observed with light scattering. When PFDs are included in solution, only by PFDα2-β2 the aggregation of CS in inhibited, the PFDα1-β1 could not prevent the CS aggregations.

The result reinforces our suggestion that the stability of chaperones in polar aprotic co-solvent related to thermal stability of chaperone at high temperature. We suggest that in the polar protic co-solvent such as methanol and ethanol the hydrogen bonding inhibited the function of chaperones, whereas no hydrogen bonding in the polar aprotic co-solvent.

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