Stability Comparisons between Natural Archaeal and Engineered Archaeal-Bacterial Heat-Shock Protein Subunits (α, β and β-cohesin) and their Oligomeric Complexes

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ABSTRACT

Hyperthermophilic archaea of family Sulfolobaceae (Order: Sulfolobales) express group II chaperonins, which are also commonly referred to as “heat-shock proteins” (HSPs) due to their upregulation upon heat shock. These HSPs interact with nascent polypeptides in the cell and subsequently assist in their folding into a more stable structure, and this interaction is often considered key in understanding the system as an enzyme protection platform for use in industrial applications. In this study, we aimed to understand the stability of the “enzyme-platform” complex.

In this study, we investigated the stability of HSP subunits (natural and engineered) and HSP complexes with different subunit compositions assessed towards improving MESP technology for use in industrial applications.

REFERENCES


HSP Subunit Structure/ Stability:

METHODS AND RESULTS

HSPs pH-dependent Stability and HSP Subunit Flexibility

CONCLUSIONS

HSPs and HSPβ are close in thermostability (up to 95°C) and their homomeric complexes have higher thermostability than heteromers. HSPα and HSPβ-cohesin were all purified to homogeneity. The CD spectra of each subunit reveals largely helical structures. Comparison of the DSC thermograms of each subunit reveals that HSPs and HSPβ-cohesin are the most thermostable with Tm = 95°C while HSPα-cohesin was slightly lower (Tm = 88°C). HSP complexation (homomeric or heteromeric) is verifiable by TEM and Native PAGE gels. HSP complexes were analyzed by DSC, evidenced by increased stability of HSP complexes. We found that the highest Tn, HSPα-cohesin was further examined. We found that HSPs retains its helical secondary structure when subjected to pH conditions from 2 to 10. HSPs was subjected to tryptic digestion and compared to BSA. HSPα is cleaved by trypsin to a larger extent than BSA indicating that HSPα has more flexible backbone structure.

Fig. 1 - SDS-PAGE and CD of HSP subunits. (A) Purity of HSP subunits are visualized on silver-stained SDS-PAGE. HSPα and HSPβ migrate to ~60 kDa. HSPα-cohesin migrates to ~75 kDa. (B) Circular Dichroism (CD) overlay of HSP subunits indicates that each has a predominately helical secondary structure with negative ellipticity bands at 222 nm and 288 nm.

Fig. 2 - DSC of HSP subunits. Differential scanning calorimetry (DSC) thermograms of: (A) HSPα, (B) HSPβ, and (C) HSPα-cohesin. DSC data indicate that HSPs has the highest melting melting temperatures at (Tm). The Tm of HSPα is significantly lower than HSPβ and HSPα-cohesin.

Fig. 3 - TEM & Native PAGE. HSP Complexes (A) Purified HSPα (1 mg/ml) forms a double-ring; (B) Purified HSPβ-cohesin (1 mg/ml) also forms double glass of archaeal-bacterial fusion protein construct; (C) equimolar concentration of HSPα and HSPβ-cohesin (0.5 mg/ml each) also forms double ring complex; (D) HSPα homomers, HSPβ-cohesin homomers, and HPSα-HSPβ-cohesin heteromers via Native PAGE.

Fig. 4 - DSC of HSP Complexes. Differential scanning calorimetry of HSP Complexes. The HSPα-HSPβ-cohesin heteromers complex indicates that the homomeric complexes are more thermostable than the heteromeric complex.

Fig. 5 - pH dependent CD spectra: HSPα, HSPβ, and HSPα-cohesin. HSPα retains its alpha helical 2 structure when subjected to pH 2 through 10.

Fig. 6 - Backbone flexibility: HSPα vs. BSA. (A) Structural comparison of HSPα (right) and BSA (left) reveals that HSPα is globally largely alpha helical with similar quantities of tryptic cleavage sites, 72 and 78, respectively. Both have similar sizes (~60 and 66 kDa), number of amino acids (560 and 670), and PI (5.34 and 5.82), respectively. (B) HSPα is less resistant to tryptic at 5%, with variable comparisons HSPα is ~30% more digested than BSA.

Fig. 7 - HSP subunit sequence alignment. Alignment of HSPα, HSPβ, and HSPα-cohesin with CLUSTAL 1.2 multiple sequence alignment. Residue color code: negative-charge (red), positive-charge (blue), polar (magenta), hydrophobic (yellow). Similar residues are shown by asterisks and periods, respectively. Cαβ cohesion residues 179-326 (red box).