SUPPLEMENTAL DATA

Analytical ultracentrifugation confirms presence of stable reduced Hsp33 dimer

Oxidized Hsp33 dimers and reduced Hsp33 monomers were shown to sediment with apparent s-values of 2.78 S and 1.74 S, respectively. In contrast, oxidized Hsp33 dimers that were incubated for 30-70 min in 5 mM DTT at 20ºC sediment with an intermediate s-value of 2.35 S. This indicated that even after a substantial incubation time in DTT, a mixed population of Hsp33 dimers and monomers still exists in solution. These experiments showed that reduced Hsp33 dimers are kinetically stable even in the absence of bound substrate proteins.

MATERIAL AND METHODS FOR SUPPLEMENTAL DATA

Analytical ultracentrifugation

Hsp33 (1 mg/ml) was analyzed either in its oxidized dimeric or short-term reduced state. For comparison, the constitutively monomeric mutant Hsp33E150R was also analyzed. To reduce Hsp33, oxidized Hsp33 dimers were incubated in the presence of 5 mM DTT at 20°C. 15 min after initiating the reduction, the determination of Hsp33's sedimentation velocity was started. Sedimentation velocity scans were measured in a Beckman XL-A analytical ultracentrifuge using double sector cells and an AnTi 50 rotor. The experiments were performed at 40,000 rpm and 10°C. Scans were recorded at 280 nm every 8 min. The s-values were calculated from scans taken 30 – 70 min after starting the experiment.

Analysis of Hsp33's complex formation

To analyze the long-term stability of Hsp33-substrate protein complexes, 0.5 □M luciferase was incubated in the presence of 0.75 □M Hsp33 dimers at 43°C in 40 mM HEPES-KOH, pH 7.5. At defined time points, aliquots were taken and the insoluble aggregates were separated by

centrifugation (13,000 rpm, 20 min, 20ºC). The supernatants were mixed with 1/5 volume of Laemmli buffer and analyzed on a 14% SDS-PAGE (Novex). The protein bands were visualized using a very fast Coomassie staining procedure (Wong *et al.*, 2000).

Thermal aggregation of luciferase

Luciferase (0.5 μM) was incubated in 40 mM HEPES-KOH, pH 7.5 at 43ºC either in the absence or presence of various amounts of dimeric Hsp33 (Jakob *et al.*, 1999). Light scattering was determined using a fluorescence spectrophotometer (Fluoromax) with $\lambda_{ex/em}$ set to 600 nm, and slit widths set to 2.5 (ex) and 5.0 (em).

Titration of BSA

The equilibrium complex formation of oxidized dimeric Hsp33 and BSA was monitored by titrating BSA to 5 µM dimeric, Oregon green labeled Hsp33. At each step of the BSA addition, the sample was incubated for 1 min before the anisotropy was measured at λ_{ex}=506 nm and λ_{em}=524nm. The titration curve was fitted according to following equations:

$$S = S_{max} - dS*(RL/R0)$$

With $RL = (R0 + L0 + K_D)/2 + (((R0 + L0 + K_D)/2)^2 - R0 *L0)^{-0.5}$

Where S is the anisotropy measured, S_{max} and dS represent the maximum anisotropy and the amplitude, respectively. R0 and L0 are the total concentrations of Hsp33 dimers and BSA, respectively. K_D is the dissociation constant of the complex formed.

LEGENDS FOR SUPPLEMENTAL FIGURES

Figure 1: Hsp33 works as an efficient chaperone holdase

Dimeric Hsp33 forms 1:1 complexes with thermally unfolding luciferase

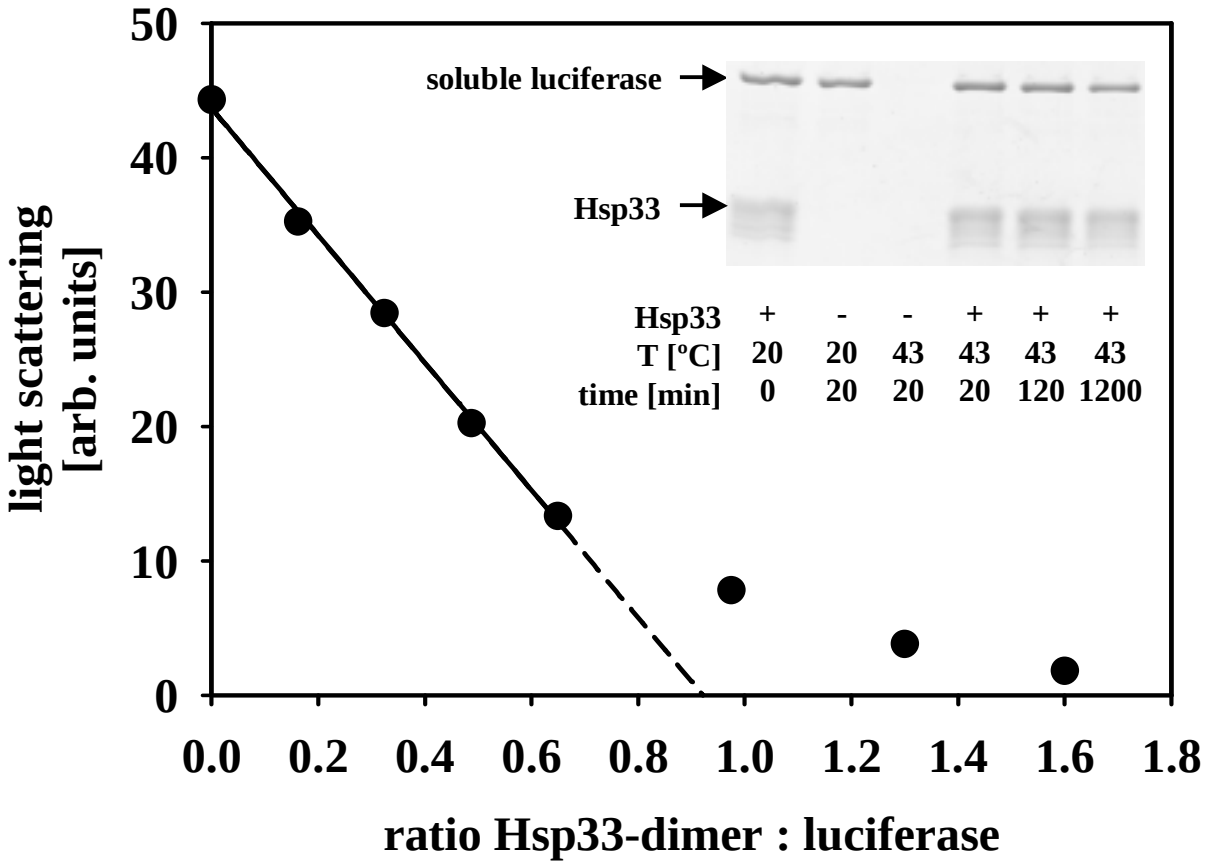
Firefly luciferase (0.5 μM) was incubated in the absence or presence of increasing concentrations of dimeric, active Hsp33 at 45ºC for 20 min. The extent of aggregation was monitored using light scattering measurements (●). Linear extrapolation of the inhibition of luciferase aggregation at substoichiometric concentrations of Hsp33 indicated that a 1:1 complex between luciferase and Hsp33 dimer is formed.

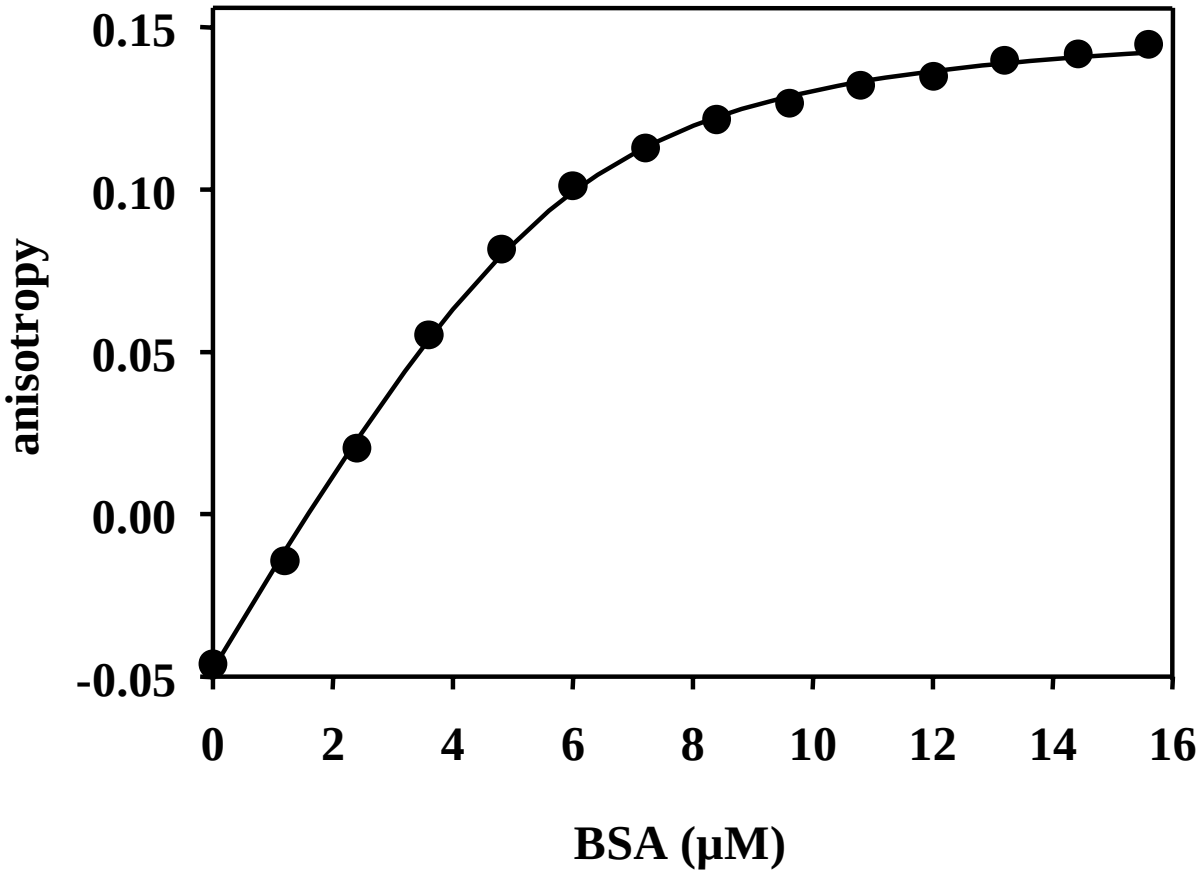
Insert: Hsp33 provides long time protection against heat induced protein aggregation.

Luciferase (0.5 □M) was incubated in the absence or presence of 0.75 μM oxidized Hsp33 dimers at either 20ºC (no heat shock) or 43ºC (heat shock). At the time points indicated, aliquots were taken and the aggregated luciferase molecules were removed by centrifugation. The soluble supernatant was analyzed by SDS-PAGE.

Figure 2: BSA binds to oxidized Hsp33 dimers with low affinity

Oregon green labeled, oxidized Hsp33 at a protein concentration of 5 µM was titrated with increasing concentrations of BSA (closed circles). After each addition, the anisotropy of the Hsp33-bound fluorescence label was quantified (λ_{ex}=506 nm and λ_{em}=524nm). The fit of the titration curve revealed a 1:1 stoichiometry of the Hsp33 dimer : BSA complex and a dissociation constant of K_D = 0.66 µM.


soluble luciferase
Hsp33
Hsp33 + - - + + +
T [°C] 20 20 43 43 43 43
time [min] 0 20 20 20 120 1200
light scattering [arb. units]
0
10
20
30
40
50
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
ratio Hsp33-dimer : luciferase


0.15
0.10
0.05
0.00
-0.05
anisotropy
0
2
4
6
8
10
12
14
16
BSA (µM)