Prevention of Protein Aggregation by Hsp104 *via* Protection of Oxidative Stress

Kyunghee Lee,[*] Youngwhan Cho, Jeonghoon Ueom, Sunmi Na, Jeong Eun Park,[†] Inho Jo,[†] and Jongmin Kang

Department of Applied Chemistry, Sejong University, Seoul 143-747, Korea
[†]Division of Cardiovascular Research, Department of Biomedical Sciences, National Institute of Health, Seoul 122-701, Korea
Received May 25, 2001

Keywords : ROS, Hsp104, Mitochondria, Aβ aggregation.

Aerobic organisms must deal with toxic reactive oxygen species (ROS) which are generated during the normal course of respiratory metabolism. They have therefore developed multiple defense mechanisms, by which they got rid of intracellular ROS to avoid oxidative damages.[1]

In this report we have focused on Hsp104 protein as a candidate molecule for a powerful protector against oxidative stress due to ROS production. We have first attempted to isolate mitochondria from both wild type and 104 mutant to confirm the unbalanced respiration pathway occurred when intracellular expression of Hsp104 protein was abolished. A functional role of Hsp104 was then demonstrated as a protector of oxidative stress due to impairment of mitochondrial integrity. The observation that Hsp104 works as an antioxidant protein led us to purify Hsp104 protein and further tested it as a molecular mediator of Aβ fibril aggregation in Alzheimer's disease (AD) for the first time.

In our previous studies, we have observed that Hsp104 protein function as a respiratory regulator in *Saccharomyces cerevisiae*, judging from comparison data of the metabolic turnover rates in the TCA cycle for both wild type and Δ104 using ^{13}C-NMR spectroscopy as well as measurements of O_2 consumption.[2] From the fact that 104 consumes more oxygen than wild type, we can envision that Hsp104 helps mitochondria efficiently accomplish metabolic functions within cells. For example, in the absence of Hsp104, the integrity of mitochondrial membrane may be lost which results in uncoupling of oxidative phosphorylation. It is also possible that oxygen can not be fully reduced due to the loss of electron transfer control through the mitochondrial inner-membrane in case that Hsp104 is absent. As a result, more ROS can be produced as by-products to cause oxidative stress within cells.

These assumptions led us to examine if mitochondria perform metabolic functions differently depending on the existence of Hsp104 at first. We isolated mitochondrial fractions from a homogenate of yeast spheroplast by sucrose differential centrifugation and tested their functions by determination of mitochondrial membrane potential (Table 1).[3] Mitochondrial fractions from both wild type and 104 were examined for uptake of the fluorescent dye 3,3'-dipropylthiocarbocyanine iodide (Molecular probe), which can be taken up by mitochondria in a potential-dependent manner. As reported in Table 1, wild type cells took more dye up to 117% as compared with Δ104 mutant. The concentration of mitochondrial proteins was higher in wild type about 2 fold,

Table 1. Functional Assays of Purified Mitochondria

	Cell Numbers	Protein Concentration[a] (mg/cell)	Fluorescence Intensity[b] (%)
Wild type	5.25×10^6	1.26×10^{-7}	117
Δ104	5.28×10^6	0.59×10^{-7}	100

[a] Determined by Bradford method. [b] Assays were performed at 100 μg/mL mitochondria concentration. Fluorescence was measured with excitation at 620 nm and emission at 670 nm.

suggesting that Δ104 has lost significant amount of functional mitochondria. From the viewpoint of the diminished membrane potentials between two strains determined at the same protein concentration, we can also conclude that oxidative phosphorylation is to some extent uncoupled in Δ104, which is in a good correlation with the higher consumption of oxygen as reported earlier.[2]

In order to check the second possibility in which Hsp104 provides a crucial protection against oxidative damage, we have examined survival rates by colony counting assays. Both wild type and Δ104 mutant were grown into a log phase using acetate as a carbon source to induce Hsp104 protein expression and treated with either 1.0 mM H_2O_2 for 10 min or 0.1 mM menadione for 20 min. Cells were washed and spread onto YPD plates and counted after 48 hr incubation at 25 °C (Figure 1A). As we expected, W303 with Hsp104 protein expressed exhibited a resonable survival rate whereas Δ104 without Hsp104 protein experienced severe oxidative damage.

We have also measured production of ROS by fluorescence of 2',7'-dichlorofluorosin diacetate (H_2DCF)[4] to verify that the cell survivals enhanced by the presence of Hsp104 protein is directly related to protection from oxidative stress. As expected, with hsp104 gene deleted, Δ104 produced the significantly increased amount of ROS in a dose dependent manner, thereby going through more severe oxidative stress (Figure 1B). Based on these data we were able to demonstrate *in vivo* for the first time that Hsp104 helps cells survive against various oxidative stress, otherwise harmful to cells. It can be further envisioned that Hsp104 may function as a molecular chaperone to prevent misaggreagtion of proteins or repair structurally altered proteins due to oxidative stress.

To date, various molecular disease, such as Alzheimer's Disease (AD), Parkinson Disease (PD) and Creutzfeldt-Jakob disease, are discovered to result from accumulation of damaged protein induced by oxidative stress.[5] Protection of these misfolded proteins by expression of molecular chaperones may be therefore used for therapeutic strategy to

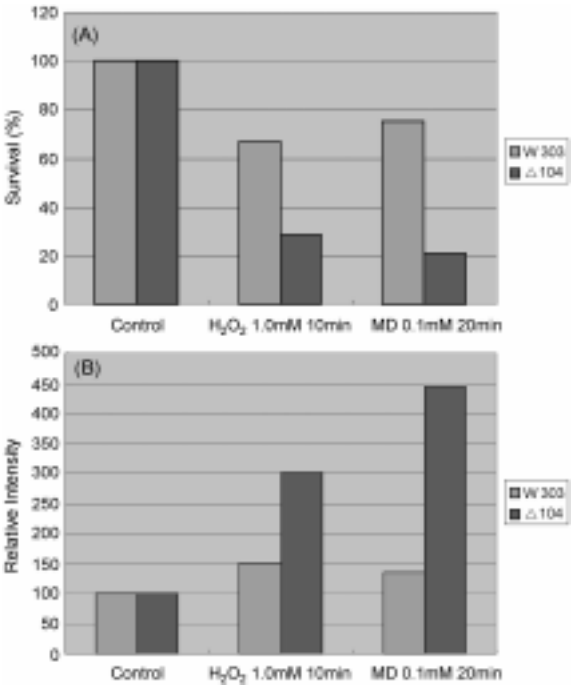

Figure 1. (A) Survival rates of wild type and Δ104 mutant upon oxidative stress. (B) Production of ROS measured by fluorescence test.

prevent or treat these diseases. Especially small heat shock proteins have been intensively studied for their potential roles in human disease by preventing aggregation of cellular proteins during various stresses.[6] In the brain of AD patients, amyloidβ (Aβ) assembles into neurotoxic fibrils and is deposited as plaques. It was found that αB-crystallin was colocalized in the plaques and prevented aggregation of Aβ protein.[7] Hsp27 was also recently reported to be localized in glial cells in the rat brain and transported into the synaptic enriched layer of the cerebellium following hyperthermia.[8] It has been strongly suggested that stress-induced Hsp27 may contribute to repair and protective mechanisms at the synapse.

Here we tested yeast Hsp104 as a protector of aggregation of Aβ protein since it has been known as a functional analog of mammalian Hsp27. More interestingly, Lindquist and coworkers have reported that Hsp104 helped cells resolubilize certain proteins which was aggregated resulting from severe heat shocks.[9] They have also found that overexpression of Hsp104 modulated aggregation of polyglutamine (polyQ) expansions found in Huntington's disease (HD) whereas the deletion of Hsp104 virtually eliminated it.[10]

Hsp104 protein was expressed from A750 strain (BJ5457/p2UG104, pG-N795) by induction with deoxycorticosterone. It was subsequently purified by serial chromatographys using Affi-Gel Blue, DEAE and Superose 6 Gel Filtration column.[11] The presence of Hsp104 in each fraction was confirmed by Western blotting using Ab_{Hsp104} (StressGen) and silver straining. In order to check if the purified Hsp104 acts as a protector of Aβ fibril formation, Congo red assays were performed in 96 well plates following 24 hr incubation and the amount of bound Aβ was calculated as Cb[M] = (A_{540}/25,295 – A_{480}/46,306).[12] Assays were performed with Hsp27

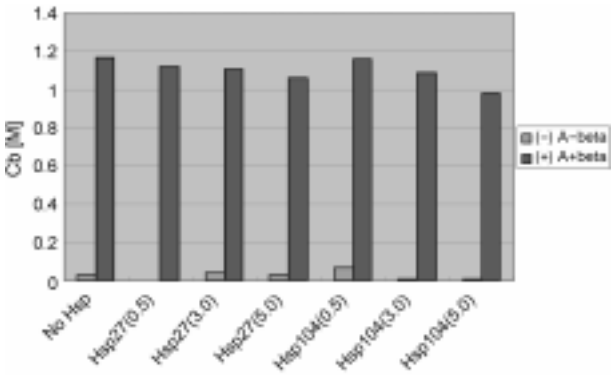

Figure 2. Effects of purified Hsp104 on Aβ aggregation determined by Congo-red assays. Either Hsp27 or Hsp104 fraction was incubated in a dose-dependent manner in the absence (left) and the presence of 100 μM Aβ (right).

control, whose preventing activity of fibril formation has been earlier demonstrated.[13] We present intriguing observations for the first time in which Hsp104 blocks aggregation of Aβ peptide (at 100 μM) in a dose-dependent manner (Figure 2). The degrees of protection by Hsp104 are comparable to those of Hsp27. Dependence of the presence of ATP on blocking of Aβ fibril formation as well as depolymerization of aggregated Aβ is currently under investigation. These findings will be applied to further elucidate the chaperone effect of Hsp104 in other types of neurodegenerative diseases closely related with oxidative stress.

Acknowledgment. We are grateful to a financial support by Korea Research Foundation Grant (KRF-99-015-DP0240). The authors give sincere thanks to Sooim Kang for technical help throughout the study.

References

1. Halliwell, B.; Gutteridge, J. M. C. *Biochem. J.* **1984**, *219*, 1.
2. Lee, K.; Kang, S.; Lindquist, S. *Bull. Korean Chem. Soc.* **1998**, *19*, 295.
3. Yaffe, M. P. *Methods in Enzymol.* **1991**, *194*, 627.
4. Griffith, O. W. *Anal. Biochem.* **1980**, *106*, 207.
5. Thomas, P. J.; Qu, B.-H.; Pedersen, P. L. *Trends in Biol. Sci.* **1995**, *20*, 456.
6. Clark, J. I.; Muchowski, P. J. *Curr Opin. Struc. Biol.* **2000**, *10*, 52.
7. Stege, G. J. J.; Renkawek, K.; Overkamp, P. S. G.; Verschuure, P.; van Rijk, A. F.; Reijnen-Aalbers, A.; Boelens, W. C.; Bosman, G. J. C. G.; de Jong, W. W. *Biochem. Biophys. Res. Commun.* **1999**, *262*, 152.
8. Bechtold, D. A.; Brown, I. R. *Mol. Brain Res.* **2000**, *75*, 309.
9. Parsell, D. A.; Kowal, A. S.; Singer, M. A.; Lindquist, S. *Nature* **1994**, *372*, 475.
10. Krobitsch, S.; Lindquist, S. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 1589.
11. Schirmer, E. C.; Queitsch, C.; Kowal, A. S.; Parsell, D. A.; Lindquist, S. *J. Biol. Chem.* **1998**, *273*, 15546.
12. Klunk, W. E.; Pettegrew, J. W.; Abraham, D. J. *J. Histochem. Cytochem.* **1989**, *37*, 1293.
13. Kudva, Y. C.; Hiddinga, H. J.; Butler, P. C.; Mueske, C. S.; Eberhardt, N. L. *FEBS Lett.* **1997**, *416*, 117.