# This student paper was written as an assignment in the graduate course

## *Free Radicals in Biology and Medicine*

**(77:222, Spring 2003)**

**offered by the**

**Free Radical and Radiation Biology Program**
**B-180 Med Labs**
**The University of Iowa**
**Iowa City, IA 52242-1181**
**Spring 2003 Term**

**Instructors:**
**GARRY R. BUETTNER, Ph.D.**
**LARRY W. OBERLEY, Ph.D.**

**with guest lectures from:**
**Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann**

**The Fine Print:**

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

# Heat Shock Protein 90
# The Shock Absorber

By
Kimberly Krager

B180 ML
Department of Free Radical and Radiation Biology
The University of Iowa
Iowa City, IA 52242-1181

For 77:222, Spring 2003

13. March 2003

Abbreviations:
AAPH: 2,2'-azo*bis*-(2-amidinopropane)-dihydrochloride
CaM: Calmodulin
DEPMPO: 5-diethoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide
EPR: Electron paramagnetic resonance
GA: Geldanamycin
Hsp 90: Heat Shock protein 90
L-NAME: *N*-nitro-L-arginine methyl ester
MDA: Malondialdehyde
NOS: Nitric oxide synthase
nNOS: Neuronal nitric oxide synthase

**Table of contents** **Page**



**Abstract**

Heat shock protein 90 is a 90 kDa cytosolic protein. It functions as a chaperone protein helping newly formed proteins and partial unfolded proteins into the correct configuration. Heat shock protein 90 forms a heterocomplex to bind the protein needing assistance and ATP. During oxidative stress, heat shock protein 90 levels are increased to assist partial degraded proteins to refold. It also assists in the reduction of superoxide by nitric oxide synthase. The reduction in superoxide ($O_2^{\bullet-}$) prevents $O_2^{\bullet-}$ from reacting with nitric oxide to form peroxynitrite and damaging cellular biomolecules. Heat shock protein 90 could also be used as a biomarker for the overall oxidative stress in an individual. This paper will focus mainly on the structure and its role in nitric oxide regulation.

## Introduction

Experiments performed on *Drosophila melanogaste*r showed cells subjected to increased temperatures induced a defined set of new transcription[1]. The discovery was then overlooked until 30 years later when two independent groups identified heat shock protein 90 (hsp 90) in 1986[1]. It was determined to be a dimeric 90 kDa non-receptor protein. Heat shock proteins are molecular chaperones that are involved in assisting newly synthesized proteins to fold in the correct orientation. It was also found to modulate the activities of transcription factors and regulates cell cycle. Hsp 90 was discovered in several different species ranging from Drosophila to humans[1]. The proteins that possessed the hsp 90 receptors belong to nuclear hormone receptors or protein kinases. Hsp 90 is induced when the cell is considered stressed, due to heat, metals, xenobiotics, and oxidative conditions [1]. This paper will discuss the role hsp 90 plays in the cell's oxidative environment.

## Characteristics of Hsp 90

Hsp 90[1] is a 90 kDa cytosolic protein that is highly abundant in higher eukaryotes. At least two isoforms exist in mammalian cells, hsp 90α and hsp 90β. Hsp 90 is a functional homodimer with three functional domains, COOH-terminal domain, the $NH_2$-terminal domain and a central charged domain[1]. The C-terminal structure appears to contain two binding sites for unfolded or partially folded polypeptides. It is also an independent ATP binding site for chaperone function and dimerization. The N-terminal domain contains a binding pocket that is the site for ADP-Mg, ATP and Geldanamycin (GA) binding[1]. The binding of ATP confers a

[1] World Wide Web at http://www.arches.uga.edu/~algraves/bcmb8010/Heat%20Shock%20Protein%2090.pdf Heat Shock Protein 90

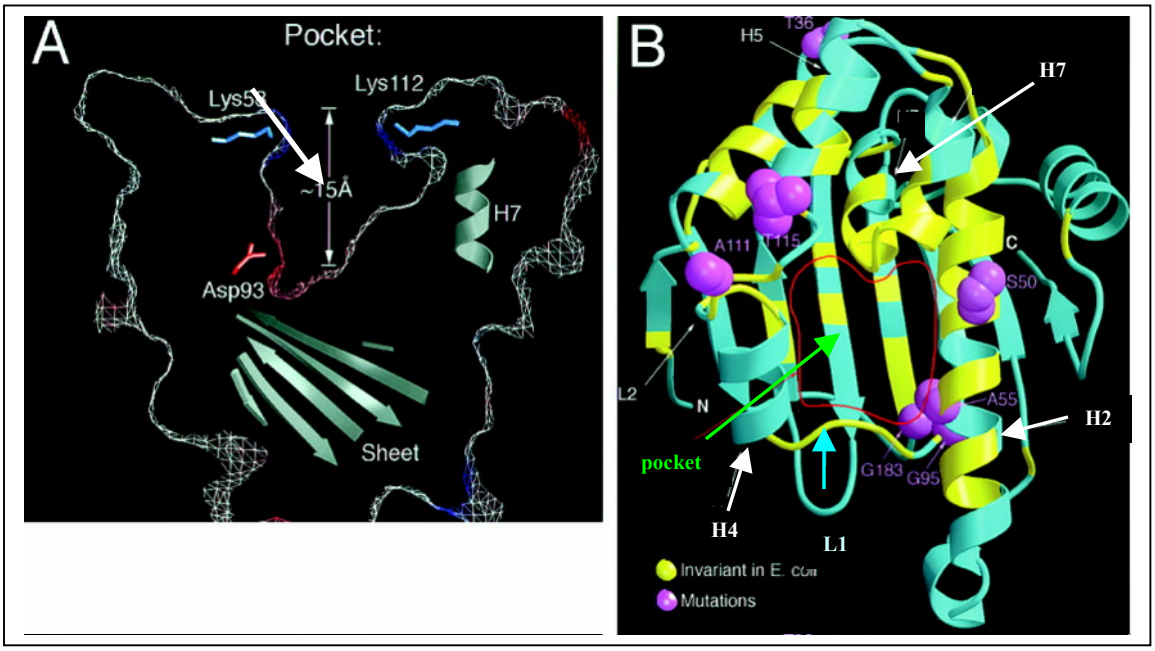


**Figure 1a**. This represents a thin slice of the hsp 90 molecular surfaces stressing the binding pocket indicated by the white arrow [1].
**Figure 1b.** Represents the beta sheets, the pocket (green) with the H2, H4, H7 helices (white) and L1 loop (blue) [1].

conformational change, which releases bound polypeptides. The cone like pocket was observed to have two configurations, open and closed, the closed configuration is incompatible with ADP. The depth of the pocket is about 15 Å and formed by an antiparallel beta sheet with H2, H4, H7 helices and L1 loop forming the walls (Figure 2) [1].

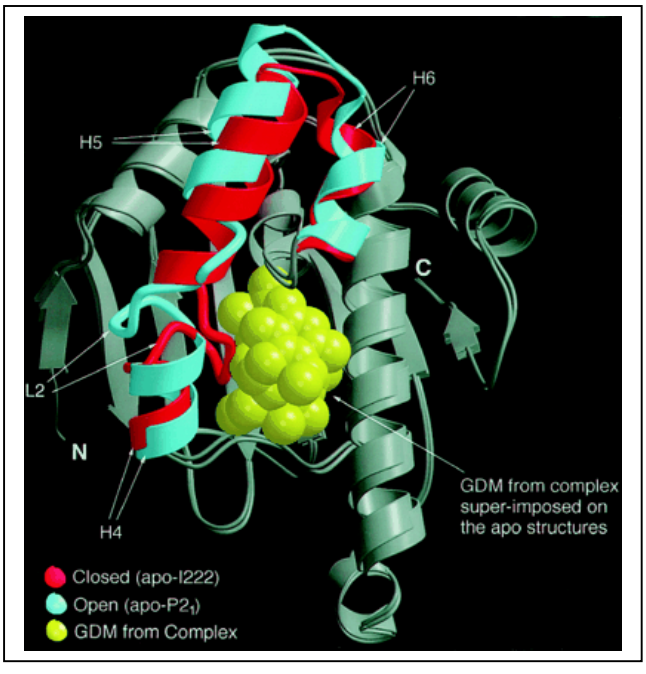


**Figure 2**. Representation of the L2 loop that can swing like a gate to the closed or open configuration [1].

The pocket has a mix of hydrophobic and polar residues, but the closer to the bottom the more hydrophobic the pocket is. The L2 loop confers the open-closed configuration, acting like a gate that constricts the actual pocket size from 12 Å to 8 Å. The movement of L2 is facilitated by the H4 and H5 helices (Figure 2) [1]. The central charged domain is about 35 kDa and facilitates the stability of the functional dimeric form.

Hsp 90 forms a heterocomplex with hsp 70, the complex is unstable and requires another protein, a heat shock co-chaperone Hop, to provide a stable link. Hop is removed after assembly to generate the ATP binding site[1].

**Nitric Oxide Synthase Regulation**

Nitric oxide ($NO^{\bullet}$) plays a key role in the cardioprotection, proliferation of endothelial cells, neuronal signal transmission and immune response [2, 3]. The observed increase of $NO^{\bullet}$ is through dual production of $NO^{\bullet}$ and superoxide by nitric oxide synthase (NOS) [2]. The dual increase of both $NO^{\bullet}$ and superoxide is a prerequisite for the formation of peroxynitrite (reaction 1) [2]. The rate constant for (reaction 1) is thought to be $6 \times 10^{9}$ $M^{-1}s^{-1}$ to $1.9 \times 10^{10}$ $M^{-1}s^{-1}$, which is very fast [4, 5]. The Gibbs energy changes for (reaction 1) is –22 kcal/mol, and is a highly favorable reaction.

$$\mathbf{NO^{\bullet} + O_2^{\bullet -} \rightarrow O{=}NOO^{-}} \qquad \mathbf{(1)}$$

It has been hypothesized that to enhance the generation of $NO^{\bullet}$ by neuronal nitric oxide synthase (nNOS) a protein-protein interaction with hsp 90 occurs [6]. The interaction plays a critical role in increasing $NO^{\bullet}$ while offsetting the increase in superoxide to reduce the oxidative environment of the cell. To determine if hsp90 interaction was affecting superoxide production, electron paramagnetic resonance (EPR) experiments were performed [6]. The

spin-trap 5-diethoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide (DEPMPO) was employed to trap superoxide, form the stable spin trap adduct DEPMPO-OOH, which a measurement of superoxide formation. The control experiments are performed without nNOS and failed to produce superoxide. However, the addition of nNOS produced a strong signal characteristic of DEPMPO-OOH spectrum. The signal was abolished with the addition of superoxide dismutase determining that superoxide was the primary oxygen free radical produced. *N*-nitro-L-arginine methyl ester, (L-NAME) a nNOS inhibitor, was used to confirm that nNOS was responsible for the production of superoxide. Hsp 90 was then incubated at varying concentration with nNOS and then the superoxide production was measured. Hsp 90 was able to inhibit the production of superoxide by NOS in a dose-dependent manner (Figure 3) [6].

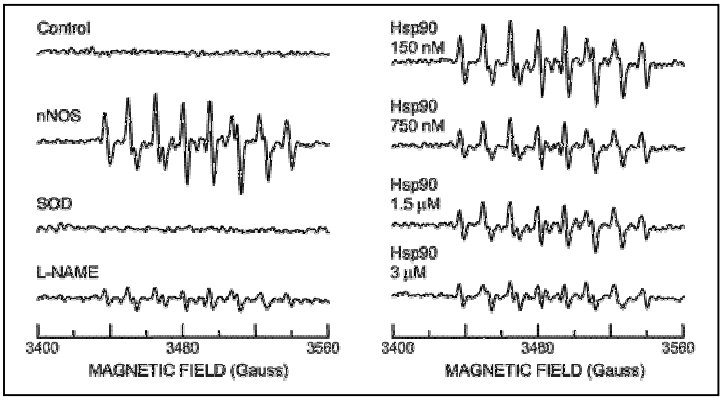


**Figure 3**. The control is without NOS and no signal is observed. NOS produces the characteristic signal of the superoxide adduct which is abolished with the addition of superoxide dismutase. L-NAME was also used and the signal decreases demonstrating that nNOS is responsible for the superoxide production. Then the addition of varying concentrations of hsp 90 produced a decrease in superoxide signal [6].

Experiments were performed to determine if the reduction of superoxide was due to scavenging mechanisms or regulation of nNOS. EPR spin-trapping was performed with xanthine oxidase generating superoxide. The control (xanthine oxidase generated superoxide) again displayed the characteristic DEPMPO-OOH signal, which was abolished with the addition of superoxide. Hsp 90 was unable to decrease the generation of superoxide as observed in Figure 4 [6]. There was no detectable decrease in the superoxide signal observed when hsp 90 was incubated with xanthine oxidase. The signal was unable to be decreased with the addition of hsp 90 demonstrating that

hsp 90 was not scavenging superoxide but directly affecting nNOS [6]. The following schematic represents several ways hsp 90 could be affecting the decrease in superoxide (Figure 5) [6].

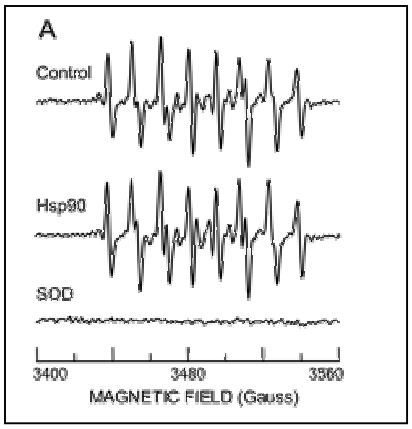


**Figure 4**. EPR spectrum of superoxide generated by xanthine oxidase. The control signal received when superoxide was generated by xanthine oxidase. The signal was unable to be decrease by the addition of hsp 90 proving that hsp 90 is not a scavenger of superoxide. The signal was abolished with the addition of superoxide dismutase [6].

Several studies have shown an increase in calmodulin (CaM) binding may be one mechanism or hsp 90 may inhibit the release of superoxide from nNOS. The second mechanism was suggested by the observations in Figures 3 and 4. The inhibition of superoxide through hsp 90 was able to enhance nNOS production of nitric oxide. The reduction of superoxide means there is less superoxide that can react with $NO^{\bullet}$. This reaction can form potent oxidants such as peroxynitrite, which is involved oxidative damage [6]. Similar interactions with hsp 90 were observed with endothelial nitric oxide synthase [3].

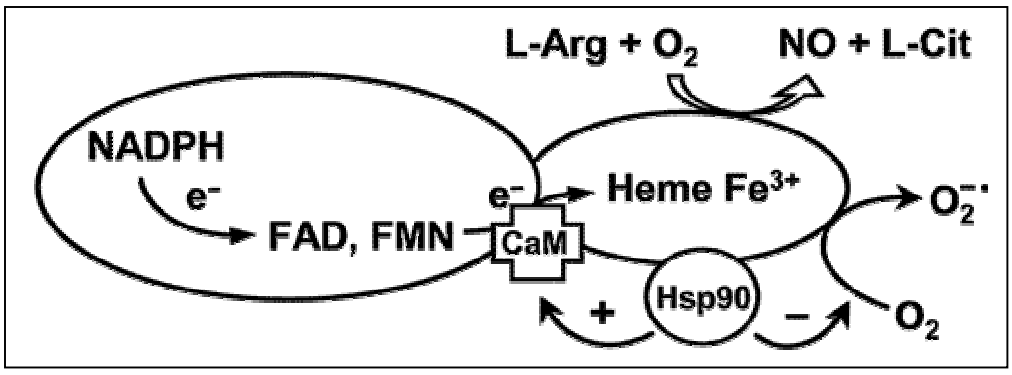


**Figure 5**. Schematic drawing of the different mechanism that hsp 90 can enhance the production of nitric oxide and reduce superoxide. One mechanism is through the CaM binding, the second is to inhibit the production of superoxide by nNOS thus increasing the nitric oxide produced [6].

**Biomarker for Oxidative Stress**

Reactive oxygen species (ROS) are both necessary for biological functions and detrimental to many biomolecules such as; lipids, DNA, and proteins [7]. An over abundance of ROS can cause mutations and the formation of more reactive ROS molecules. The oxidative status of the biological system is a careful balance between antioxidants and pro-oxidants. The antioxidants are being increased through dietary intake and supplements [7]. Unfortunately, a technique to measure the beneficial increase in the overall oxidative status does not exist yet. A study examined biomarkers for monitoring the cellular defense as influenced by the increase in antioxidant intake [7]. The stress protein synthesis in lymphocytes was correlated with the free radical damage in response to the antioxidant supplement to determine the overall antioxidant status of health volunteers. Heat shock proteins are known to be induced not only by heat but also through oxidative stress. Lymphocytes isolated from individuals given supplements were subjected to heat shock in the presence or absence of 2,2'-azo*bis*-(2-amidinopropane)-dihydrochloride, (AAPH) a free radical generator. The cells were labeled with $^{35}$S-methionine and western blots were performed to identify any increase in the protein levels. Lymphocytes that were heat shocked in the presence of AAPH showed and enhancement of heat shock proteins compared to heat shock alone. One of the key heat shock proteins was hsp 90. There were no detectable differences observed in superoxide dismutase or glutathione specific activity when measured with the Randox Kit [7]. Malondialdehyde (MDA) was measured using thiobarbituric acid assay to determine the levels of lipid peroxidation in the lymphocytes. A decrease in the lipid peroxidation was observed in the lymphocytes from the individuals receiving the supplements. This study was able to show that an increase in oxidative stress to lymphocytes enhanced the response of key heat shock proteins including hsp 90 [7].

**Conclusions**

Heat shock protein 90 belongs to a family of chaperone proteins. When hsp 90 is coupled with heat shock 70, they are able to aid in the folding of newly formed proteins [2]. Hsp 90 levels are also increased in stressful situations to assist partially unfolded proteins to refold. Stress to the cell could be in many forms like heat or oxidative stress. Hsp 90 is able to assist in the enhancement of nitric oxide through inhibition of superoxide either through the binding of CaM or inhibiting the release of superoxide by nNOS [6]. The coupling of hsp 90 and nNOS prevents the overproduction of superoxide and reaction with nitric oxide to form peroxynitrite. This inhibition prevents cellular damage from occurring to important biomolecules [7]. Hsp 90 is also an important marker for overall oxidative status, when an increase in antioxidant supplements are taken. Hsp 90 is important in many facets of cellular function whether in stressed or normal environments of the cell.

References

1 Stebbins C, Russo A, Schneider C, Rosen N, Hartl F, Ravletich N. (1997) Crystal structure of an hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. *Cell*. **89**:239-250.

2 Ou J, Ou Z, Ackerman A, Oldham K, Pritchard K. (2003) Inhibition of heat shock protein 90 (hsp90) in proliferating endothelial cells uncouples endothelial nitric oxide synthase activity. *Free Radic Biol Med*. **34**:269-276.

3 Shi Y, Baker J, Zhang C, Tweddell J, Su J, Pritchard K. (2002) Chronic hypoxia increases endothelial nitric oxide synthase generation of nitric oxide by increasing heat shock protein 90 association and serine phosphorylation. *Circ Res* **91**:300-306.

4 Huie R, Padmaja S, (1993) The reaction of no with superoxide. *Free Radicl Res Com*. **18**:195-199.

5 Koppenol W. (1998) The basic chemistry of nitrogen monoxide and peroxynitrite. *Free Radic Biol Med*. **25**:385-391.

6 Song Y, Cardounel A, Zweirer J, Xia Y. (2002) Inhibition of superoxide generation from neuronal nitric oxide synthase by heat shock protein 90: implications in NOS regulation. *Biochem J*. **41**:10616-10622.

7 Peng J, Jones G, Watson K. (2000) Stress proteins as biomarkers of oxidative stress: effects of antioxidant supplements. *Free Radic Biol Med*. **28**:1598-1606.