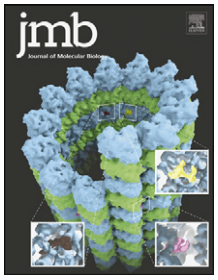

Isoform-Specific Phosphorylation in Human Hsp90β Affects Interaction with Clients and the Cochaperone Cdc37

Minh T.N. Nguyen [1], Robert A. Knieß [1], Soumya Daturpalli [1], Laura Le Breton [1], Xiangyu Ke [2], Xuemei Chen [2] and Matthias P. Mayer [1]

*1 - **Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH),** DKFZ-ZMBH-Alliance, D-69120, Heidelberg, Germany*
*2 - **School of Public Health,** Southern Medical University, Guangzhou, 510515, Guangdong, China*

***Correspondence to Matthias P. Mayer:** ZMBH, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany.*
m.mayer@zmbh.uni-heidelberg.de
http://dx.doi.org/10.1016/j.jmb.2017.01.011
Edited by J. Buchner

Abstract

The 90-kDa heat shock proteins (Hsp90s) assist the maturation of many key regulators of signal transduction pathways and cellular control circuits like protein kinases and transcription factors and chaperone their stability and activity. In this function, Hsp90s cooperate with some 30 cochaperones and they are themselves subject to regulation by numerous post-translational modifications. In vertebrates, two major isoforms exist in the cytosol, Hsp90α and Hsp90β, which share a high degree of sequence identity and are expressed in tissue- and environmental condition-dependent manner. We identified an isoform-specific phosphorylation site in human Hsp90β. This phosphorylation site seems to be linked to vertebrate evolution since it is not found in invertebrata but in all tetrapoda and many but not all fish species. We provide data suggesting that this phosphorylation is important for the activation of Hsp90 clients like glucocorticoid receptor and a protein kinase. Replacement of the phosphorylation site by glutamate affects the conformational dynamics of Hsp90 and interaction with the kinase-specific cochaperone Cdc37.

Introduction

The 90-kDa heat shock proteins (Hsp90s) are abundant molecular chaperones, comprising 1%–2% of cellular proteins that are essential in all eukaryotic organisms investigated so far. In the cytosol of mammalian cells coexist two isoforms of Hsp90, Hsp90α (gene HSP90AA1/HSPC1), which is highly inducible by many types of conditions, and the constitutive and, in most cells, more abundant Hsp90β (gene HSP90AB1/HSPC2) [1]. Despite high functional conservation in evolution, as exemplified by the fact that human Hsp90 complements the deletion of the Hsp90-encoding genes in yeast, and despite of 86% sequence identity and 93% similarity, the two human Hsp90 isoforms do not seem to be completely redundant. There appear to be Hsp90α-specific functions as it was reported to participate specifically in antigen processing [2] and block caspase-2 activation [3]. Hsp90α is also secreted to stimulate cell migration in wound healing [4] and assist the maturation of matrix metalloprotease MMP2, promoting cell invasiveness and metastasis [5]. Mice lacking Hsp90α but having normal levels of Hsp90β exhibit male sterility due to arrest of spermatogenesis at the pachytene stage of meiosis and thus complete absence of functional sperms but no other obvious phenotype [6]. In contrast, mouse embryos lacking Hsp90β die at implantation due to failure of the allantois mesoderm to induce trophoblast differentiation [7].

Eukaryotic Hsp90s do not work alone but cooperate with the Hsp70 system and more than 30 cochaperones to chaperone some 300 protein substrates called clients [8,9]. Many Hsp90 clients are receptors, transcription factors and kinases, and key regulators of cell homeostasis, proliferation, differentiation, and cell death [10,11]. Hsp90s are believed to assist late folding steps during maturation and control the activity and stability of its clients. Many clients only become responsive to upstream activating signals when they interact with the Hsp90 machinery.

Hsp90s are highly dynamic homodimeric proteins that consist of an N-terminal nucleotide binding domain, a highly charged unstructured region, a middle domain, and a C-terminal dimerization domain. The chaperone cycle, as originally proposed for progesterone receptor, assumes that the client first interacts with Hsp70 and is subsequently transferred to Hsp90, involving an intermediate Hsp70–Hsp90–client complex. The mature Hsp90–client complex dissociates with a client-specific half-life, and the rebinding of the client to Hsp70 ensues. Cochaperones regulate the Hsp90 cycle by acting as client targeting factors like Cdc37, the kinase-specific cochaperone, or Hop/Sti1, which aids client transfer from Hsp70 to Hsp90 by stabilizing the intermediate Hsp70–Hsp90–client complex. Cochaperones also regulate progression through the Hsp90 cycle by inhibiting (Hop/Sti1, Cdc37, p23/Sba1) and stimulating the ATPase activity (Aha1) or by orchestrating the succession of cochaperone binding [12,13]. Hsp90 chaperones are additionally regulated by many post-translational modifications, which also affect the interaction with clients and cochaperones [12,14–18]. In a study on serine/threonine phosphorylation of yeast Hsp90, 10 sites were identified in MD and DD and found that the phosphorylation of these sites was not essential, as an Hsp90 variant with all sites replaced by alanine supported yeast growth like the a strain containing the wild-type Hsp90 protein [19]. On the contrary, replacement of a single site by glutamate compromised viability in yeast and replacement of a second site induced a growth defect. The situation is much more complex for human Hsp90α and β, for which currently 75 and 69 phosphorylation sites have been identified, respectively, mostly by proteomics studies[†]. Only few of these sites have been characterized in detail, demonstrating that phosphorylation can be important for the interaction with and activation of client proteins [15,16,18]. In return, some of the kinase clients phosphorylate Hsp90 [20,21].

Since many of the clients of Hsp90 are oncogenic kinases and tumor suppressors, Hsp90 became a prime target for antitumor therapy in the recent years and several clinical trials are currently underway [22–24]. Interestingly, tumor cells are more susceptible to Hsp90 inhibitors, presumably due to their addiction to the activity of tumor-driving oncoproteins, which are Hsp90 clients, opening a therapeutic window for targeting these essential proteins. More surprisingly, Hsp90s isolated from tumor cells have a higher affinity for several different Hsp90 inhibitors, most likely due to differences in post-translational modifications, and the inhibitors are accumulated in the tumor tissue [14,20,25 ,26].

To better understand the regulation of Hsp90, we searched for novel post-translational modifications in a liver carcinoma cell line (HepG2). In this study, we characterize one Hsp90β-specific phosphorylation site. We provide *in vivo* and *in vitro* evidence suggesting that phosphorylation at this site influences interaction with cochaperones and affects the activation of clients.

Results

Identification of a previously uncharacterized phosphorylation site

Through several proteomic studies, a large number of post-translational modifications have been identified for Hsp90α and Hsp90β (see www.phosphosite.org). In many proteomic studies, modified peptides are specifically enriched before mass spectrometric analysis [27]. These studies therefore identify sites that can be modified but not the relative abundance of the modification at steady-state conditions. To identify sites that are modified in a significant proportion of the Hsp90 molecules in HepG2 liver carcinoma cells, we lysed the cells using phosphatase inhibitors only in the lysis buffer to avoid post-lysis dephosphorylation and we precipitated Hsp90 using specific antisera or Geldanamycin-agarose beads but did not use modification-specific enrichment strategies. Among the different modifications identified, a previously uncharacterized phosphorylation in the middle domain of Hsp90β (S365) caught our attention since the corresponding residue in Hsp90α was asparagine, which cannot be phosphorylated (Fig. 1). Interestingly, the corresponding residue in the two yeast Hsp90 homologs is glutamate, generally considered to mimic phosphorylation. In fact, a blast search using the yeast Hsp82 sequence as query against the UniRef90 database of sequence clusters that are 90% identical yielded 100 clusters representing 688 sequences. A Clustal Ω alignment revealed that of these sequences, all but one contained an acidic residue in the position corresponding to S365 in human Hsp90β. However, in the single *Caenorhabditis elegans* and *Drosophila melanogaster* Hsp90 proteins, this residue is asparagine as in human Hsp90α. We therefore performed a similar blast search using *C. elegans* Hsp90 as query against the UniRef90 database, yielding 100 clusters representing 1024 sequences, which were aligned using Clustal Ω. Remarkably, only 5 of the 100 clusters, representing 315 sequences, contained serine. All of these sequences belonged to vertebrate organisms. All other sequences in this alignment had an asparagine in this position. To better understand the frequency of the phosphorylatable serine as compared to the non-phosphorylated asparagine residues, we performed a blast search using the human Hsp90β sequence as query against all sequences of vertebrate organism, retrieving 500 sequences, which were aligned using Clustal Ω. After the removal of partial and obviously wrongly assembled protein sequences, we retained 265 Hsp90 sequences that segregated nicely in Hsp90α