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Research article

Structural and functional differences of cytosolic 90-kDa heat-shock proteins (Hsp90s) in *Arabidopsis thaliana*☆

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ABSTRACT

The seven members of the 90-kDa heat shock protein (Hsp90) family encode highly conserved molecular chaperones essential for cell survival in *Arabidopsis thaliana*. Hsp90 are abundant proteins, localized in different compartments with AtHsp90.1-4 in the cytosol and AtHsp90.5-7 in different organelles. Among the AtHsp90, *AtHsp90.1*, is stress-inducible and shares comparatively low sequence identity with the constitutively expressed *AtHsp90.2-4*. Even though abundant information is available on mammalian cytosolic Hsp90 proteins, it is unknown whether cytosolic Hsp90 proteins display different structural and functional properties. We have now analyzed two *A. thalianas* cytosolic Hsp90s, AtHsp90.1 and AtHsp90.3, for functional divergence. AtHsp90.3 showed higher holdase chaperone activity than AtHsp90.1, although both AtHsp90s exhibited effective chaperone activity. Size-exclusion chromatography revealed different oligomeric states distinguishing the two Hsp90 proteins. While AtHsp90.1 exists in several oligomeric states, including monomers, dimers and higher oligomers, AtHsp90.3 exists predominantly in a high oligomeric state. High oligomeric state of AtHsp90.1 showed higher holdase chaperone activity than the respective monomer or dimer states. When high oligomeric forms of AtHsp90.1 and AtHsp90.3 are reduced by DTT, activity was reduced compared to that found in the native high oligomeric state. In addition, ATP-dependent foldase chaperone activity of AtHsp90.3 was higher with strong intrinsic ATPase activity than that of AtHsp90.1. As a conclusion, the two *A. thaliana* cytosolic Hsp90 proteins display different functional activities depending on structural differences, implying functional divergence although the proteins are localized to the same sub-cellular organelle.

1. Introduction

Heat stress can initiate severe decreases in crop production worldwide [1]. High temperatures provoke cellular injury including the inactivation of enzymes in cytosol, chloroplasts and mitochondria, inhibition of protein synthesis, protein denaturation and aggregation, and loss of membrane integrity in plants [2]. Among several groups of stress proteins, heat shock proteins (Hsps) exist in prokaryotes and eukaryotes alike [3]. They are rapidly synthesized and accumulate in diverse sub-cellular compartments during heat stress.

The 90-kDa heat shock protein (Hsp90) includes a family of Hsps that are abundant and highly conserved molecular chaperones [4]. Hsp90s function in protein folding, maintenance of protein stability, and activation and maturation of cellular proteins through cooperation with more than 200 client proteins and co-chaperones to regulate numerous cellular processes [5]. Under thermal stress

Abbreviations: Hsps, heat shock proteins; HS, heat shock; MDH, malate dehydrogenase; SEC, size-exclusion chromatography; G6PDH, glucose-6-phosphate dehydrogenase.

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conditions, Hsp90 also prevent the denaturation of substrate proteins in a holdase chaperone function, and promotes the refolding of heat-denatured proteins in a foldase chaperone function [4,5]. Hsp90s are well characterized as ATP-dependent molecular chaperones with an ATPase domain acting in ATP hydrolysis, an essential requisite of foldase chaperone proteins. In human genome, 17 genes encoding Hsp90s have been identified. They are found in different cellular compartments, such as cytoplasm, endoplasmic reticulum (ER) and mitochondria [6]. Human Hsp90s primarily exist as homodimers, but higher oligomeric states, tetramers, hexamer and even higher oligomers have been reported [7]. Heat shock (HS) induces oligomerization of mammalian cytosolic Hsp90, which then enhances substrate binding that then prevents irreversible aggregation [8,9]. Thus, Hsp90 self-oligomerization in HS-exposed cells protects by preventing denaturation and aggregation of many sub-cellular substrates.

Hsp90 have been identified in many plant species, including tomato, maize, orchardgrass, rice, rape and *Arabidopsis thaliana* [10–15]. In *A. thaliana*, seven members of the Hsp90 family have been identified by genome sequence analysis. They are localized in different sub-cellular compartments, with four HSPs found in the cytoplasm, and one each in mitochondria, chloroplasts, and ER membranes [16]. Sequence identities among *A. thaliana* cytosolic Hsp90 proteins are higher than 85%, whereas those between cytosolic and other sub-cellular localized Hsp90 proteins are 45–53%. However, two domains of Hsp90s at the N-terminus (nucleotide binding) and middle in the protein (substrate binding), respectively, are highly conserved in all *A. thaliana* Hsp90 isoforms [16].

Recently, AtHsp90.3 has been shown to complement the function of yeast Hsp82 under HS conditions. However, over-expression of AtHsp90.2, AtHsp90.3, AtHsp90.5 and AtHsp90.7 in *A. thaliana* lowers tolerance to heat stress. As one possible interpretation, disturbing the ratio between Hsp90 enzymes and their substrates, in combination with the involvement of Hsp90s in different functions and locations, may lead to imbalances that interfere with the regulation of the HS-response [17,18].

Another distinction between *Hsp90* isoforms is based on transcript expression. One distinction between the four cytosolic *A. thaliana* Hsp90 proteins is the expression of their transcripts either under normal growth conditions (*Hsp90.2-4*) or following HS (*Hsp90.1*) [19]. To date, differences of expression among these cytosolic *Hsp90* genes have not been correlated with possible functional differences. We now depict structural and functional differences between the HS-inducible *AtHsp90.1* and the constitutively expressed *AtHsp90.3*. Functional diversification between the two *A. thaliana* cytosolic Hsp90s are based on distinct structural states.

2. Results and discussion

2.1. Functional differences in holdase activity between A. thaliana cytosolic Hsp90.1 and Hsp90.3

Among cytosolic AtHsp90 proteins, AtHsp90.1 exhibits lower sequence identity (approximately 87%) with the three other cytosolic Hsps, AtHsp90.2-4, that share identities of at least 96%. Distinct are also different transcriptional expression patterns with respect to heat stress [16,19]. We have confirmed the consistent expression patterns of *AtHsp90.1* which is HS-induced, and *AtHsp90.3*, which is constitutively expressed (data not shown). Missing however are experiments clarifying whether the differences among cytosolic AtHsp90s affect differences in how they may be regulated or show different functional activities. Thus, we compared holdase chaperone activities, a canonical activity of Hsp90 proteins, between AtHsp90.1 and AtHsp90.3. As shown in Fig. 1, both recombinant AtHsp90.1 and AtHsp90.3 proteins exhibited an effective holdase chaperone activity

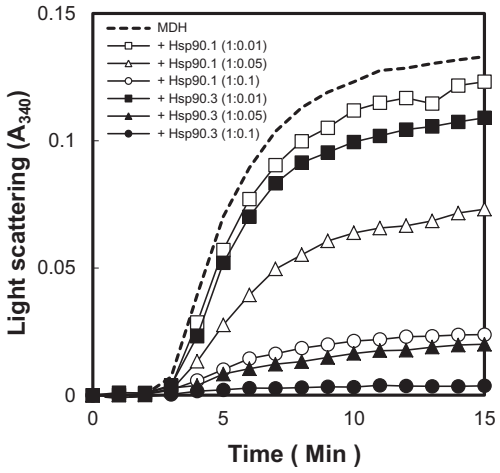

Fig. 1. Comparative holdase chaperone activities of recombinant AtHsp90.1 and AtHsp90.3. MDH (1 μM) was incubated in the absence (*dotted line*) or presence of 0.01 μM (*square*), 0.05 μM (*triangle*), 0.1 μM (*circle*) AtHsp90.1 (*open symbol*) and AtHsp90.3 (*closed symbol*) at 45 °C for 15 min. Light scattering of samples was monitored at 340 nm.

in protecting malate dehydrogenase (MDH) enzyme activity under thermal denaturing conditions in a dose-dependent manner, whereas BSA (as a negative control; data not shown) did not protect. Interestingly, AtHsp90.3 (80% MDH protection from aggregation; *closed triangles*) displayed much higher activity compared to AtHsp90.1 (50%; *open triangles*) at a molar ratio of MDH to AtHsp90 of 1:0.05 (Fig. 1). These results suggest that differences in sequence and transcriptional expression between AtHsp90.1 and AtHsp90.3 could cause the different functional activity.

2.2. Different oligomeric states between AtHsp90.1 and AtHsp90.3

Two human cytosolic Hsp90 isoforms, originally termed α and β, are encoded by two separate genes and their transcriptional expression patterns are different. *Hsp90*α is stress-inducible, however *Hsp90*β is constitutively expressed [6]. It has been reported that Hsp90α and Hsp90β proteins predominantly exist as either homodimers or as monomers, however two proteins had also been observed to associate into tetramers, hexamers, and even high oligomers [7,20]. Based on these different gene expression patterns and oligomeric states of two human Hsp90 isoforms, *A. thaliana* cytosolic Hsp90s may be existed as different oligomeric states.

To probe for putative differences in oligomeric states distinguishing AtHsp90.1 and AtHsp90.3, we carried out size-exclusion chromatography analysis. As shown in Fig. 2A, AtHsp90.1 and AtHsp90.3 show different oligomeric pattern. Highest amounts of proteins after size exclusion chromatography for AtHsp90.1 is seen with size classes ranging from monomers to high oligomeric sizes while AtHsp90.3 showed mainly high oligomers. AtHsp90.1 existed as various oligomeric states, monomer, dimer, and high oligomer and AtHsp90.3 mainly as high oligomer on Native-PAGE, while monomeric AtHsp90s are detected as a single band on denaturing SDS-PAGE, (Fig. 2B and C). Thus, *A. thaliana* cytosolic Hsp90 proteins are divided by oligomeric states, but the oligomerization patterns differed compared to human cytosolic Hsp90s [7,20].

2.3. Different holdase chaperone activity of AtHsp90.1 depending on their oligomeric states

Mammalian Hsp90 display oligomerization and enhanced chaperone activity as a result of heat treatment, and Hsp90 functional activity appears to depend on the dynamically varying amount of