Regulation of Heat Shock Protein 90 ATPase Activity by Sequences in the Carboxyl Terminus*

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hsp90, in addition to being an abundant and pivotal cytoplasmic chaperone protein, has been shown to be a weak ATPase. In an effort to characterize the ATPase activity of hsp90, we have observed marked differences in activities among various species of hsp90. Chicken or human hsp90 hydrolyzed ATP with a \( k_{\text{cat}} \) of 0.02 min\(^{-1} \) and a \( K_m \) greater than 300 \( \mu \)M. In contrast, yeast hsp90 and TRAP1, a hsp90 homologue found in mitochondria, were 10–100-fold more active as ATPases. Sedimentation studies confirmed that all are dimeric proteins. Chicken hsp90 mutants were then analyzed to identify regions within the protein that influence ATPase activity. A truncation mutant of chicken hsp90, N1–573, was found to be monomeric, and yet the catalytic efficiency \( (k_{\text{cat}}/K_m) \) was greater than 100 times that of the full-length protein \( (k_{\text{cat}} \text{ of } 0.24 \text{ min}^{-1} \text{ and } K_m \text{ of } 60 \text{ } \mu\text{M}) \). In contrast, an internal deletion mutant, Δ661–677, was also monomeric but failed to hydrolyze ATP. Finally, deletion of the last 30 amino acids resulted in a dimeric protein with an ATPase activity very similar to full-length hsp90. These data indicate that sequences within the last one-fourth of hsp90 regulate ATP hydrolysis.

Heat shock protein 90 (hsp90)\(^1\) has been demonstrated to be an important chaperone for a vast array of proteins involved in cell regulation, such as transcription factors and protein kinases (1–6). Interestingly, hsp90 seems to assist in the late stages of folding its substrate and may be important for modulating specific interactions such as ligand or co-factor binding or covalent modifications such as phosphorylation. hsp90 functions optimally in a multicomponent complex of chaperone proteins including hsp40, hsp70, Hop, p23, and one of a variety of immunophilins (1–6).

Until recently, the evidence for nucleotide binding and hydrolysis by hsp90 was controversial. There is now direct evidence that hsp90 binds ADP and ATP and that the conformational state of hsp90 differs substantially depending upon which nucleotide is bound (7–11). The crystallization of the amino-terminal 220 amino acids of hsp90 with either nucleotide or an hsp90-specific inhibitor, geldanamycin, revealed that hsp90 possesses a unique nucleotide-binding site that differs substantially from that of hsp70 (8, 9). It has thus been proposed that hsp90 belongs to the GHKL superfamily of ATP-binding proteins that includes the DNA-repair protein MutL, DNA gyrase, topoisomerase II, and histidine kinases (12). The proposed mechanism of action for DNA gyrase and topoisomerase II requires transient ATP-mediated amino-terminal dimerization to form a “molecular clamp” that allows single-stranded DNA strand passage to occur. Binding of single-stranded DNA increases the ATPase activity of the enzyme by severalfold, and ATP hydrolysis is required to reset the “clamp” (13, 14). It has been suggested that hsp90 may interact with ATP and its substrate (client protein) through a similar molecular clamp mechanism (4–6).

Although the binding site for adenine nucleotides has been localized to the amino terminus of hsp90, nucleotide binding has a profound impact on more distal parts of the protein. Specifically, the hsp90 co-chaperone p23 associates with hsp90 only when it is bound to ATP but not when it is bound to ADP or the ansamycin inhibitor geldanamycin (7). The two nucleotide-dependent conformational states of hsp90 are perhaps similar, therefore, to the “open” and “closed” forms of either hsp70 (15, 16), topoisomerase II (13, 14), or MutL (17, 18).

The nucleotide binding and hydrolysis properties of the hsp90 class of molecular chaperones are not as thoroughly characterized as they are for hsp70. hsp70 binds ATP with a \( K_m \) of around 50 \( \mu \)M and hydrolyzes it slowly with a \( k_{\text{cat}} \) of 0.5 \( \text{min}^{-1} \) in the absence of co-chaperones and substrate (19). In the presence of substrate, or the co-chaperone hsp40, hsp70 hydrolyzes ATP 10–20 times more efficiently, and the resulting hsp70-ADP complex binds substrate more tightly (20–22). Disassociation of the substrate from the hsp70-ADP complex is promoted by a nucleotide exchange factor (23). The mechanism of action of the nucleotide exchange factor has recently been deduced. Upon binding hsp70, the nucleotide exchange factor opens the nucleotide-binding pocket, thereby releasing ADP and stabilizing the nucleotide-free state of hsp70 (24).

Unlike either hsp70 or the DNA repair enzymes, an in vitro substrate binding reaction has not been discovered that affects the ATP-hydrolyzing capabilities of hsp90. Additionally, a nucleotide exchange factor has not been identified. However, based on the available crystal structure data and recognized co-factor requirements for hsp90 function as a chaperone, a hypothesis in which nucleotide and most likely co-factor and/or substrate binding alter the conformation of hsp90 is plausible. The impact that these structural alterations have on ATP hydrolysis activity is unknown.

Initial studies with yeast hsp90 indicate that the full-length protein is required for maximal ATPase activity (25). More specifically, although amino acids 1–221 are capable of binding nucleotide, additional residues (1–450) are needed to observe tight (i.e. committed) ATP binding, and residues beyond 450 are necessary for efficient ATP hydrolysis (26). ATP binding has been shown to induce dimer interactions near the amino

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‡ The abbreviations used are: hsp, heat shock protein; Hop, hsp-organizing protein; DTT, dithiothreitol.
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Kₐ and V_max values were calculated directly with standard deviations. A replot of the kinetic data of v/[S] versus v, using the Eadie-Hofstee equation (Equation 2), yielded 1/Kₐ as the slope and V_max/Kₐ as the y intercept.

\[ v/[S] = V_{\text{max}}/K_{\text{a}} - v/K_{\text{a}} \]  

(Eq. 2)

**Sedimentation Equilibrium Experiments—**Sedimentation equilibrium experiments were performed using the Beckman Optima XL-I analytical ultracentrifuge. All proteins were equilibrated in 20 mM HEPES, pH 7.4, 100 mM KCl, 5 mM MgCl₂, and 2 mM DTT buffer using BioSpin 6 chromatography columns (Bio-Rad). Samples (300 μl) of each protein at ~1 mg/ml (5–10 μM) were analyzed in double-sector cells fitted with sapphire windows against a reference composed of the BioSpin column equilibration buffer. To visualize the protein at the bottom of the cell, 20 μl of the immiscible fluorocarbon FC-43 was added to each sample containing hsp90 or derivative protein. All hsp90 full-length proteins and truncations were analyzed at 10,000 rpm and N1–220, N1–573, and N1–692 were also analyzed at 18,000 rpm. Full-length chicken hsp90 was also analyzed at 8,000 and 12,000 rpm at protein concentrations ranging from 0.1 to 1.5 mg/ml. Samples were either run in an An60Ti (4-hole) or An50Ti (8-hole) rotor at 20°C. Data were obtained using Rayleigh interference optics as scans of fringe displacement (protein concentration, C) versus radial distance (r). Equilibrium was achieved when scans taken 3 h apart after an initial 24 h of centrifugation were superimposable.

Primary data were fit to a self-association model using a Levenberg-Marquardt non-linear, least squares fitting routine which minimizes the x² residuals between the function (see below, Equation 3) and the data (XL-I UltraScan-Origin, Beckman Instruments, Fullerton, CA).

The monomer-polymer model is represented by Equation 3,

\[ C_r = a_1 \exp(M_b(1 - v_p/2RT)^2 - r_p^2)) + a_2 \exp(n \text{M}^2) \]

\[ (1 - v_p/2RTv - r_p^2)) + b \]  

(Eq. 3)

Where \( C_r \) is the concentration at radial position \( r \) in the cell, and \( r \) the radial position at the meniscus. \( a_1 \) and \( a_2 \) are the absorbance values of the monomer and dimer, respectively, at the meniscus. \( M \) is the molecular weight of the monomer, \( R \) is the gas constant, \( T \) the temperature (in degrees Kelvin), \( \omega \) the angular velocity, \( e \) the partial specific volume of the protein, and \( \rho \) the density of the buffer. The number of monomers in the polymer assembly is \( n \), and \( b \) is the base-line absorbance.

**RESULTS**

**Analysis of hsp90 Species and Deletion Mutants for ATPase Activity—**Fig. 1 illustrates the constructs used in this study and lists their relative ATPase activities based on \( k_{\text{cat}}/K_{\text{m}} \). Each species is indicated by a uniquely shaded block to emphasize that although all proteins are hsp90 family members, there are substantial species-specific differences. Residues 1–220 are delineated by the first block for each protein and represent the most conserved region between species. TRAP1–220 are delineated by the first block for each protein and represent the minimal ATP-binding domain. Each species is indicated by a uniquely shaded block to emphasize that although all proteins are hsp90 family members, there are substantial species-specific differences. Residues 1–220 are delineated by the first block for each protein and represent the most conserved region between species. TRAP1–220 are delineated by the first block for each protein and represent the minimal ATP-binding domain. Each species is indicated by a uniquely shaded block to emphasize that although all proteins are hsp90 family members, there are substantial species-specific differences. Residues 1–220 are delineated by the first block for each protein and represent the most conserved region between species. TRAP1–220 are delineated by the first block for each protein and represent the minimal ATP-binding domain.
E. coli

1. N1

573 hydrolyzed ATP with a $K_m$ of 100 times better than the full-length protein (see Fig. 1). N1–692 removes the last 30 amino acids from the chicken hsp90 that are not found in either HtpG or TRAP1. Finally, the internal deletion mutant, Δ661–677, removes one of the proposed dimerization domains from the carboxyl terminus of chicken hsp90, yet Δ661–677 has been shown to confer viability in a yeast strain lacking endogenous hsp90 (36).

ATPase activity for each protein was assayed by incubating with [α-32P]ATP and measuring the direct conversion to [α-32P]ADP as described under “Experimental Procedures”. Full-length chicken hsp90 hydrolyzed ATP poorly with a $K_m$ for ATP of 1.5 mM and a $k_{cat}$ of 0.02 min$^{-1}$ at 30 °C (Fig. 2). In contrast, yeast hsp90 hydrolyzed ATP at nearly 10 times this rate, whereas HtpG and TRAP1 were still more efficient ATPases (Fig. 1 and Table I). These results imply that chicken hsp90 does not hydrolyze ATP efficiently in the absence of either cofactors and/or substrate.

Somewhat surprisingly, the N1–573 construct hydrolyzed ATP over 100-fold better than the full-length protein (see Fig. 1). N1–573 hydrolyzed ATP with a $K_m$ of 100 μM and a $k_{cat}$ of 0.24 min$^{-1}$ (Fig. 3). Thus, the kinetics of this carboxyl-terminally truncated chicken protein were more efficient than those found for full-length yeast hsp90 and similar to the kinetics for the mitochondrial homologue, TRAP1 (see below and Ref. 31). These results are particularly interesting because all carboxyl-terminal truncations tested in the yeast protein to date have resulted in decreases of ATPase activity (26). Likewise, our efforts to generate a similar truncation mutant in TRAP1 have resulted in proteins with substantially reduced ATPase activities. Specifically, the carboxyl-terminal truncation of TRAP1 at amino acid 529, which coincides with amino acid 573 of the chicken sequence, resulted in a decrease in ATPase activity to less than 5% of the full-length protein. A more conservative carboxyl-terminal deletion mutant, TRAP1 N1–578, was slightly more active as an ATPase but still had less than 20% of the activity of wild-type TRAP1 (data not shown).

Removal of the last 30 amino acids of chicken hsp90 (N1–692) had little effect on ATPase activity (Fig. 1 and Table I). The deletion mutant Δ661–677, which had one of the proposed dimerization domains removed, showed ATPase activity that was approximately one-tenth that of the full-length chicken hsp90. Similarly, and in agreement with studies in yeast, the N1–220 truncation mutant of chicken hsp90 also had less than 1/10 the ATPase activity of the full-length protein.

The observation that truncating full-length hsp90 to residue.
was analyzed at 8,000, 10,000, and 12,000 rpm using protein concentrations that ranged from 0.5 to 2.5 mg/ml or 2.9 to 15.7 μM, as shown in Table II. The average molecular weight determined from these measurements was 166,993 ± 890 (n = 9). The calculated molecular mass for chicken hsp90, based on primary amino acid sequence, is 84,060 (monomer) or 168,120 (dimer). Therefore, in solution, full-length chicken hsp90α exists as an unambiguous dimer over a 5-fold range of protein concentration.

Table III summarizes the results of the sedimentation equilibrium analysis of the different hsp90 species and mutant chicken proteins. In addition to the full-length hsp90 species, deletion mutant N1–692 and the thrombin cleavage product N1–611 were found to be dimers in solution. In contrast, N1–220, N1–573, and Δ661–677 were monomeric. The monomeric deletion mutants of hsp90 (N1–220, N1–573, and Δ661–677) were all analyzed at moderate protein concentrations, i.e. 0.3–1.2 mg/ml. This represents a concentration similar to that used in our kinetic analysis. It is possible that some of these proteins can exist as dimers in solution at much higher protein concentrations.

**DISCUSSION**

It has been recognized for some time that hsp90 not only binds but hydrolyzes ATP (8–10, 33, 37). These actions are thought to alter the conformation of hsp90 and to affect its interactions with substrate proteins and co-chaperones. Indeed, the co-chaperone p23 binds to hsp90 only when hsp90 is bound to ATP (7). hsp-organizing protein (Hop), however, will bind hsp90 in either its aponucleotide or ADP-bound state. Under conditions that promote p23 binding (i.e. ATP, molybdate, and Nonidet P-40), Hop binding is reduced (38). Neither Hop nor p23 appears to bind directly to the minimal ATP-binding domain (amino acids 1–220), indicating that nucleotides induce more global conformational changes in hsp90. The ATPase activity of hsp90 is very low, and in parallel with some other molecular chaperones, it seems likely that some specific interactions with co-chaperones or substrates would stimulate this ATPase activity. Thus, ATP hydrolysis would occur only at a point in the chaperone process where energy expenditure is required, such as substrate and/or co-factor release. In this regard, Hop inhibits the ATPase activity of hsp90, but the activity is not altered by p23 binding (27, 29). The influence of substrate on the ATPase activity of hsp90 has yet to be determined.

To investigate the possibility of intramolecular regulation of the hsp90 ATPase, we assayed ATPase activity as a function of hsp90 structure using both divergent hsp90 family members and carboxy-terminal deletion mutants of chicken hsp90. By taking advantage of natural variations among hsp90 proteins derived from different species, we were able to construct a hierarchy of organism complexity versus hsp90 ATPase activity. The range of activity as measured by kcat alone was over 10-fold, with TRAP1, a human mitochondrial hsp90 homologue, being the most efficient enzyme and chicken hsp90 being the least efficient. We have found the activity of human hsp90β to be similar to that of chicken. Yeast hsp90 and the E. coli HtpG were somewhat moderate in activity.

The catalytic efficiencies (kcatal/Km) of these divergent hsp90 family members actually differed by nearly 200-fold. This is quite remarkable, considering that the eukaryotic cytosolic hsp90 proteins have a minimum of 60% identity to each other (39). There are, however, several differences that may account for the varied ATP hydrolysis rates of these proteins. In the chicken and yeast proteins, a charged domain exists that starts at approximately amino acid 220. This charged domain has been shown to influence the binding of the amino-terminal

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**Fig. 4. Analysis of carboxyl-terminal truncation mutants of chicken hsp90α.** A, relative rates of ATP hydrolysis. Each mutant protein (5 μM) was assayed with 1 μM ATP at 30 °C, and activity (×10⁶) is reported as μmole of ATP hydrolyzed per min per μmol of hsp90 protein. B, SDS-PAGE of carboxy-terminal truncation mutants. Each protein (1–5 μg) was analyzed by reduced 7.5% SDS-PAGE and stained with Coomassie Blue. 1st lane, wild type (WT) hsp90; 2nd lane, Δ661–677; 3rd lane, N1–584, proteolytically cleaved Δ661–677 isolated by Mono-Q chromatography; 4th lane, N1–692; 5th lane, N1–607; 6th lane, N1–573; and 7th lane, N1–220.

573 dramatically enhanced ATPase activity suggested that the carboxyl terminus negatively regulates activity. To test this hypothesis, we generated two additional truncation mutants by proteolysis. Bovine thrombin cleaves chicken hsp90 between residues Lys-611 and Ala-612, as determined by carboxyl-terminal sequencing of the thrombin-cleaved product, generating residues Lys-611 and Ala-612, as determined by carboxyl-ter-

proteolysis. Bovine thrombin cleaves chicken hsp90 between carboxyl terminus negatively regulates activity. To test this hypothesis, we generated two additional truncation mutants by proteolysis. Bovine thrombin cleaves chicken hsp90 between residues Lys-611 and Ala-612, as determined by carboxyl-terminal sequencing of the thrombin-cleaved product, generating a homogeneous truncation mutant of N1–611 (Fig. 4B). This mutant, which has only 35 additional carboxy-terminal amino acids than N1–573, hydrolyzed ATP in a manner similar to full-length hsp90 and N1–692 (Fig. 4A).

During purification, the internal deletion mutant, Δ661–677, displayed an increased susceptibility to proteolysis. We mapped the susceptible site by mass spectrometry to within 1200 mass units carboxyl-terminal to residue 574. Carboxy-terminal amino acid sequencing confirmed the proteolytic cleavage site to be after amino acid Val-584. Fortuitously, the parental and cleaved Δ661–677 proteins were separable by Mono-Q chromatography (Fig. 4B). The cleaved product, N1–584, had an ATPase activity that was 10 times that of the full-length chicken hsp90 protein and approximately one-third that of N1–573 protein (Fig. 4A). Taken together, these findings suggest that the amino acid sequence found between residues 574 and 611 suppresses the ATPase activity of full-length, dimeric chicken hsp90.

**Determination of Oligomerization States of hsp90 Proteins—**

Equilibrium sedimentation analysis was used to determine the oligomerization state of the full-length or deletion mutant hsp90 proteins in solution. To determine whether full-length chicken hsp90 behaved ideally and homogeneously in solution over the concentrations used for ATPase assays, the protein was analyzed at 8,000, 10,000, and 12,000 rpm using protein concentrations that ranged from 0.5 to 2.5 mg/ml or 2.9 to 15.7 μM, as shown in Table II. The average molecular weight determined from these measurements was 166,993 ± 890 (n = 9). The calculated molecular mass for chicken hsp90, based on primary amino acid sequence, is 84,060 (monomer) or 168,120 (dimer). Therefore, in solution, full-length chicken hsp90α exists as an unambiguous dimer over a 5-fold range of protein concentration.

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Three different concentrations of full-length chicken hsp90α were analyzed at three different speeds in the analytical ultracentrifuge in the same buffer used for ATPase assays.

| Protein          | Speed (rpm) | Mₑ (measured) | Root mean square | Mₑ (theoretical) | Oligomer status |
|------------------|-------------|----------------|------------------|------------------|-----------------|
| Chicken, α (2.9 μM) | 8,000       | 168,854        | 3.5              | 84,058           | Dimer           |
|                  | 10,000      | 162,257        | 0.36             |                  |                 |
|                  | 12,000      | 168,367        | 0.74             |                  |                 |
| Chicken, α (8.9 μM) | 8,000       | 168,954        | 1.3              | 84,058           | Dimer           |
|                  | 10,000      | 167,435        | 2.53             |                  |                 |
|                  | 12,000      | 162,700        | 0.69             |                  |                 |
| Chicken, α (15.7 μM) | 8,000       | 170,341        | 2.3              | 84,058           | Dimer           |
|                  | 10,000      | 168,151        | 1.58             |                  |                 |
|                  | 12,000      | 169,852        | 2.0              |                  |                 |

Recent studies (9, 25, 26, 34, 44) indicate that ATP binding by hsp90 induces dimer contacts near the amino terminus, and this may be a key conformational transition relating to substrate-chaperone interaction. Also, monomeric yeast hsp90 fragments lacking regions toward the carboxy terminus exhibit very low ATPase activity (25, 26, 45), and it was suggested that transient dimer contacts near the amino terminus are required for tight binding of ATP and significant ATPase activity (45). In light of these findings, the relatively high activity we observed with the monomeric fragment N1–573 is surprising. However, a parallel finding has been reported for topoisomerase II wherein a mutation that disrupts amino-terminal dimerization increased ATP hydrolysis 10-fold, and this hydrolysis was no longer regulated by DNA substrate (46). In another example, the human MutLα protein is a heterodimer that contains an ATPase activity that is stimulated by binding DNA. An amino-terminal fragment of hPMS2, one subunit of MutLα, was shown to be monomeric and still active in binding DNA and as an ATPase (47). However, the influence of DNA binding on ATPase activity was lost. Together these findings suggest that although dimer interactions may be important for regulation of ATPase activity in the full-length hsp90 molecule, monomeric forms of hsp90 can exhibit substantial ATPase activity if regions that normally suppress this activity are removed.

The ATPase activity of chicken N1–573 was very similar to that of the human mitochondrial homologue TRAP1. TRAP1, however, is dimeric, and the TRAP1 equivalent of chicken N1–573 (TRAP N1–529), which is a monomer, had less than 5% of the ATPase activity of full-length TRAP1 (data not shown). The internal deletion mutant of hsp90 Δ661–677 was also monomeric when analyzed by sedimentation equilibrium. This mutant, however, had one-tenth the ATPase activity of full-length chicken hsp90, showing clearly that the enhanced ATPase activity of N1–573 is not simply due to dimer disrup-

domain to denatured protein substrates (40). But, TRAP1, which lacks this charged domain, and yeast hsp90 hydrolyze ATP with similar kcat values. Thus, this domain, per se, does not necessarily affect efficient ATP hydrolysis. Another region that is present in all species of hsp90 (except HtpG, TRAP1, and the endoplasmic reticulum cognate grp94) is the last 30 amino acids. This region has been shown to be important for the binding of Hsp and other co-chaperones (41). Removal of these residues from the chicken protein did not affect ATPase activity. Therefore, the most obvious differences in the structural organization of these various hsp90 family members do not account for the large variation in ATPase activities. We can only surmise that in the context of each full-length protein these regions may influence some aspect(s) of ATP binding or hydrolysis and may be particularly relevant when substrate is bound.

Another point to be drawn from our studies relates to the importance of hsp90 dimerization and the carboxyl terminus to ATPase activity. hsp90 functions as a homodimer, and it has been widely accepted that the primary dimer contacts are near the carboxyl terminus (42). The last 30 amino acids of chicken hsp90 have been speculated to be important for dimerization (36). Our data clearly show that this is not the case because even at low micromolar protein concentrations, N1–692 was still dimeric. These findings are not completely unexpected however, because both HtpG and TRAP1 were found to be dimeric, and they lack this particular sequence. Although this region has no impact on ATPase activity or the default, carboxyl-terminal dimerization, it may, however, affect changes in conformation within the context of the full-length protein. It also remains a formal possibility that this region affects dimer interactions or ATPase activity only in the presence of co-chaperones and/or substrate. For example, mutations in the terminal EEVD sequence of hsp70 can dramatically influence the stimulation of ATPase activity by the hsp40 protein HDJ-1 (43).
tion. The thrombin cleavage of chicken hsp90 resulted in the creation of N1–611, an extension of 38 amino acids carboxy-terminal to the N1–573 mutant. N1–611 was dimeric and hydrolyzed ATP poorly like full-length hsp90 and N1–692.

Overall, the effect of dimerization on ATPase activity might therefore be dependent upon amino acid sequences present within the rest of the protein. But these data make it probable that the region defined by amino acids 574–611 in higher eukaryotic hsp90 proteins, in addition to maintaining carboxy-terminal dimerization, is responsible for negatively regulating ATPase activity. Indeed, this hypothesis is also supported by the increased ATPase activity of the N1–584 proteolytic cleavage fragment generated from Δ661–667 during purification. The region surrounding amino acids 574–611 also appears to contribute to the binding of some hsp90 co-chaperones (41), and it may be part of a second nucleotide-binding site reported recently to exist within amino acids 539–728 (48). These studies by Marcu et al. (48) indicate an intriguing intramolecular regulation of the ATP-binding sites. The second carboxy-terminal site was suppressed in the presence of the amino-terminal binding site and the charged domain. On the other hand, blocking the second site with novobiocin resulted in inhibition of the amino-terminal ATP-binding site (48). In a similar manner, the enhanced ATPase activity of our N1–573 mutant might be explained by the removal of regulation by the second ATP-binding site. Given the importance of hsp90 residues 574–692 that the region defined by amino acids 574–611 in higher eukaryotic hsp90 proteins, in addition to maintaining carboxy-terminal dimerization, is responsible for negatively regulating ATPase activity. Indeed, this hypothesis is also supported by the increased ATPase activity of the N1–584 proteolytic cleavage fragment generated from Δ661–667 during purification. The region surrounding amino acids 574–611 also appears to contribute to the binding of some hsp90 co-chaperones (41), and it may be part of a second nucleotide-binding site reported recently to exist within amino acids 539–728 (48). These studies by Marcu et al. (48) indicate an intriguing intramolecular regulation of the ATP-binding sites. The second carboxy-terminal site was suppressed in the presence of the amino-terminal binding site and the charged domain. On the other hand, blocking the second site with novobiocin resulted in inhibition of the amino-terminal ATP-binding site (48). In a similar manner, the enhanced ATPase activity of our N1–573 mutant might be explained by the removal of regulation by the second ATP-binding site. Given the importance of hsp90 residues 574–692.

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REFERENCES

1. Pratt, W. B. (1998) Proc. Soc. Exp. Biol. Med. 217, 429–434
2. Kaplow, A. J. (1999) Trends Cell Biol. 9, 262–268
3. Manger, M. F., and Bukau, B. (1999) Curr. Biol. 9, R322–R325
4. Richter, K., and Buchner, J. (2001) J. Biol. Chem. 276, 283–289
5. Pearl, L. H., and Proumou, C. (2000) Curr. Opin. Struct. Biol. 10, 46–51
6. Young, J. C., Moarefi, I., and Hartl, F.-U. (2000) Mol. Cell. Biol. 20, 3882–3892
7. Proumou, C., Roe, S. M., O’Brien, R., Ladbury, J. E., Piiper, P. W., and Pearl, L. H. (1998) J. Cell Biol. 143, 901–910
8. Cheng, S., Sullivan, W. P., Toft, D. O., and Smith, D. F. (1998) Cell Stress Chaperones 3, 118–129
9. Nemoto, T., Ohara-Nemoto, Y., Ota, M., Takagi, T., and Yokoyama, K. (1995) Eur. J. Biochem. 233, 1–8
10. Freeman, B. C., Myers, M. P., Schumacher, R., and Morimoto, R. I. (1995) EMBO J. 14, 2281–2289
11. Maruyama, S., Sameshima, M., Nemoto, T., and Yahara, I. (1999) J. Mol. Biol. 283, 903–907
12. Richter, K., Muschler, P., Hainzl, O., and Buchner, J. (2001) J. Biol. Chem. 276, 33689–33696
13. Bjergbaek, L., Kinna, P., Nielsen, I. S., Wang, Y., Westergaard, O., Osharoff, N., and Andersen, A. H. (2000) J. Biol. Chem. 275, 26873–26878
14. Guarnieri, A., Junop, M. S., and Yang, W. (2001) EMBO J. 20, 5521–5531
15. Maruyama, M. G., Chadli, A., Bouhouche, I., Catelli, M., and Neckers, L. M. (2000) J. Biol. Chem. 275, 37181–37186
16. Scheibel, T., Weikl, T., and Buchner, J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 1495–1499
17. Young, J. C., Schneider, C., and Hartl, F. U. (1997) FEBS Lett. 418, 139–143
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