Hsp90 is an abundant molecular chaperone involved in a variety of cellular processes ranging from signal transduction to viral replication. The function of Hsp90 has been shown to be dependent on its ability to hydrolyze ATP, and in vitro studies suggest that the dimeric nature of Hsp90 is critical for this activity. ATP binding occurs at the N-terminal domains of the Hsp90 dimer, whereas the main dimerization site resides in the very C-terminal domain. ATP hydrolysis is performed in a series of conformational changes. These include the association of the two N-terminal domains, which has been shown to stimulate the hydrolysis reaction. In this study, we set out to identify regions in the N-terminal domain that are important for this interaction. We show that N-terminal deletion variants of Hsp90 are severely impaired in their ability to hydrolyze ATP. However, nucleotide binding of these constructs is similar to that of the wild type protein. Heterodimers of the Hsp90 deletion mutants with wild type protein showed that the first 24 amino acids play a crucial role during the ATPase reaction, because their deletion abolishes the trans-activation between the two N-terminal domains. We propose that the turnover rate of Hsp90 is decisively controlled by intermolecular interactions between the N-terminal domains.

Hsp90 is a molecular chaperone that has been linked to the function of several signaling proteins such as Src kinase, steroid hormone receptors, and p53 among others (for review see Refs. 1 and 2). It may stabilize an otherwise unfavored conformation of its substrate molecules such as the open binding site for steroid hormone receptors (3). For the function of several signaling proteins such as Src kinase, steroid hormone receptors, and p53 among others (for review see Refs. 1 and 2). It may stabilize an otherwise unfavored conformation of its substrate molecules such as the open binding site for steroid hormone receptors (3). For the essential function of Hsp90 in vivo, ATP binding and hydrolysis by Hsp90 are a prerequisite (4, 5). Furthermore, studies with steroid hormone receptors revealed that ATP is necessary to form a stable complex between Hsp90 and its substrate (6).

The ATPase of Hsp90 is very slow in vitro with values of one ATP hydrolyzed/min for the yeast Hsp90 homologue (7, 8). ATP hydrolysis has been proposed to drive a cycle of conformational changes in Hsp90 that involve the entire Hsp90 dimer, although ATP binding occurs only at the N-terminal domains (7, 9, 10). The ATP molecules, although bound weakly, are trapped during these conformational changes inside the Hsp90 dimer, and it was shown that these changes require the presence of the middle domain of Hsp90 and at least in part the presence of the C-terminal dimerization domain (7). During the ATPase cycle, the N-terminal domains of the dimer associate. This seems to be a prerequisite for an efficient hydrolysis reaction. This N-terminal association is weak. Therefore, dimerization of the C-terminal Hsp90 is required to increase the local concentration of the N-terminal domains (11, 12). Experiments with heterodimers consisting of C-terminal Hsp90 fragments and WT protein support the view that the C-terminal dimerization is the requirement for an efficient N-terminal dimerization.

The complexity of the ATPase cycle led us to further investigate the mechanism of the N-terminal dimerization. As for Hsp90, no conclusive structural data were available for the N-terminal dimerized conformation. We used the structures of two weakly homologues proteins (GyraseB and MutL) as starting points to design several mutants of Hsp90 that might influence the dimerization. In these proteins, approximately 30 amino acids interact between the N-terminal subunits to form a dimer (13–15). These amino acids correspond to the first 24 amino acids of Hsp90, representing the first β-strand and the following α-helix.

Based on the known structure for the N-terminal domain (8, 16), we decided to delete 8, 16, or 24 amino acids from the N terminus in Hsp90 and compare the ATPase activities of these proteins with that of the WT Hsp90. The results obtained shed light on the mechanism of stimulation in the ATPase cycle of Hsp90. We found that this region is involved in the intermolecular interactions of the N-terminal domains. In addition, we conclude that these interactions control the turnover of the overall ATPase cycle.

EXPERIMENTAL PROCEDURES

Materials—Radicicol was purchased from Sigma. Geldanamycin was a kind gift of the Experimental Drug Division (National Institutes of Health, Bethesda, MD). All other chemicals were purchased from Merck (Darmstadt, Germany).

Hsp90 Constructs—Deletion mutants of yeast Hsp90 were constructed using the plasmid pET28-Hsp82 containing the full-length Hsp82 gene of Saccharomyces cerevisiae with an N-terminal His tag as a template. All of the PCR fragments were cloned into the pET28b vector, resulting in the constructs pET28b-ΔHsp82, pET28b-Δ16-Hsp82, and pET28b-Δ24-Hsp82. The other fragments mentioned in the text are as described in Richter et al. (11). Mutagenesis, leading to the E33A mutation and the D79N mutation of full-length Hsp90, was done.
by overlap extension PCR in N210-Hsp82 using two primers containing the mutation and subsequent insertion of this fragment into the full-length gene in the vector pQE30 using an N-terminal BamHI restriction site and the internal XbaI site. The identity of all of the constructs was confirmed by DNA sequencing.

**Protein Expression and Purification**—His-Hsp90 and its deletion mutants were expressed in the strain BL21 (DE3) cop + (Stratagene, La Jolla) at 37 °C in LB ampicillin and induced with 1 mM isopropyl-1-thio-

β-D-galactopyranoside. Cells were lysed using a cell disruption system (Constant Systems, Warwick, United Kingdom). Protein purification was done according to the protocol described in Richter *et al.* (11). Following nickel-nitrilotriacetic acid chromatography, an anion exchange chromatography (Resource Q, Amersham Biosciences) and a gel filtration column (Superdex 200 HiLoad, Amersham Biosciences) were used. Proteins were stored in 40 mM HEPES, pH 7.5, 20 mM KCl at concentrations of 1.5–9 mg/ml at ~80 °C. Electrospray ionization time-of-flight mass spectrometry was used to verify the integrity and purity of the proteins.

**CD Spectroscopy**—Far-ultraviolet CD spectroscopy was used to confirm the secondary structure of the fragments. The measurements were performed in 40 mM potassium phosphate, pH 7.0, at protein concentrations of 200 μg/ml in 1-mm cuvettes at 20 °C. CD spectra were collected between 195 and 250 nm in a J-715 spectropolarimeter (Jasco, Groß-Umstadt, Germany).

**Protein Stability**—GdmCl transitions were performed with 20 μg/ml protein and GdmCl concentrations ranging from 0 to 5 M in 40 mM HEPES, pH 7.5, 20 mM KCl. Changes in tertiary structure were detected by fluorescence measurements in a FluoroMax-2 fluorometer (SPEX, Edison) at 25 °C. The excitation wavelength was set to 280 nm, whereas the emission spectra were collected from 300 to 400 nm. The midpoint of the GdmCl transition (Cm) was obtained from a plot of the fluorescence signal at 325 nm against the GdmCl concentration.

**ADP Binding Assay**—Nucleotide binding to the isolated N-terminal domains was monitored using the change in fluorescence signal. The protein was excited at a wavelength of 295 nm, and the emission was detected at 328 nm using a FluoroMax-2 fluorometer. The temperature was set to 20 °C. Protein concentrations were 20 μg/ml for the three proteins tested. The buffer used was 40 mM HEPES, pH 7.5, 150 mM KCl, 5 mM MgCl2. ADP was added, and the change in fluorescence was recorded. The obtained values were corrected for dilution and inner filter effect. Both were small compared with the signal change at the conditions used. Dissociation constants were derived from the corrected binding curve by non-linear least square fitting with the program SigmaPlot (SPSS Science, Chicago).

**Stopped-flow Analysis**—Stopped-flow measurements were performed with a HiTech SF-61 DX2 instrument in 40 mM HEPES, pH 7.5, 150 mM KCl, 5 mM MgCl2. The excitation slit was set to 0.5 nm, the excitation wavelength was set to 334 nm for direct excitation of (P4)MABA-ATP or 296 nm for tryptophan/MABA energy transfer, and emission was detected through a cut-off filter of 418 nm. The temperature was set to 25 °C unless indicated otherwise. The concentrations indicated refer to the concentrations in the mixing chamber.

Dissociation rate constants were measured directly by the displacement of a preformed Hsp90(P4)MABA-ATP complex with excess unlabeled ligand. They followed single exponential equations. Association rate constants were derived from a series of experiments where either the concentrations of fluorescent ligand or the enzyme were varied. The individual time traces were analyzed with single exponential equations. Replots of these series of experiments with the observed rate constant (koff) as a function of ligand concentration followed straight lines, which is consistent with a simple one-step binding mechanisms. The rate constant for dissociation (koff) could be derived from the intercept and checked for consistency with the directly measured rate constant, whereas koff is represented by the slope. A replot of the observed amplitudes of the individual time traces versus concentration directly gives the dissociation constant (Koff), which can be compared with the one derived from the kinetic constants (koff/kon).

**ATPase Activity**—ATPase activities were measured using a regenerating ATPase assay as described by Ali *et al.* (17). The assays were performed in 120-μl cuvettes, and the reduction of NADH concentration was detected by the decrease of absorbance at 340 nm using a Pharmacia 40/60 spectrophotometer (Amersham Biosciences). The temperature was set to 37 °C. Assays were performed in 40 mM HEPES, pH 7.5, 150 mM KCl, 5 mM MgCl2, 2 mM ATP. Typical protein concentrations were 2.5 μM for Hsp90 and up to 50 μM for the less active deletion mutants. To determine contaminating ATPase activities that could co-purify with Hsp90, Radicicol, a specific inhibitor of the Hsp90 ATPase, was used at severalfold excess. The remaining ATPase activity in the presence of Radicicol was interpreted to be background and was subtracted from the total activity.

For competition experiments, Hsp90 concentrations were 2.5 μM, whereas the concentrations of fragments were varied between 1 and 16 μM. To allow equilibration between homodimers and heterodimers, the
samples were incubated for 10 min at 37 °C, which was sufficient to reach equilibrium before ATP was added. Again, Radiellic was added later on to ensure that the detected ATPase activity is attributed to ATP hydrolysis by Hsp90. The data analysis has been performed as described in Richter et al. (11).

Size Exclusion HPLC Analysis—An analysis of the dimerization properties of the full-length proteins was carried out by size exclusion chromatography. A Superdex 200HR column (Amersham Biosciences) was used on a Jasco HPLC system with fluorescence detection. The buffers used were either 40 mM HEPES, pH 7.5, 150 mM KCl or 40 mM HEPES, pH 7.5, 150 mM KCl, 1.5 mM MgCl2, 2 mM ATP. Different protein concentrations ranging from 30 µM to 10 mM were injected, and the elution time was monitored by fluorescence. The excitation wavelength was set to 295 nm to minimize inner filter effects resulting from the ATP, and the emission wavelength was 328 nm. The analysis of the SEC data allowed us to determine the apparent dimerization constants for the different constructs as described in Richter et al. (11).

RESULTS

Structural Characterization of the Mutated N-Terminal Domains—To investigate the importance of the N-terminal amino acids for the function of yeast Hsp90 in vitro, we constructed several mutants based on the crystal structure of the N-terminal domain (Fig. 1). These mutants lack the first 8 amino acids (Δ8-Hsp90), the first 16 amino acids (Δ16-Hsp90), or the first 24 amino acids (Δ24-Hsp90) of Hsp90. In addition to creating the truncated full-length proteins, we constructed the respectively deleted regions to analyze the ATPase activities of the full-length proteins as the tryptophan/MABA energy transfer signal observed upon binding of the (Pγ)MABA-ATP was sufficiently strong. The tryptophan/MABA energy transfer signal of a series of experiments with varying protein concentrations (Δ16-Hsp90) is shown in Fig. 4B. A plot of the observed rate constants versus protein concentration resulted in a straight line. This indicates a simple one-step binding mechanism and allows the extraction of a rate constant for association ($k_{on}$) and dissociation ($k_{off}$).

These experiments revealed that the on and off rates of all variants were not influenced significantly by the mutations (Table I). The kinetic values for the isolated N-terminal domains did not differ by more than a factor of two from the values obtained for the full-length proteins (data not shown). Taken together, these experiments demonstrate that in general the nucleotide binding properties of Hsp90 do not change significantly if N-terminal amino acids are deleted. One notable exception appears to be Δ8-Hsp90, which binds nucleotide approximately four times tighter because of a reduced $k_{off}$ value.

The ATPase Activity of the Deletion Mutants Is Affected—We next analyzed the ATPase activities of the full-length proteins. Δ8-Hsp90 showed an ATPase activity, which was ~1.5 times higher than that of WT Hsp90 (Fig. 5A). Surprisingly, the other deletion mutants showed severely reduced ATP hydrolysis. Neither for Δ16-Hsp90 nor for Δ24-Hsp90 could ATPase activity be observed. These experiments demonstrate that the deleted regions are important for the ATPase activity of Hsp90, although they are neither involved in ATP binding nor are they part of the active center. This seems to be especially true for amino acids 8–16, as their deletion already results in a greatly reduced ATPase activity.

Because dimerization of the N-terminal domains during the ATPase cycle is an important step for efficient hydrolysis, we were interested to determine whether the N-terminal deletions were defective in this respect. It has been observed previously that Hsp90 can form heterodimers (11). Heterodimers consisting of one WT protein and one fragment lacking the N-terminal domain exhibit severely reduced ATPase activities. However, a point mutant unable to bind ATP was shown to be able to form active heterodimers with WT Hsp90 (11). We used this observation to determine whether the deletion constructs were still effective in stimulating the WT protein in a heterodimer. We added increasing concentrations of Δ16-Hsp90 or Δ24-Hsp90 to constant concentrations of WT Hsp90 and determined the effect of heterodimerization on ATPase activity.

| Cm of the unfolding transition of the N-terminal domain | $k_{on}$ for ADP | $k_{off}$ | $k_p$ ($k_{on}$/$k_{off}$) |
|-----------------|------------------|----------|-----------------|
| Hsp90            | 1.15 ± 0.1       | 8.8      | 0.6             |
| Δ8-Hsp90         | 1.2 ± 0.1        | 11.1     | 0.6             |
| Δ16-Hsp90        | 1.2 ± 0.1        | 11.1     | 0.6             |
| Δ24-Hsp90        | 1.2 ± 0.1        | 11.1     | 0.6             |

To test whether the mutations change the dynamic properties of nucleotide binding and release, we performed kinetic studies employing rapid mixing techniques and fluorescent labeled ATP analogues as described previously (see “Experimental Procedures” (7)). Dissociation rate constants of (Pγ)MABA-ATP were measured directly by displacing (Pγ)MABA-ATP with an excess of ADP (data not shown).

To further evaluate the binding kinetics of the labeled ATP to various constructs, we performed experiments in which we followed the interaction at variable protein or nucleotide concentrations. These experiments were carried out with the full-length proteins as the tryptophan/MABA energy transfer signal observed upon binding of the (Pγ)MABA-ATP was sufficiently strong. The tryptophan/MABA energy transfer signal of a series of experiments with varying protein concentrations (Δ16-Hsp90) is shown in Fig. 4B. A plot of the observed rate constants versus protein concentration resulted in a straight line. This indicates a simple one-step binding mechanism and allows the extraction of a rate constant for association ($k_{on}$) and dissociation ($k_{off}$).

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1. Regulation of the Hsp90 ATPase

2. Size Exclusion HPLC Analysis

3. Structural Characterization of the Mutated N-Terminal Domains

4. ATPase Activity of the Deletion Mutants

5. Effect of Heterodimerization

6. Table I: Stability and Nucleotide Binding Properties of Hsp90 and N-terminal deletion constructs

7. Experimental Procedures
ATPase activities. Interestingly, the activity was unchanged or even slightly increased in the case of \( \text{Hsp90}_{16} \), whereas it was reduced in the case of \( \text{Hsp90}_{24} \) (Fig. 5B). The observed decrease in the case of \( \text{Hsp90}_{24} \) is similar to the effect that has been observed for a fragment lacking the entire N-terminal domain (11), indicating that the \( \text{Hsp90}_{24} \) mutant is unable to activate the intact N-terminal domain. Therefore, we conclude that regions required for efficient N-terminal dimerization include the first 24 amino acids. In contrast, the deletion of only 16 amino acids does not result in a disruption of the N-terminal dimerization properties, because activation of WT Hsp90 is still possible here. In the case of homodimers, however, neither \( \text{Hsp90}_{16} \) nor \( \text{Hsp90}_{24} \) is active.

\( \text{Hsp90}_{16} \) Stimulates ATP Hydrolysis in the Full-length Partner—To explain the function of \( \text{Hsp90}_{16} \) in the heterodimer, two possibilities exist. The inactive domain (\( \text{Hsp90}_{16} \)) could become activated in the heterodimer as a result of the contact with WT Hsp90, or the hydrolysis in the wild type domain is stimulated upon contact with \( \text{Hsp90}_{16} \). To discriminate between these possibilities, we formed heterodimers

![Fig. 4. Nucleotide binding to different Hsp90 constructs](image1)

**Fig. 4.** Nucleotide binding to different Hsp90 constructs. A, fluorescence titrations were used to determine the affinity of the truncated N-terminal domains for ADP. Data analysis was performed as described under “Experimental Procedures.” The data presented here are for N210. Similar curves were obtained for Δ16-N210 and Δ24-N210. The values for Δ16-N210 and Δ24-N210 are included in Table I. B, the fluorescence change upon binding of (Py)MABA-ATP was used to determine the on and off rates of nucleotide binding to the different full-length proteins (see “Experimental Procedures”). Here the data for Δ16-Hsp90 are shown. Similar experiments were used to determine the binding kinetics of the other full-length constructs (Table I). The concentrations of Δ16-Hsp90 were as follows: (○) 1 μM, (●) 2 μM, (▲) 3.5 μM, (△) 5 μM, and (■) 7.5 μM. C, the observed rate constants from Fig. 4B were replotted against the concentration of Δ16-Hsp90 to obtain the on and off rates of the binding reaction.

![Fig. 5. ATPase activities in homodimers and heterodimers](image2)

**Fig. 5.** ATPase activities in homodimers and heterodimers. A, ATPase activities of the N-terminal truncated Hsp90 constructs in comparison to Hsp90. The assay was performed as described under “Experimental Procedures” (see also Table II). B, influence of Δ16-Hsp90 (○) and Δ24-Hsp90 (●) on the ATPase activity of WT Hsp90. Data analysis was performed as described in Richter *et al.* (11). Statistical evaluation of the induced inhibition in the case of Δ24-Hsp90 resulted in the ATPase activity of the heterodimer of approximately 0.16 μmol/min/μgHsp90 and an n-value of 1.4. An n-value of 1 indicates equal probabilities of homodimer and heterodimer formation.

ATPase activities. Interestingly, the activity was unchanged or even slightly increased in the case of Δ16-Hsp90, whereas it was reduced in the case of Δ24-Hsp90 (Fig. 5B). The observed decrease in the case of Δ24-Hsp90 is similar to the effect that has been observed for a fragment lacking the entire N-terminal domain (11), indicating that the Δ24-Hsp90 mutant is unable to activate the intact N-terminal domain.

Therefore, we conclude that regions required for efficient N-terminal dimerization include the first 24 amino acids. In contrast, the deletion of only 16 amino acids does not result in a disruption of the N-terminal dimerization properties, because activation of WT Hsp90 is still possible here. In the case of homodimers, however, neither Δ16-Hsp90 nor Δ24-Hsp90 is active.

Δ16-Hsp90 Stimulates ATP Hydrolysis in the Full-length Partner—To explain the function of Δ16-Hsp90 in the heterodimer, two possibilities exist. The inactive domain (Δ16-Hsp90) could become activated in the heterodimer as a result of the contact with WT Hsp90, or the hydrolysis in the wild type domain is stimulated upon contact with Δ16-Hsp90. To discriminate between these possibilities, we formed heterodimers...
of deletion constructs and specific point mutants of Hsp90. We used the mutants D79N-Hsp90, which does not bind ATP, and E33A-Hsp90, which binds but does not hydrolyze ATP (4, 5). For D79N-Hsp90, it had been reported that it is able to sustain an increase in activity (Table II). Thus, we conclude that the mutant is fully active in the stimulation of an ATPase-active protein.

For E33A-Hsp90, we observed in this study an increase in the ATPase activity of the WT protein in a heterodimer (11). For stimulation-inactive subunits like 24-Hsp90, 262C-Hsp90, or 527C-Hsp90, the effect on the —

increase in the case of WT Hsp90 has been only a factor of 1.5. For stimulation-inactive subunits like 24-Hsp90, 262C-Hsp90, or 527C-Hsp90, the effect on the 

**Fig. 6. Heterodimers of Δ8-Hsp90.** Heterodimers were formed here with Hsp90-262C (○) and E33A-Hsp90 (●). The statistical evaluation resulted in the ATPase activity of the heterodimer for E33A-Hsp90 and Δ8-Hsp90 of 5.35 and an n-value of 1.1. The heterodimer activity of 262C-Hsp90 and Δ8-Hsp90 was found to be approximately 0.4 μM/min/μM-Hsp90, and the n-value was ~7, indicating that it is significantly more difficult to form heterodimers with Δ8-Hsp90 than it is with Hsp90 where the corresponding n-value was 1.3 (11).

**Fig. 7. SEC-HPLC of WT Hsp90 and Δ8-Hsp90.** SEC-HPLC runs were performed as described under “Experimental Procedures.” Different concentrations of WT Hsp90 and Δ8-Hsp90 were applied to the column. The straight lines represent the fit to obtain the dissociation constants for the buffer without ATP. Data analysis was performed as described in Richter et al. (11). Elution maxima of the single peaks were plotted against the concentration, which was corrected for dilution on the column. The corresponding curve was fitted to a monomer/dimer model. A, dissociation of Hsp90 with (○) and without ATP (●) in the running buffer. B, dissociation of Δ8-Hsp90 with (○) and without (●) ATP in the running buffer.
To address this directly, we employed SEC-HPLC to compare the dimerization constant of Δ8-Hsp90 and WT Hsp90 in the absence and presence of ATP. In the absence of ATP, both proteins dissociate at low protein concentrations and can be found in a monomer-dimer equilibrium on the SEC column. Their dissociation constants are both in the range of 80–100 nM. In the presence of ATP, the dissociation behavior of WT Hsp90 is not affected (Fig. 7A). However, with ATP, dissociation of Δ8-Hsp90 is no longer observed, even at very low protein concentrations (Fig. 7B). These data show that in particular in the presence of ATP the dimerization of Δ8-Hsp90 is stronger than that of WT Hsp90.

**DISCUSSION**

ATP binding and hydrolysis were shown to be essential for the *in vivo* function of the molecular chaperone Hsp90 (4, 5). Previous studies revealed that the ATPase cycle includes significant conformational changes that involve the entire Hsp90 molecule (7). These movements lead to a conformation in which the entire subunit in the heterodimer with WT Hsp90 is only able to hydrolyze ATP on its own. Therefore, the first 16 amino acids are required for a domain to become an activable subunit. The deletion of the first eight amino acids leads to an active protein that hydrolyzes ATP with a turnover rate of 1.5 times that of WT Hsp90. This implies that the first eight amino acids are not required for the stimulation reaction. In contrast to amino acids 8–16, the first eight amino acids in the WT protein seem to have a slightly inhibitory effect on the ATPase cycle.

For a structural interpretation, it is interesting to correlate our results with the known structures of GyraseB and MutL. Here, the corresponding N-terminal regions are involved in the N-terminal dimerization reaction. In both cases, the first β-strand and the first α-helix are swapped with the other subunit in the dimeric protein upon dimerization of the N-terminal domains (13–15). Based on our data, we would like to extend this model to Hsp90. Assuming strand swapping, the loss in dimerization properties of Δ16-Hsp90 and Δ24-Hsp90 can be explained as more and more of the dimerization site is lost. The observed increase in homodimerization and ATPase activity upon deletion of the first eight amino acids could be also explained by the proposed mechanism of strand swapping. This requires that the interactions are broken in one domain, because the exchanged amino acids normally are a stronger bound part of the N-terminal domain (8, 16). Therefore, the activation energy is first to be invested to disrupt these interactions before new ones can be formed with the other subunit. The deletion of amino acids 1–8 could lead to a weaker intramolecularly bound N-terminal peptide, facilitating the exchange process and subsequently the activation. Interestingly, the increase in the overall dimerization properties of Δ8-Hsp90 is dependent on ATP, consistent with the higher affinity of Δ8-Hsp90 for nucleotides and the increased ATPase activity compared with other Hsp90 constructs. Strand swapping also explains how Δ16-Hsp90 can be used to stimulate the WT protein in the heterodimer. It is reasonable to assume that the exchange of the strands, which occurs in the heterodimer only for the WT subunit, is required for the stimulation reaction, implying that the swapped strand is used to activate the stimulation activity. In addition, our results give insight into the basis for the extremely slow turnover number for the ATP hydrolysis. The activity of yeast Hsp90 was found to be one ATP hydrolyzed/min, whereas human Hsp90 turns over one ATP every 16 min (18), which prompts the question about the rate-limiting step in the ATPase cycle. Given the strong influence that can be observed once active monomer is bound to different inactive monomers, we propose that the step controlling the turnover must involve both subunits of the dimeric protein. This would be either the N-terminal dimerization reaction itself as the rate-limiting step or a subsequent step that involves an N-terminal dimerized form. Our results show that this step is greatly influenced by interactions within the first 24 amino acids.

In previous studies, C-terminal mutations and truncations were found to influence the ATPase activity, which suggests the involvement of C-terminal regions in the ATPase cycle (7, 8, 19). This involves the dimerization site in the C-terminal part and regions in the middle domain, which are required for the trapping of the ATP molecule. The analysis of heterodimers between WT and mutant subunits demonstrated the importance of the communication between the two subunits in the dimer and during the ATPase cycle. Thus, we would like to propose that the catalytic efficiency of Hsp90 is tightly regulated by intramolecular interactions mediated by N-terminal amino acids.

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N-terminal Residues Regulate the Catalytic Efficiency of the Hsp90 ATPase Cycle
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