Coordinated ATP Hydrolysis by the Hsp90 Dimer*

Received for publication, April 30, 2001, and in revised form, June 13, 2001
Published, JBC Papers in Press, July 5, 2001, DOI 10.1074/jbc.M103832200

Klaus Richter, Paul Muschler, Otmar Hainzl, and Johannes Buchner‡

From the Institut für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstr. 4, Garching 85747, Germany

The Hsp90 dimer is a molecular chaperone with an unusual N-terminal ATP binding site. The structure of the ATP binding site makes it a member of a new class of ATP-hydrolyzing enzymes, known as the GHKL family. While for some of the family members structural data on conformational changes occurring after ATP binding are available, these are still lacking for Hsp90. Here, we set out to investigate the correlation between dimerization and ATP hydrolysis by Hsp90. The dimerization constant of wild type (WT) Hsp90 was determined to be 60 nM. Heterodimers of WT Hsp90 with fragments lacking the ATP binding domain form readily and exhibit dimerization constants similar to full-length Hsp90. However, the ATPase activity of these heterodimers was significantly lower than that of the wild type protein, indicating cooperative interactions in the N-terminal part of the protein that lead to the activation of the ATPase activity. To further address the contribution of the N-terminal domains to the ATPase activity, we used an Hsp90 point mutant that is unable to bind ATP. Since heterodimers between the WT protein and this mutant showed WT ATPase activity, this mutant, although unable to bind ATP, still has the ability to stimulate the activity in its WT partner domain. Thus, contact formation between the N-terminal domains might not depend on ATP bound to both domains. Together, these results suggest a mechanism for coupling the hydrolysis of ATP to the opening-closing movement of the Hsp90 molecular chaperone.

Hsp90 is an abundant cytosolic protein that belongs to the class of molecular chaperones. Interaction with nonnative and destabilized proteins has been shown in vitro (1–5). In vivo an ever increasing number of proteins have been found to be associated with Hsp90 (6, 7). Most of the known in vivo substrates are involved in signal transduction pathways, like tyrosin and serine/threonine kinases, steroid hormone receptors, helix-loop-helix transcription factors, and tumor suppressor proteins (6, 7, 8). Other proteins, like reverse transcriptase (9) or telomerase (10), were found to be dependent on Hsp90 action as well. Consistent with the importance of the substrate proteins, Hsp90 was found to be an essential protein in yeast (11). Although the mechanism of Hsp90 is still far from being understood, Hsp90 is thought to maintain an otherwise unstable conformation of the substrate (7). ATP binding and hydrolysis were found to be essential for the function of Hsp90 (12, 13), and competitive inhibitors for ATP binding like geldanamycin were shown to be potent antiproliferative agents (14, 15).

The first indications that Hsp90 conformation is influenced by ATP were reported by Csermely et al. (16) and Grenert et al. (17). Experiments using spin-labeled ATP showed that Hsp90 binds ATP weakly (18). The nucleotide binding site was identified in the crystal structure of the N-terminal domain of yeast Hsp90 in the presence of ADP (19, 20). This binding site exhibits a new type of fold similar to that of DNA gyrase B and MutL (21). The nucleotide is bound in an unusual kinked conformation with the adenosine base and the ribose buried inside the protein in a cleft. The β-phosphate and probably even more the γ-phosphate of ATP are solvent-accessible in the crystal structure. Interestingly, the crystal structures of the domain in the presence or absence of nucleotide were almost identical (19). The kinetic analysis of the ATPase cycle of Hsp90 revealed that, after ATP binding, a conformational change occurs in Hsp90 that traps the ATP molecule (22). This trapped ATP molecule proved to be committed to hydrolysis, since it was impossible to exchange it for unbound ATP. This conformational change requires parts of the protein that are C-terminal to the binding site. A conformational change was also observed in the crystal structure of the homologous protein MutL (23, 24). MutL traps ATP by contacts between the γ-phosphate of ATP and a domain further C-terminal from the ATP binding site. Conformational changes of Hsp90 upon ATP binding were also reported by Prodromou et al. (25). Here, cross-linking data indicate that the N-terminal domains associate in the presence of AMP-PNP, which had been suggested previously based on electron microscopic data (26). This seems to be a prerequisite for the association of Hsp90 with the co-chaperone p23, which is known to occur after ATP-binding (27, 28, 29). In the studies of Weikl et al. (22) and Prodromou et al. (25), fragments of Hsp90 lacking C-terminal domains were found to be considerably less active than wild-type Hsp90. These fragments are thought to be monomeric, in contrast to full-length Hsp90, which is a dimer. The dimerization site was shown to reside in the very C-terminal domain of Hsp90 (30, 31).

To gain further insight into the ATPase mechanism of Hsp90, we investigated the importance of dimer formation for the ATPase activity. To this end, we used a set of Hsp90 deletion mutants and analyzed their quaternary structure as well as their ATPase activities. Hsp90 as well as C-terminal fragments were shown to be dimeric with dissociation con-
stains of 0.06 μM. Analysis of the ATPase activity of these fragments demonstrated that the presence of two N-terminal domains is required to stimulate the ATPase activity, since heterodimers formed between C-terminal fragments and the WT protein had diminished ATPase activities. Surprisingly, the stimulation of hydrolysis does not require ATP bound on both sides of the dimer. The presence of two N-terminal domains is sufficient to activate ATP-hydrolysis.

**EXPERIMENTAL PROCEDURES**

**Materials**—Geldanamycin (GA) was a kind gift of the NCI Experimental Drug Division, National Institutes of Health (Bethesda, MD).

**Hsp90 Constructs**—Proteins 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 polymerase chain reaction fragments were cloned into the Qiagen vectors pQE30, resulting in the constructs pQE30-N529, pQE30-N599, and pQE30-527C. In addition, fragments constructed by Scheibl et al. (5) were used. The mutagenesis leading to the D79N mutation in full-length Hsp90 was done by overlap extension polymerase chain reaction in N210 using two primers containing the mutation (5′-GGAATTCGAGCATTTGTTCTGATTTCC-3′ and 5′-GGAAATCAGAAACTCTGGTATTG-3′) and subsequent insertion of this fragment into the full-length gene, using an N-terminal BamHI restriction site and the internal XhoI site. The identity of all constructs was confirmed by DNA sequencing.

**Protein Expression and Purification**—His-Hsp90 was expressed in the strain BL21 (DE3) cop † (Stratagene, La Jolla, CA) in LB_kan and induced with 1 mM isopropyl-1-thio-β-galactopyranoside. For all other constructs, the strain M15 prep and 2 mM isopropyl-1-thio-β-galactopyranoside were used. Cells were lysed using a cell disruption system (Constant Systems, Warwick, UK). The fragments and the His-tagged full-length protein were purified on a chelating Sepharose column (Amersham Pharmacia Biotech) depending on protein size, was run in 40 mM Potassium phosphate, pH 7.0, at protein concentrations of 200–1000 μM. The column was washed with wash buffer (40 mM KPO4, pH 8.0, 400 mM KCl, 20 mM imidazole) before elution was performed in a step gradient with washing buffer containing 300 mM imidazole. A Resource Q column (Amersham Pharmacia Biotech) was used to further purify the proteins. The protein was loaded in 50 mM Tris, pH 8.0, 20 mM KCl and eluted with a gradient from 20 to 1000 mM KCl. As a final purification step, a Superdex 200 HiLoad column matrix was observed (with the exception of 527C), since the protein is diluted severalfold during the course of the experiment. Interaction of monomeric and dimeric species with the column matrix was observed (with the exception of 527C), since the peak shapes were similar for the dimeric and the monomeric species. The analysis of the dissociation curves was based on the assumption that at any time monomeric and dimeric species were in equilibrium. Data were fitted using the Scientist program (MicroMath, Salt Lake City, UT) and Equation 2.

\[
ET = ET_{\text{mono}} - (ET_{\text{mono}} - ET_{\text{dimer}}) \times \frac{[\text{Protein}]}{[\text{Protein}] + K_{\text{app}}} \quad (\text{Eq. 2})
\]

where ET represents the elution time, ETmono is the elution time of the monomeric species, and ETdimer is the elution time of the dimeric species. The obtained dissociation constant K_{app} is only an approximation, since the protein is diluted severalfold during the course of the experiment. To determine the dilution factor, the peak area was analyzed. At the time of injection, the width of the peak was 0.2 min, which corresponds to a 0.1-ml injected volume. Dividing the peak area by 0.2 gives a signal that is about 7 times as high as the maximum of the originally observed peak. This factor was used as the dilution factor for the calculation of the "real" K_{app} by dividing K_{app} (with the dilution factor. Since the dilution factors were found to be similar for the differently Hsp90 fragments, they allowed us to directly compare their association behaviors. All of the following values are already corrected for dilution.

**Analytical Ultracentrifugation**—Data for sedimentation equilibrium runs were collected on a Beckman XL-A analytical ultracentrifuge using a Ti60 rotor. All runs were performed at 4°C for 48 h with the addition of protease inhibitors (Complete; Roche Molecular Biochemicals) to avoid degradation. The protein gradient was detected at 280 nm. Data analysis was done with the program Origin (Beckman, Fullerton, CA).

**RESULTS**

**N-terminal Fragments of Yeast Hsp90 Show Reduced ATPase Activity**—Earlier studies showed that truncation mutants of Hsp90 that contain the N-terminal ATP-binding site
but lack C-terminal regions are considerably less active in ATP hydrolysis than the WT protein (5, 22, 25). We were interested in further defining the requirements for full ATPase activity of Hsp90. For this purpose, we designed a set of Hsp90 fragments based on proteolytic digests (5), sequence alignments with homologous proteins, and hydropathy plots (Fig. 1). The fragments, varying in size from 210 amino acids (positions 1–210) to 599 amino acids (positions 1–599), were purified, and their structures and stabilities were analyzed using CD spectroscopy and urea transitions. The midpoint of the unfolding transition was between 3 and 5 M urea for all fragments tested. This compares with the stability of the WT protein. All domains used were thus shown to be folded and stable (data not shown). As reported previously, Hsp90-N210 and Hsp90-N272 showed extremely weak ATPase activities, while Hsp90-N529 and, more pronounced, Hsp90-N599 exhibited increased ATPase activities. However, these activities are still by a factor of 6 lower than the ATPase activity of wild-type Hsp90 (Fig. 2A).

We were unable to detect any concentration dependence of the ATPase activity for Hsp90-N210 and for the full-length protein. However, Hsp90-N529 and Hsp90-N599 showed a reproducible increase of activity with protein concentration (Fig. 2B).

**C-terminal Fragments and WT Hsp90 Share Similar Dimerization Properties**—To determine the dissociation constant (K_d) for Hsp90, we decided to employ size exclusion HPLC (SEC-HPLC) with fluorescence detection at different protein concentrations. Using Hsp90 concentrations of 2 nM to 1 μM, only one peak was observed at every concentration tested. However, the elution times differed, depending on the protein concentration used (Fig. 3A). A shift could be observed starting from an elution time of 20 min (high protein concentration) to an elution time of 23 min (low protein concentration). The presence of one peak at every concentration is consistent with fast equilibration compared with the time scale of the experiment. Using a model that assumes a monomer-dimer equilibrium at every time point of the experiment, we fitted the data points and obtained a K_d of 60 ± 12 nM (Fig. 3B).

Having calculated the K_d for WT Hsp90, we were interested to investigate whether C-terminal fragments containing the dimerization site show similar dimerization characteristics. Two fragments were constructed, one ranging from amino acid 262 to 708 (Hsp90-262C) and the other containing amino acids 527–709 (Hsp90-527C). Hsp90-262C gave a transition curve similar to Hsp90 (Fig. 3C). The K_d obtained for Hsp90-262C was 45 ± 12 nM. For Hsp90-527C, no titration curve was obtained, since this protein interacted with the gel filtration matrix, resulting in significant peak broadening.

These data clearly show that Hsp90 and its C-terminal fragments are dimeric under conditions used for the ATPase assay, where protein concentrations usually are by at least 1 order of magnitude higher than the dimerization constant of Hsp90.

**N-terminal Hsp90 Fragments Are Monomeric**—To analyze the quaternary structure of N-terminal fragments, the same experimental setup was used. For Hsp90-N529, no changes in the elution time were observed at concentrations ranging from 0.04 to 5 μM. This, together with equilibrium sedimentation data from analytical ultracentrifugation collected for Hsp90-N529 (data not shown) led us to conclude, that Hsp90-N529 is monomeric within the concentration range tested. Similarly, SEC-HPLC experiments were performed with Hsp90-N599. Here no change in quaternary structure was observed within the concentration range tested (0.04–2 μM) as well (Fig. 3C).

Next, we investigated whether the oligomerization properties of the N-terminal fragments change in the presence of ATP. Hsp90-N529 as well as Hsp90-N599 showed a slight concentration dependence in SEC-HPLC experiments (data not
In agreement with the ATPase assays (Fig. 2B), which were performed at concentrations between 2 and 10 μM for Hsp90-N599, these data indicate that this could be the beginning of a monomer-dimer transition curve but still be far away from the actual dimerization constant.

Taken together, these data suggest that the fragments lacking the C-terminal domain are monomeric and that ATP does not alter the oligomerization behavior of these proteins in the concentration range tested. At higher concentrations, as used in the ATPase assays (Fig. 2B), they dimerize with dimerization constants in the range of 10⁻⁶ M for Hsp90-N599 and about 70⁻⁶ M for Hsp90-N530, as obtained by analysis of the concentration-dependent ATPase assays.

Heterodimers Can Be Formed between Hsp90 and C-terminal Hsp90 Fragments—Having established that the dimerization properties for Hsp90 and its C-terminal fragment 262C are similar, we were interested in whether heterodimers could be obtained by mixing Hsp90 with C-terminal fragments. To detect dimers, we used glutaraldehyde, which is known to efficiently cross-link dimeric Hsp90 (32). Cross-linking is not complete, since about 20% of monomers can be detected. (Fig. 4). Hsp90-527C was added to WT Hsp90. In the presence of Hsp90-527C, a decrease in the intensity of the dimeric WT Hsp90 band was visible on SDS-polyacrylamide gel electrophoresis, while an additional band appeared, consisting of one molecule Hsp90-527C and one molecule Hsp90 (Fig. 4A). Thus, it is possible to form heterodimers between Hsp90 and its C-terminal fragments in a concentration-dependent manner. Although the concentration of the C-terminal fragment was much higher than the concentration of WT Hsp90 in these experiments, the sample still seems to contain a fraction of homodimers of WT Hsp90. A quantitative densitometric analysis of the scanned SDS gels showed that the cross-linked sample at the highest concentration of 527C-Hsp90 contains about equal concentrations of monomeric Hsp90, dimeric Hsp90, and heterodimeric Hsp90. This might reflect a higher

![Analysis of Hsp90 dimerization by SEC-HPLC. A, size exclusion HPLC of Hsp90. The running buffer contained 40 mM HEPES, 150 mM KCl, pH 7.5, at 20 °C. Protein was detected by fluorescence with an excitation wavelength of 280 nm and an emission wavelength of 328 nm. The shift of the peaks from 20 to 24 min of elution time represents the dissociation of Hsp90. Solid line, 1 μM Hsp90; dotted line, 0.03 μM Hsp90; dashed line, 5 nM Hsp90. B, dissociation curve of the Hsp90 dimer. The data points of the SEC analysis were fitted according to Equation 1 (see “Experimental Procedures”). The resulting dissociation constant is 60 ± 12 nM. C, dissociation curve of the C-terminal fragment Hsp90-262C (●) and Hsp90-N529 (○) and Hsp90-N599 (▪). The dissociation constant for Hsp90-262C is 45 ± 12 nM. The elution time of Hsp90-529, which is suggested to be monomeric, does not change over a wide range of concentrations, as does the elution time of Hsp90-N599.

![Demonstration of heterodimer formation between Hsp90 and Hsp90-527C by cross-links. A, SDS-polyacrylamide gel electrophoresis of cross-linked Hsp90 complexes in a buffer containing 40 mM HEPES, pH 7.5, 150 mM KCl, 5 mM MgCl₂, and 2 mM AMP-PNP. Hsp90-527C was added at a stoichiometry of 8:1, 4:1, 2:1, 1:1, 1:2, and 1:4 (lanes 4–9) to Hsp90. Cross-linking was performed for 2 min at 30 °C with glutaraldehyde. Lane 1, high molecular weight standard; lane 2, Hsp90 without cross-linking; lanes 3 and 10, cross-linking of 527C-Hsp90 and WT Hsp90, respectively. D, dimer; M, monomer; HD, heterodimer; D2, homodimer of Hsp90-527C. B, densitometric analysis of the SDS-polyacrylamide gel electrophoresis. ▼, WT Hsp90 dimer; ●, WT Hsp90 monomer; ○, heterodimer of WT Hsp90 and Hsp90-527C.](http://www.jbc.org/)

Coordinated ATP Hydrolysis of Hsp90

![Fig. 3](http://www.jbc.org/)
Cross-linking efficiency of the homodimer compared with the heterodimer and the C-terminal fragment. Cross-links made in the absence and presence of AMP-PNP did not show detectable differences in the intensity of the heterodimer band (Fig. 4B). Thus, the N-terminal association induced by AMP-PNP does not contribute significantly to the ability of 527C-Hsp90 to disrupt Hsp90 homodimers.

**Heterodimers of Hsp90 and C-terminal Hsp90 Fragments Show Reduced ATPase Activity**—Next, ATPase assays were performed with Hsp90 and Hsp90-527C. Hsp90-527C itself was found to have no ATPase activity. When the concentration of Hsp90-527C was increased in the presence of a constant amount of Hsp90, a decrease in ATPase activity was observed (Fig. 5A). This result suggests that the heterodimers have reduced ATPase activity. A statistical model that assumes equal probabilities for the formation of homodimers and heterodimers gave the expected increase in activity (Fig. 5A).

The addition of longer C-terminal fragments containing the middle domain in addition to the C-terminal domain (amino acids 262–709; cf. Fig. 1) also led to decreased ATPase activities (Fig. 5B). The inhibition curves were very similar to that of Hsp90-527C, and again the statistical model gave a good fit to the data points. The model used to fit the data points also provides information on the ATPase activity of the heterodimer. The calculated activity of the heterodimer is one-third of the WT activity. This indicates that the ATPase is not completely inhibited in the heterodimer, at least not to the same extent as in the isolated N-terminal domains. The fact that a further increase in the concentrations of N-terminal truncated mutants did not result in additional decrease of the ATPase activity suggests that the end point of the titration is reached, as would be expected based on the concentrations of the two proteins. This suggests that beside the N-terminal dimerization, C-terminal regions seem to be important for efficacy of the ATPase cycle. Also, fast dissociation and reassociation of dimers could result in temporary formation of homodimers, which might be sufficient to stimulate the ATPase activity and thus prevent complete inhibition.

Taken together, the data suggest that the presence of two N-terminal domains is required to activate the ATPase activity in WT Hsp90. Additionally, they confirm that the dimerization properties of Hsp90-262C and Hsp90-527C are very similar to that of wild-type Hsp90, indicating that interactions in the N-terminal part of the protein do not contribute significantly to the dissociation constant of the wild-type protein.

**Hsp90-D79N Does Not Inhibit the ATPase Activity of Hsp90**—Next, we wanted to know, whether ATP binding is required in both N-terminal domains of the homodimer to achieve wild-type activity. To test this possibility, we used a point mutant of Hsp90, Hsp90-D79N, which does not bind or hydrolyze ATP (12, 13).

Competition experiments performed with increasing concentrations of Hsp90-D79N and a fixed amount of Hsp90 showed that Hsp90-D79N did not affect the ATPase activity of Hsp90, even if an 8-fold excess of the Hsp90 mutant was used (Fig. 6A). This implies that the activity of an individual WT monomer in the dimer context is the same, independent of whether the N-terminal partner domain is active or inactive. Thus, a defect in ATP binding does not compromise stimulation of ATP hydrolysis in the partner domain.

Since we could not detect any change in ATPase activity, we wanted to rule out the possibility that formation of heterodimers did not occur in the competition experiment. Hsp90 is known to be very sensitive to C-terminal proteolysis, and the loss of even a small fragment from the C-terminal end affects the dimerization properties significantly (data not shown). Thus, we determined the dimerization constant for Hsp90-D79N by SEC-HPLC. Here we obtained a dimerization constant of 85 ± 20 nM (Fig. 6B). This value indicates that the dimerization of Hsp90-D79N does not differ significantly from that of WT Hsp90.

**Dimerization of Hsp90 Is a Dynamic Process**—We used the inhibition of the ATPase activity of Hsp90 by formation of heterodimers to obtain insight into the dynamics of the dimerization reaction. To this end, we added Hsp90-262C to the ATPase-suppressed heterodimeric form of Hsp90 and Hsp90-527C to the ATPase reaction while monitoring the progress of ATP hydrolysis. As expected, the formation of heterodimers resulted in a pronounced decrease in ATP hydrolysis immediately after mixing (Fig. 7A).

Additionally, we monitored the kinetics of formation of active dimers by adding Hsp90-D79N to an ATPase-suppressed heterodimeric form of Hsp90 and Hsp90-262C. This experiment gave the expected increase in activity (Fig. 7B), since the addition of Hsp90-D79N leads to the formation of Hsp90/Hsp90-D79N heterodimers, in which the intact N-terminal domain exhibits wild type ATPase activity (cf. Fig. 6A). Again, the increase in activity was obtained within the first seconds of the experiment.

These results indicate that the Hsp90 dimer is a highly dynamic structure.
The x-ray structure of an N-terminal fragment of Hsp90 in complex with ADP showed a nucleotide binding site that buries the ribose backbone and the adenine base inside a cleft but leaves the γ-phosphate pointing toward the surface of the molecule (19). It is obvious from this structure that for ATP the γ-phosphate would be completely solvent-exposed. More importantly, no conformational changes were observed in the crystal structure of the N-terminal domain upon binding of ADP or GA (19, 20). Subsequent studies showed that conformational changes occur in other parts of the protein, which trap the ATP molecule and commit it to hydrolysis (22). In addition, the N-terminal domains come in close contact (25). The functional consequences of these changes are still largely unclear. One of the few functional correlations of these rearrangements is that binding of the cofactor p23 requires ATP bound to Hsp90 (28) and the dimeric form of the N-terminal domains (22, 29).

In this study, the Hsp90 dimer was shown to exhibit a dissociation constant of about 60 nM. The major dimerization site resides in the C-terminal region, since an Hsp90 fragment consisting of amino acids 527–709 formed heterodimers with a affinity comparable with the native dimer. This is in agreement with previous qualitative studies, using truncated fragments or deletion mutants of Hsp90. For human Hsp90, Jibard et al. (31) mapped sites important for dimerization to amino acids 548–567, 661–677, and 679–728. These sites can be found in fragment 527C of yeast Hsp90, indicating that this domain is responsible for dimerization. In addition to this, our results suggest that much weaker (K_d > 70 µM) interaction sites are present within amino acids 1–529.

Our approach to obtaining the dimerization constant of Hsp90 differs from other methods in that it does not require labeling. The quantitative analysis of gel filtration allows a wide concentration range to be covered using sensitive fluorescence detection. Previously, dimerization constants for homodimers were obtained for enzymes like lactate dehydrogenases using enzymatic activity, as the probe for the native tetrameric state (33, 34). The value obtained is 1 nM. A dissociation constant of 60 nM for Hsp90 may reflect the requirement for a dynamic monomer/dimer equilibrium. Most interestingly, the dissociation constant for the dimer of CheA, a histidine kinase with an ATP-binding site homologous to Hsp90, was determined by enzymatic assays and found to be in the range of 200–400 nM (35). Dissociation and association in the Hsp90 dimer occurs fast, since we were unable to measure the rates of subunit exchange in experiments with a dead time of about 20 s. This shows that the dynamic of the dimerization of Hsp90 is at least in the same range as ATP hydrolysis, leading to the interesting possibility that Hsp90 might be able to dissociate during the ATPase cycle.

The data of this study, combined with earlier data (22, 25),
allow us to define the key steps of the Hsp90 ATPase as summarized below (Fig. 8).

In the first step of our model, ATP binding occurs independently at both N-terminal domains of the dimer in a fast reaction (Fig. 8, step 1). Cooperativity does not seem to be involved in the binding reaction, since the binding constant for AMP-PNP to WT Hsp90 is identical to that of Hsp90-N529 or Hsp90-N210 (data not shown).

Following the binding of ATP, a conformational change occurs in Hsp90 (Fig. 8, step 2), which requires amino acids 1–451 and was suggested to use Lys-342 as the acceptor for the γ-phosphate of the ATP (22). These movements seem to be a prerequisite for a functional N-terminal dimerization reaction (25) that we consider to be the next step (Fig. 8, step 3).

This dimerization reaction was shown to be possible for Hsp90-N529 but much more efficient for Hsp90-N599, indicating that major interaction sites exist between amino acids 529 and 599. Our results show that the N-terminal dimerization is the prerequisite for an efficient ATP hydrolysis reaction. Formation of heterodimers with fragments that compete for the dimerization site on the WT protein leads to a significant inhibition of the Hsp90 ATPase activity. A heterodimer comprising one wild-type protein and one fragment lacking the first 261 amino acids shows reduced ATPase activity, suggesting that the two N-terminal fragments hydrolyze their ATP molecules in a cooperative manner (Fig. 8).

In the case of Hsp90, surprisingly, the addition of Hsp90-D79N did not change the activity of the wild-type protein, indicating that the WT monomer in complex with Hsp90-D79N is as active as the WT monomer in the homodimer. This implies that Hsp90-D79N fulfills all of the requirements needed to activate the ATPase activity of WT protein, although its part is only a passive one. Thus, in principle, Hsp90 is able to work even with only one ATP bound to the dimer, although this case might be unphysiological, given the high ATP concentrations inside the cell and the fact that ATP binding is known to be very fast compared with the following steps in the hydrolysis cycle (22). After hydrolysis, Hsp90 presumably opens and releases the ADP molecule (Fig. 8, step 6), which brings Hsp90 back to the conformation competent to bind ATP.

Additional evidence for this model comes from studies of homologous proteins, known to share a structurally related ATP-binding site with Hsp90. N-terminal fragments of gyrase B show a concentration-dependent ATPase activity that closely resembles that observed for Hsp90. Most striking is the crystal structure of an N-terminal fragment of gyrase B (38). Here, the structural organization seems to be very similar to that of Hsp90. The model proposed for the ATPase mechanism of gyrase involves the transient association of the N-terminal domains in their ATP-bound state (39). This may lead to changes in the active center of the enzyme, which are the basis for the cooperative hydrolysis of ATP (40). A cooperative mechanism of this kind would guarantee that ATP hydrolysis is closely coupled to the coordinated movement of the two N-terminal domains. It might be envisioned that Hsp90 this way couples the energy of ATP hydrolysis to the coordinated movement of domains.

It remains to be seen how these movements are influenced by the partner proteins of Hsp90 and how they affect the conformational processing of client proteins.

Acknowledgments—We thank Dr. Stefan Walter for stimulating discussions and Alex Frenzl and Martin Haslbeck for help with the artwork.

REFERENCES
1. Wieh, H., Buchner, J., Zimmermann, R. & Jakob, U. (1992) Nature 358, 169–170
2. Miyata, Y. & Yahara, I. (1992) J. Biol. Chem. 267, 7042–7047
3. Freeman, B. C. & Morimoto, R. I. (1996) EMBO J. 15, 2969–2979
4. Jakob, U., Lilie, H., Meyer, I. & Buchner, J. (1995) J. Biol. Chem. 270, 7288–7294
5. Scheibb, T., Weikl, T. & Buchner, J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 1495–1499
6. Pratt, W. B. (1998) Proc. Soc. Exp. Biol. Med. 217, 420–434
7. Buchner, J. (1999) Trends Biochem. Sci. 24, 136–141
8. Richter, K. & Buchner, J. (2001) J. Cell. Physiol. 188, 281–290
9. Hu, J. & Seeger, C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1060–1064
10. Holt, S. E., Aisner, D. L., Baur, J., Tesmer, V. M., Dy, M., Ouellette, M., Trager, J. B., Morin, G. B., Toft, O. D., Shay, J. W., Wright, W. E. & White, M. A. (1999) Genes Dev. 13, 817–826
11. Nathan, D. F. & Lindquist, S. (1995) Mol. Cell. Biol. 15, 3917–3925
12. Obermann, W. M. J., Sondermann, H., Russo, A. A., Pavlevitch, N. P. & Hartl, F. U. (1998) J. Cell Biol. 143, 903–910
13. Panaretou, B., Prodromou, C., Roe, S. M., O’Brien, R., Ladbury, J. E., Piper, P. W. & Pearl, L. H. (1998) EMBO J. 17, 4829–4836
14. Whitesell, L., Minnaugh, E. G., DeCosta, B., Myers, C. E. & Neckers, L. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8324–8328
15. An, W. G., Schnur, R. C., Neckers, L. & Biggskonny, M. V. (1997) Cancer Chemother. Pharmacol. 40, 60–64
16. Csermely, P., Rajtar, J., Hallasi, M., Jalszovszky, G., Holly, S., Kahn, C. R., Gergely, P., Stei, C., Mihaly, K. & Somogyi, J. (1993) J. Biol. Chem. 268, 1901–1907
17. Grenert, J. P., Sullivan, W. P., Fadden, P., Haystead, T. A., Clark, D.,
Coordinated ATP Hydrolysis of Hsp90

Mimnaugh, E., Krutzsch, H., Ochel, H. J., Schulte, T. W., Sausville, E., Neckers, L. M. & Toft, D. O. (1997) *J. Biol. Chem.* **272**, 23832–23850

18. Scheibl, T., Neuhefen, S., Weikl, T., Mayr, C., Reinstein, J., Vogel, P. D. & Buchner, J. (1997) *J. Biol. Chem.* **272**, 18608–18613

19. Prodomain, C., Roe, S. M., O'Brien, R., Ladbury, J. E., Piper, P. W. & Pearl, L. H. (1997) *Cell* **90**, 65–75

20. Stebbins, C. E., Russo, A. A., Schneider, C., Rosen, N., Hartl, F. U. & Pavletich, N. P. (1997) *Cell* **90**, 65–75

21. Prodromou, C., Roe, S. M., O'Brien, R., Ladbury, J. E., Piper, P. W. & Pearl, L. H. (1997) *Cell* **90**, 65–75

22. Stebbins, C. E., Russo, A. A., Schneider, C., Rosen, N., Hartl, F. U. & Pavletich, N. P. (1997) *Cell* **90**, 65–75

23. Prodromou, C., Panaretou, B., Chohan, S., Siligardi, G., O’Brien, R., Ladbury, J. E., Roe, S. M., Piper, P. W. & Pearl, L. H. (1997) *EMBO J.* **16**, 4383–4392

24. Ban, C., Junop, M. & Yang, W. (1999) *Cell* **97**, 85–97

25. Jakob, U., Meyer, I., Bugl, H., Andre, S., Bardwell, J. C. & Buchner, J. (1995) *J. Biol. Chem.* **270**, 14412–14419

26. Stebbins, C. E., Russo, A. A., Schneider, C., Rosen, N., Hartl, F. U. & Pavletich, N. P. (1997) *Cell* **90**, 65–75

27. Johnson, J. L. & Toft, D. O. (1994) *J. Biol. Chem.* **269**, 24989–24993

28. Johnson, J. L., Corbisier, R., Stensgard, B. & Toft, D. O. (1996) *J. Steroid Biochem. Mol. Biol.* **56**, 31–37

29. Chadli, A., Bouhouche, I., Sullivan, W., Stensgard, B., McMahon, N., Catelli, M. G. & Toft, D. O. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 12524–12529

30. Nemoto, T., Ohara-Nemoto, Y., Ota, M., Takagi, T. & Yokoyama, K. (1995) *Eur. J. Biochem.* **233**, 1–8

31. Jihard, N., Meng, X., Leclerc, P., Rajkowski, K., Fortin, D., Schweizer-Greyer, G., Catelli, M. G., Baudieu, E. E. & Cadepond, F. (1999) *Exp. Cell. Res.* **247**, 461–474

32. Jakob, U., Meyer, I., Bugl, H., Andre, S., Bardwell, J. C. & Buchner, J. (1995) *J. Biol. Chem.* **270**, 14412–14419

33. Bartholmes, P., Duran, P., Buchner, J. (1973) *Eur. J. Biochem.* **39**, 101–108

34. Berr, K., Wassenberg, D., Lilie, H., Behlke, J. & Jaenicke, R. (2000) *Eur. J. Biochem.* **267**, 5413–5420

35. Surette, M. G., Levit, M., Liu, Y., Lukat, G., Ninfa, E. G., Ninfa, A. & Stock, J. B. (1996) *J. Biol. Chem.* **271**, 939–945

36. Todd, M. J., Viitanen, P. V. & Larimer, G. H. (1994) *Science* **265**, 659–666

37. Weissman, J. S., Rye, H. S., Fenton, W. A., Beechem, J. M. & Horwich, A. L. (1996) *Cell* **84**, 481–490

38. Wigley, D. B., Davies, G. J., Dodson, E. J., Maxwell, A. & Dodson, G. (1991) *Nature* **351**, 624–629

39. Kampranis, S. C. & Maxwell, A. (1998) *J. Biol. Chem.* **273**, 26305–26309

40. Brino, L., Urzhumtsev, A., Mousli, M., Bronner, C., Mitschler, A., Oudet, P. & Moras, D. (2000) *J. Biol. Chem.* **275**, 9468–9475
