Structural Basis for Inhibition of the Hsp90 Molecular Chaperone by the Antitumor Antibiotics Radicicol and Geldanamycin

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The cellular activity of several regulatory and signal transduction proteins, which depend on the Hsp90 molecular chaperone for folding, is markedly decreased by geldanamycin and by radicicol (monorden). We now show that these unrelated compounds both bind to the N-terminal ATP/ADP-binding domain of Hsp90, with radicicol displaying nanomolar affinity, and both inhibit the inherent ATPase activity of Hsp90 which is essential for its function in vivo. Crystal structure determinations of Hsp90 N-terminal domain complexes with geldanamycin and radicicol identify key aspects of their nucleotide mimicry and suggest a rational basis for the design of novel antichaperone drugs.

Introduction

Radicicol (monorden) is an antitumor antibiotic which is able to revert the tumor phenotype of *src*[1] and *ras*[2,3] transformed cell lines and disrupts mitogenic signaling via Ras- and Raf-1-dependent signal transduction pathways.[4] Although apparently able to inhibit tyrosine kinase activities in vivo, radicicol has no inhibitory activity against the serine/threonine kinases of the mitogen-activated pathway.[5] Rather, it appears to promote accelerated turnover and degradation of Raf-1, in a manner very similar to that of ansamycin antibiotics such as geldanamycin or herbimycin-A,[6−8] although it has no significant structural similarity to these (Figure 1). The action of ansamycins was also originally attributed to direct inhibition of kinase activity,[9] but it has subsequently been shown to result from inhibition of folding and activation of the target proteins by the Hsp90 molecular chaperone.[6,7,10−14] Ansamycin antibiotics bind in the ATP-binding site in the N-terminal domain of Hsp90s[15,16] and disrupt the ATPase activity of Hsp90 which is essential for its chaperone activity in vivo.[17] The similarity in behavior of geldanamycin and radicicol in disrupting the folding of protein kinases dependent on Hsp90 has prompted us to examine whether radicicol might act in a similar manner at the molecular level.

Here we show that radicicol is a potent and specific inhibitor of the ATPase activity of Hsp90, with nanomolar affinity, and we have determined the crystal structure of a complex between radicicol and the N-terminal domain of yeast Hsp90. Comparison of this structure with structures for yeast Hsp90 N-domain complexes with ADP, and with geldanamycin, defines the key nucleotide mimetic interactions made by these distinct antibiotics and provides a structural basis for the design of new Hsp90 inhibitors.

Results

Hsp90 Binding and ATPase Inhibition. Using isothermal titration calorimetry (see Experimental Section), we analyzed the thermodynamics of radicicol and geldanamycin binding to Hsp90 (Figure 2A and Table 1). Geldanamycin binds to full-length dimeric Hsp90 with a $K_d = 1.2\ \mu$M, while radicicol shows significantly higher affinity with a $K_d = 19$ nM. Both compounds bind to intact dimeric yeast Hsp90 with stoichiometries of approximately one antibiotic molecule per Hsp90 monomer. Comparable affinities were measured for binding to the isolated N-domain of Hsp90 (residues 1−220), but no detectable binding occurred between either antibiotic and the isolated C-terminal region of Hsp90 (residues 251−706) confirming the binding site for both antibiotics as the N-terminal nucleotide-binding domain. Binding of both compounds is accompanied by major favorable enthalpy changes although the weaker-binding geldanamycin has a more favorable enthalpic contribution on binding than radicicol. However, the entropic contributions to binding for the two inhibitors are of opposite signs suggesting significantly different modes of binding. Binding of geldanamycin incurs an entropic penalty, whereas radicicol binds with a favorable entropy change.

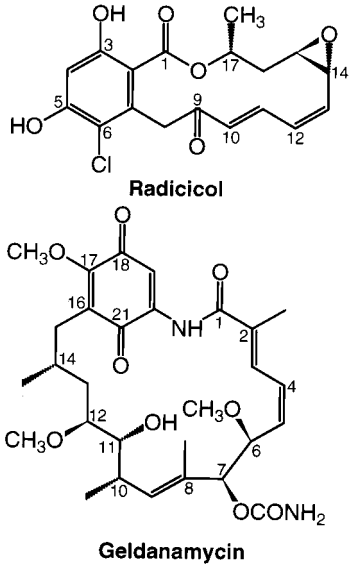

Figure 1. Molecular structures of radicicol and geldanamycin.

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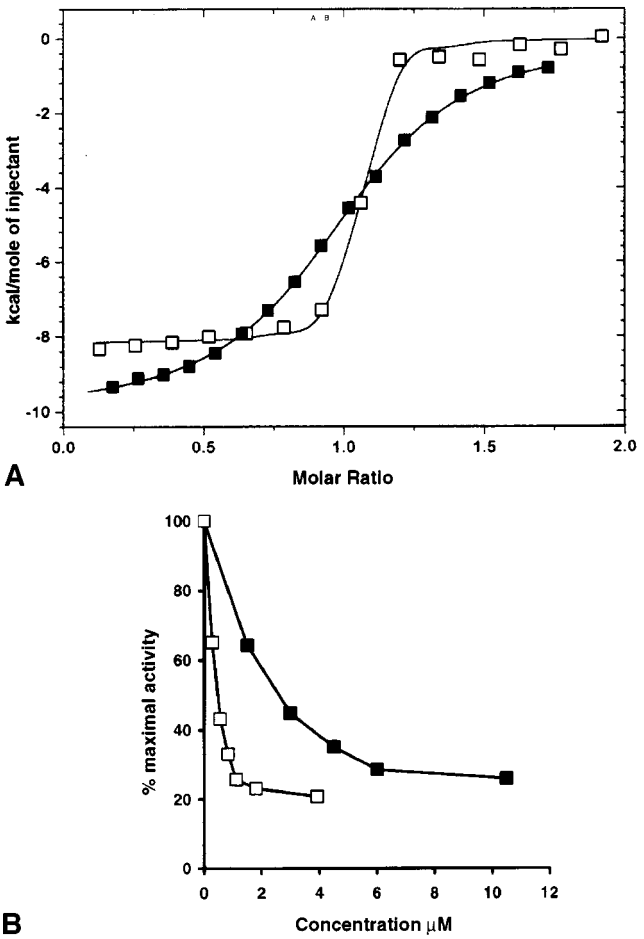


Figure 2. Binding to Hsp90 and ATPase inhibition. (A) Isothermal titration calorimetry of binding of radicicol (open squares) and geldanamycin (filled squares) to full-length dimeric Hsp90. The molar ratio is with respect to Hsp90 monomer. (B) Inhibition of Hsp90 ATPase activity by radicicol (open squares) and geldanamycin (closed squares). A residual activity is present in Hsp90 preparations, which is attributable to contamination with copurifying ATPases but which is resistant to both the Hsp90-specific inhibitors.

Table 1. ITC Binding Data for Geldanamycin and Radicicol

	K_d (nM)	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1})
	Geldanamycin			
Hsp90	1215	−33.8	−41.8	−26.8
N-domain (1−220)	775	−34.8	−44.9	−33.9
	Radicicol			
Hsp90	19	−44.1	−33.7	34.9
N-domain (1−220)	2.7[a]	−48.9	−22.8	87.5

[a] ITC binding curves at this high affinity become too steep for accurate fitting, and the estimation of contingent parameters is consequently less reliable. However the measured binding of radicicol to the isolated N-domain is at least as tight as for intact Hsp90.

Using a sensitive coupled enzyme system[18] to detect the weak inherent ATPase activity of Hsp90,[17] both geldanamycin and radicicol were found to be potent inhibitors (Figure 2B), consistent with their high affinity for the nucleotide-binding site.

Crystal Structures of Hsp90 Antibiotic Complexes. The crystal structures of yeast Hsp90 N-domain complexed with radicicol and with geldanamycin were determined by molecular replacement with the free N-domain structure,[19] and both were refined at 2.5 Å resolution. For both complexes, clear positive electron density features are present in difference Fourier maps which can be readily interpreted in terms of the known chemical structures of the antibiotics (Figure 3A,B). In both cases the antibiotics bind within the deep pocket formed between the α-helices from 85−97, 28−51, and 123−130, previously identified as the binding site for geldanamycin in human Hsp90[20] and ATP/ADP in yeast Hsp90[15] (Figure 3C). Consistent with the high amino acid sequence homology between human and yeast Hsp90s, the structure of the yeast Hsp90−geldanamycin complex is very similar to that described for human Hsp90−geldanamycin.[20] However, as geldanamycin was interpreted as a peptide mimetic in that study, a reassessment of its interactions in terms of nucleotide mimicry and a comparison with the interactions made by radicicol are essential for understanding of its structure/function relationships.

Geldanamycin is orientated with the macrocyclic ansa ring and pendant carbamate group directed toward the bottom of the binding pocket and the benzoquinone ring directed toward the top of the pocket as it opens to the surface of the domain. In contrast to the extended structure adopted by unliganded geldanamycin,[21] the bound antibiotic is almost folded over, so that the planes of the benzoquinone and of the macrocycle are close to parallel (Figure 4A).

Radicicol is orientated in the opposite sense to geldanamycin, so that the aromatic ring is directed toward the bottom of the pocket and the macrocycle containing the conjugated bond system and epoxide are toward the top of the pocket. The orientation of the aromatic ring is confirmed by a strong electron density feature, present at greater than 5σ in difference Fourier maps (Figure 3A), attributable to the exocyclic chlorine. Like geldanamycin, radicicol adopts a folded structure, but less dramatically, with the aromatic ring and the macrocycle approximately perpendicular to each other (Figure 4B). However, unlike geldanamycin,[21] this is also the conformation observed in the crystal structure of the unliganded antibiotic.[22]

ADP Mimicry by Geldanamycin and Radicicol. Mg^{2+}-ATP/ADP binds to the N-domain of Hsp90 in an unusual compacted conformation, which brings the sugar and α-phosphate group into close proximity to the five-membered ring of the adenine and precludes the binding of 8-substituted analogues.[15] The binding site is unusual in that it provides relatively few direct polar contacts with the nucleotide and instead makes a substantial number of solvent-mediated interactions. Some of these interactions, particularly those with the N1 of adenine and the magnesium, involve water molecules with low crystallographic temperature factors, which must be considered as implicit in the structure of the site. A common superimposition of the Hsp90-bound structures of geldanamycin, radicicol, and Mg^{2+}-ADP (Figure 5) reveals a remarkable nucleotide mimicry by these antibiotics, both in shape complementarity and in the interactions they make both with the protein and with the tightly bound water molecules implicit in the structure (Figure 6).

The major specific interaction between the adenine base and the protein involves a direct hydrogen bond from the exocyclic N6 of adenine to the carboxylate side

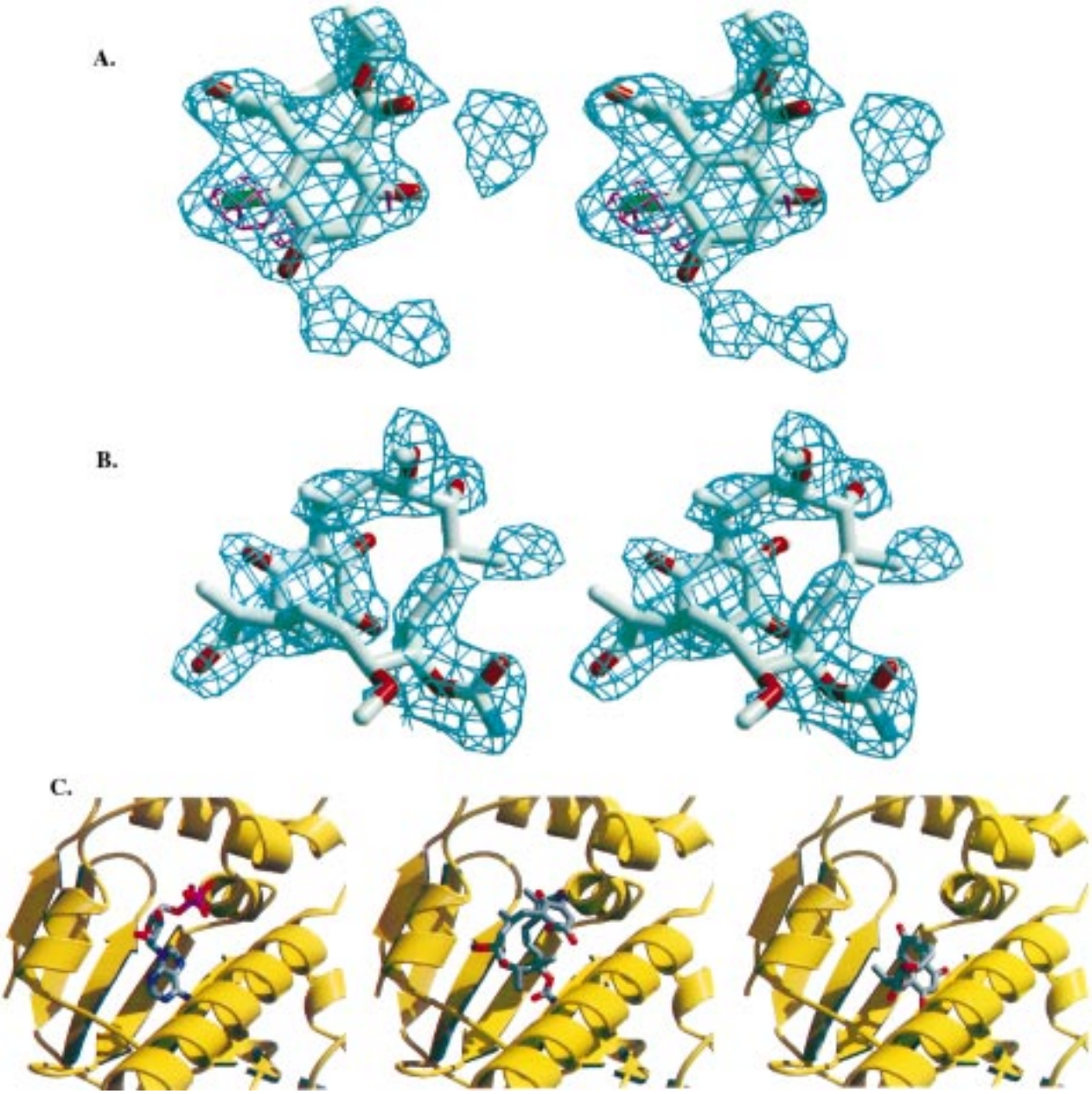


Figure 3. (A) Stereopair showing electron density for radicicol bound to the N-terminal domain of yeast Hsp90. The electron density is from an 'omit' map with phases calculated from protein atoms only. The blue mesh is contoured at 2σ; the magenta mesh is at 5σ and indicates the position of the electron-dense exocyclic chlorine. (B) Stereopair showing electron density for geldanamycin bound to the N-terminal domain of yeast Hsp90. The electron density is from an 'omit' map with phases calculated from protein atoms only. The blue mesh is contoured at 1σ. (C) Colocalization of the binding sites for ADP/ATP (left), geldanamycin (center), and radicicol (right).

chain of Asp 79. Simultaneously, Asp 79 provides one of three protein ligands for a tightly bound water molecule which donates a hydrogen bond to the N1 imino nitrogen of the adenine. These interactions are critical to specific binding of adenine nucleotides, and mutation of Asp 79 to asparagine is sufficient to abolish Hsp90 function in vivo.[17] In the geldanamycin complex, equivalent interactions are provided by the carbamate group pendant on the macrocycle. Radicicol reproduces the direct hydrogen bond to Asp 79 via the 3-hydroxyl of the aromatic ring which also participates with the ester carbonyl of the macrocycle in a bidentate interaction with the bound water. The position of the bound water changes by less than 0.5 Å between the three complexes (Figure 4).

The remainder of the hydrogen-bonding interactions made by adenine are with bound water molecules. Those providing hydrogen bonds to N6 and N7 of adenine are retained in the geldanamycin complex, but the water bridging N3 of the base and O2′ of the sugar is displaced, and the side chain of Asn 92 which provides the protein anchor for that water rotates around 90° and packs against a methoxy group of the macrocycle. Radicicol also retains the water that binds to N6 of adenine but replaces the N7 binding water with the 5-hydroxyl of its aromatic ring. The N3−O2′ bridging water is also displaced by radicicol, but unlike the geldanamycin complex, the conformation of Asn 92 is not disturbed.

The two hydrophobic faces of bound adenine experi-

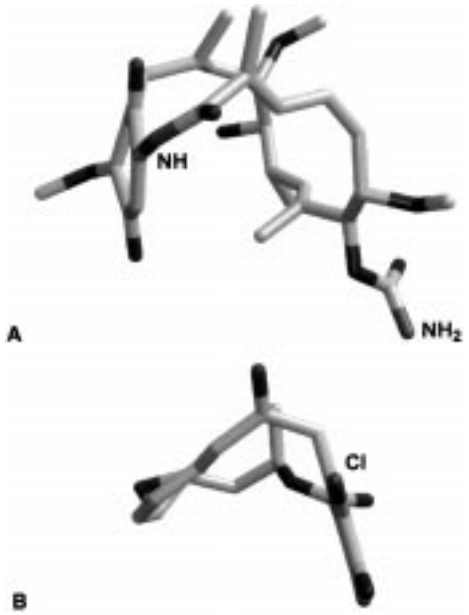


Figure 4. Hsp90-bound conformations of (A) geldanamycinand (B) radicicol.

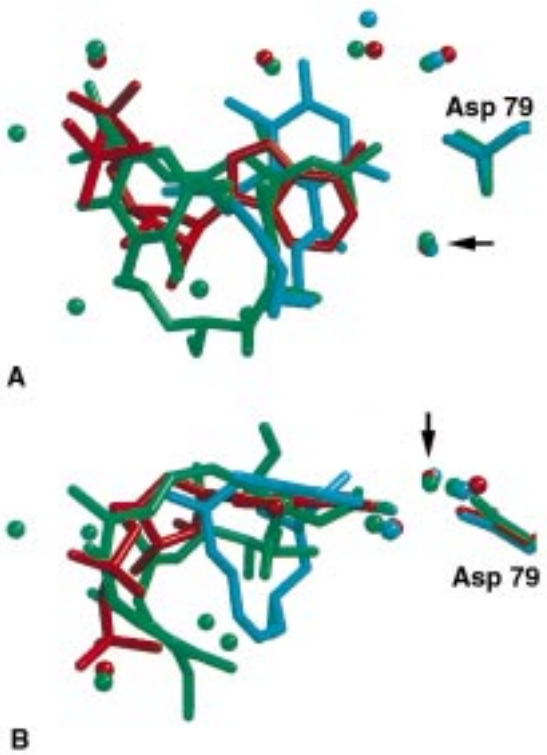


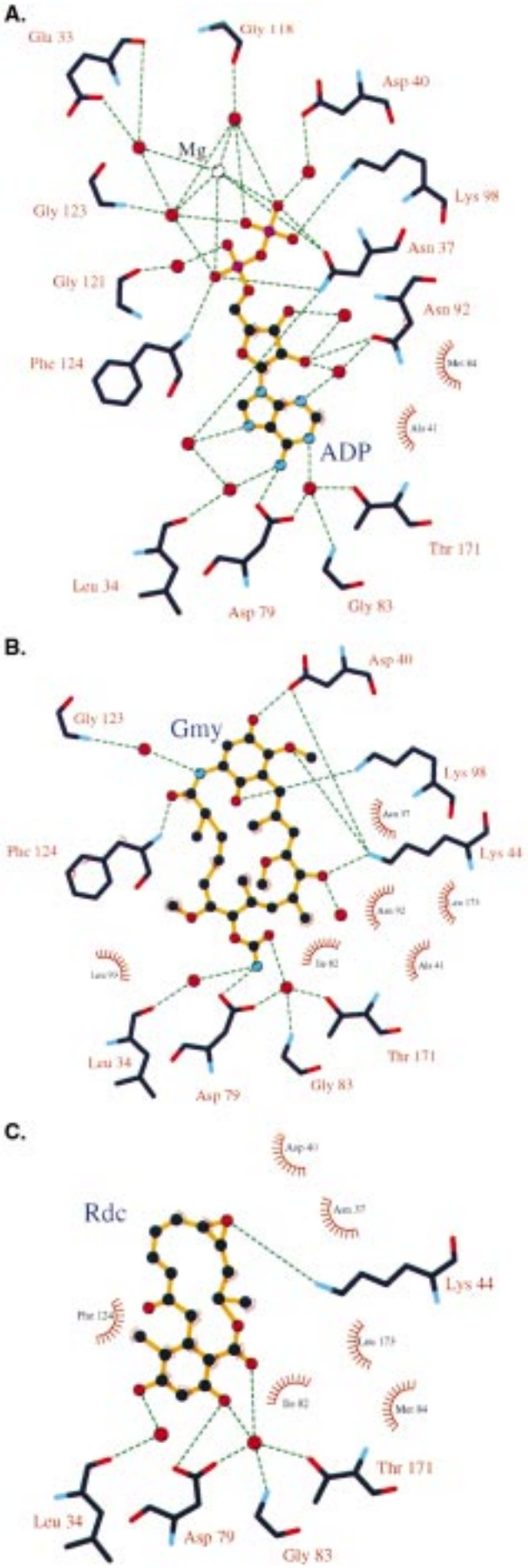
Figure 5. Perpendicular views of the superimposition of bound ADP, geldanamycin, and radicicol, based on alignment of protein Cα positions from three complexes. ADP and associated water molecules are red, geldanamycin is green, and radicicol is blue. The side chain of Asp 79 from each complex is also shown. The tightly bound water (see text) which forms an integral part of the adenine specificity is indicated by an arrow.

ence very different environments, with one being substantially buried against a hydrophobic surface provided by the side chains of Met 84, Leu 93, Phe 124, Thr 171, and Leu 173. The other face is exposed and coated by a network of bound water molecules which connect to the waters and Mg^{2+} bound by the phosphates of the ADP toward the open mouth of the pocket. Binding of geldanamycin displaces much of this extended water network, occupying the volume with part of the macrocycle and the benzoquinone ring, quinone, and methoxy oxygens of which replace two of the water positions associated with the β-phosphate of a bound ADP. This water network is also disrupted by binding of radicicol, which occupies the space with its macrocycle and epoxide group, but without any direct replacement of water sites by functional groups. The groups forming the hydrophobic face of the adenine binding site make extensive hydrophobic interactions with the macrocycle of geldanamycin and with the face of the aromatic ring of radicicol. This ring is approximately coplanar with the adenine of bound ADP and is orientated such that the exocyclic chlorine is in van der Waals contact with the edge of the phenyl ring of Phe 124. The conformations of all the protein residues involved in forming the hydrophobic face of the nucleotide-binding pocket are unaffected by binding geldanamycin or radicicol.

The phosphate groups of bound ADP make only two direct contacts with the protein; the peptide nitrogen of Gly 123 at the N-terminus of the α-helix from 123 to 130 donates a hydrogen bond to the α-phosphate group, while the β-phosphate is involved in an ion pair/hydrogen bond with the side chain of Lys 98. Both of these interactions are reproduced by geldanamycin, with Gly 123 hydrogen bonding to the macrocycle peptide oxygen and Lys 98 to a quinone oxygen. The smaller macrocycle of radicicol in contrast, is directed away from the phosphate-binding region and instead accepts a hydrogen bond from the side chain of Lys 44, to the epoxide oxygen. Lys 44 is also involved in an interaction with the macrocycle hydroxyl of geldanamycin but makes no interactions with a bound nucleotide.

Structure−Activity Relationships. Geldanamycin has been subjected to an extensive synthetic program in an attempt to improve its potency in reducing the activity of the ErbB-2 oncogenic tyrosine kinase in vitro and in vivo.[23,24] At the time of those studies, it was not appreciated that the effects of geldanamycin on cellular ErbB-2 activity was mediated by binding to Hsp90. It now becomes possible to rationalize the effects of some of the modifications made in that program, in terms of the structure of the yeast Hsp90−geldanamycin complex. However, as the potency of these derivatives was primarily assessed in terms of ErbB-2 levels in cells or whole animals, factors such as stability and bioavailability will clearly also have played a part.

As expected from its role in reproducing the functions of the exocyclic amino and imino nitrogens of adenine, all modifications of the carbamate group resulted in substantial loss of potency. The significant loss of activity resulting from the reduction of the 2−3-double bond is less easily understood but may result from the loss of the rigidifying effect of this bond in stabilizing the folded-over conformation of bound geldanamycin. The only improvements that were obtained over unmodified geldanamycin resulted from modifications of the ansa ring hydroxyl and the methoxy of the benzoquinone ring, both of which participate in a hydrogen bond network involving Asp 40 and Lys 44. In the ADP complex, Asp 40 provides a hydrogen bond to one of the ligand waters of the Mg^{2+} cation, and its carboxyl headgroup is directed away from Lys 44, which makes



no contacts to the bound nucleotide and is relatively disordered. In the geldanamycin complex, the side chain of Asp 40 rotates toward Lys 44 and forms a hydrogen bond to its ϵ-amino group, stabilizing its conformation. The ϵ-amino group of Lys 44 is simultaneously hydrogen bonded to the ansa ring hydroxyl of geldanamycin. Oxidation of this hydroxyl to a ketone would provide a better hydrogen bond acceptor for this interaction and would explain the increase in potency observed with ketone derivatives and the substantial loss of potency when amines were introduced at this position. As well as interacting with Lys 44, the carboxyl of Asp 40 is in hydrogen-bonding contact with both the benzoquinone methoxy oxygen and the adjacent quinone oxygen. Replacement of the methoxy oxygen, a poor hydrogen bond acceptor, with an amino group would provide a much better hydrogen-bonding interaction for Asp 40. Consistent with this, introduction of a variety of alkylamino groups at this position gave improved potency.

By contrast, little medicinal chemistry has been reported for radicicol, and the essential pharmacophores have not previously been identified. In light of our structural data, the reasonable presumption that reactivity of the epoxide would be an essential feature of its mechanism of action seems unlikely, at least with regard to its inhibition of Hsp90, as the epoxide oxygen only serves to provide a polar interaction for the ϵ-amino group of Lys 44.

Discussion

The structural and biochemical data presented here confirm the suggestion[4] that radicicol, like geldanamycin, achieves at least some of its antitumor activity by disruption of Hsp90-mediated activation of protein kinases. Both antibiotics bind to the N-terminal nucleotide-binding domain of Hsp90, and both antibiotics inhibit the inherent ATPase activity of Hsp90, which is essential to its in vivo function.[17]

The structural data cannot provide a simple explanation of the greater affinity of radicicol over geldanamycin, in terms of strengths of interactions made by the antibiotics in the nucleotide-binding site. However, the ITC data indicate that the major difference in affinity results from entropic rather than enthalpic factors, with geldanamycin paying a significant entropic penalty on binding. Consistent with this, binding of radicicol appears to cause the release of more ordered water molecules from the protein into bulk solvent than does binding of geldanamycin; radicicol binding involves no significant conformational changes or increased ordering in protein side chains, unlike geldanamycin, and radicicol binds in the same conformation observed in crystals of the free antibiotic, unlike geldanamycin.

Hsp90 chaperones are involved in the folding and activation of a wide range of 'client' proteins, including steroid hormone receptors,[25] tyrosine kinases such as pp60^{v-src},[26] Her-2/neu,[27] p56^{lck}[13], and p185^{erbB2},[28] serine/threonine kinases such Cdk4[29] and c-Raf,[30] and mutant forms of p53.[31] Downregulation of any one of these

Figure 6. Schematic diagrams of the interactions of (A) ADP, (B) geldanamycin, and (C) radicicol in the N-terminal domain of Hsp90, generated using Ligplot.[38] Hydrogen-bonding interactions are shown as broken lines. Atoms in van der Waals contact have 'spokes' directed at each other.

would have positive antiproliferative effects, so that Hsp90, on which all of these depend to greater or lesser extents for their activity, is an attractive target for the development of antitumor drugs. Geldanamycin and other ansamycin antibiotics have shown potent antitumor activity in cell culture and animal models,[32] and correlation between the downregulation of mutant p53, p185^{erbB2}, and Raf-1 and the antiproliferative activity of such compounds[33] suggests that they act via Hsp90. Clinical application of geldanamycin, however, is limited by a substantial hepatotoxicity.[34]

Conclusion

The involvement of Hsp90 in the activation of proteins involved in cell-cycle regulation, signal transduction, and steroid hormone response makes it an attractive target for antitumor drug development. The recent demonstration of Hsp90's direct role in activation and regulation of epithelial nitric oxide synthase[14] may also make it a target for development of drugs modulating vascular function. So far, medicinal chemistry has been restricted to geldanamycin and related ansamycin antibiotics. The structural data presented here should provide a rational basis for further developments of ansamycins, which may allow the elimination of features responsible for toxicity and instability, while retaining the essential nucleotide-mimetic pharmacophores. The demonstration that radicicol also inhibits Hsp90 function by binding to the nucleotide-binding site of the chaperone, but with some 50-fold greater affinity, together with a detailed structural description of the essential interactions should inagurate a new stage in the development of Hsp90 inhibitors.

Experimental Section

Isothermal Titration Calorimetry (ITC) of Nucelotide Binding. The titration experiments were performed using the MSC system (MicroCal Inc., MA). In each experiment, 16 aliquots of 15 μL of radicicol (310 μM in 1% DMSO) or geldanamycin (300 μM in 1% DMSO) were injected into 1.3 mL of protein (31 μM in 20 mM Tris-HCl, pH 7.5, 1 mM EDTA) at 25 °C, and the resulting data were fit after subtracting the heats of dilution as described previously.[15] Heats of dilution were determined in separate experiments from addition of radicicol or geldanamycin into buffer and buffer into protein. No evidence for binding of DMSO in the nucleotide binding site was observed. Titration data were fit using a nonlinear least-squares curve-fitting algorithm with three floating variables: stoichiometry, binding constant ($K_b = 1/K_d$), and change of enthalpy of interaction ($\Delta H°$). Dissociation constants estimated for radicicol bind and for geldanamycin binding to intact yeast Hsp90 are 19 nM and 1.22 μM, respectively, and for binding to Hsp90 N-terminal domain are 2.7 nM and 0.78 μM, respectively (see Table 1). No meaningful heats were observed with either compound binding to the C-terminal fragment.

ATPase Assay. The ATPase assay was based on a regenerating coupled enzyme assay,[18] in which the phosphorylation of ADP by pyruvate kinase at the expense of phosphoenolpyruvate is coupled to the reduction of the resulting pyruvate by lactate dehydrogenase at the expense of NADH. Oxidation of NADH to NAD^+ produces a loss of optical density at the NADH absorbance maximum of 340 nm, in direct stoichiometry to the amount of ADP phosphorylated. Each 1-mL assay contained 100 mM Tris-HCl (pH 7.4), 20 mM KCl, 6 mM $MgCl_2$, 0.8 mM ATP (Sigma), 0.1 mM NADH (Boehringer Mannheim), 2 mM phosphoenolpyruvate (Boehringer Mannheim), 0.2 mg of pyruvate kinase (Boehringer Mannheim), 0.05 mg of L-lactate dehydrogenase (Boeringer Mannheim), and between 2 and 3.5 nmol of Hsp90. Sufficient NADH was added to give an initial absorbance of 0.3 at 340 nm prior to addition of Hsp90s or fragments, and activity was detected as a decrease in absorbance. Inhibition of ATPase activity by radicicol or geldanamycin was achieved by the addition of 1−10 μL of antibiotic dissolved in 100% DMSO to a final concentration of 1.5, 9, 15, and 30 μM. In control experiments, 1% DMSO present alone did not affect the measured ATPase activities, and stoichiometric rephosphorylation of ADP directly added to the assay system was unaffected by 1% DMSO or by either of the antibiotics at all concentrations tested. Geldanamycin was kindly provided by the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD. Radicicol was a kind gift of Dr. Phillip Hedge, Zeneca Pharmaceuticals PLC. All measurements were made on a Shimadzu UV-240 spectrophotometer.

Crystallization, X-ray Diffraction, Structure Determination, and Refinement. The expression, purification, and crystallization of the N-terminal domain of yeast Hsp90 has been described previously.[35] For cocrystallization with radicicol or geldanamycin, the antibiotic was dissolved in 100% DMSO and diluted into protein stock solution. Procedures for X-ray diffraction data collection and processing, manual interpretation of electron density maps, and structure refinement for all crystals are as described in ref 19. The structure of the radicicol and geldanamycin complexes were determined by molecular replacement with the coordinates of the native tetragonal crystal form of the yeast HSP90 N-domain (Protein Data Bank code 1AH6) as previously described for nucleotide complexes.[15] Refinement of the geldanamycin complex converged after the addition of 153 solvent molecules, with an *R*-factor for all reflections in the resolution range 30−2.5 Å of 0.206. Refinement of the radicicol complex converged after the addition of 94 solvent molecules, with an *R*-factor for all reflections in the resolution range 30−2.5 Å of 0.193. Figures 3−5 were produced using Robert Esnouf's adaptation of Molscript[36] and Raster3D.[37]

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References

(1) Kwon, H. J.; Yoshida, M.; Abe, K.; Horinouchi, S.; Beppu, T. Radicicol, an Agent Inducing the Reversal of Transformed Phenotypes of Src-Transformed Fibroblasts. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 538−539.

(2) Shimada, Y.; Ogawa, T.; Sato, A.; Kaneko, I.; Tsujita, Y. Induction of Differentiation of Hl-60 Cells By the Antifungal Antibiotic, Radicicol. *J. Antibiot.* **1995**, *48*, 824−830.

(3) Zhao, J. F.; Nakano, H.; Sharma, S. Suppression of Ras and Mos Transformation By Radicicol. *Oncogene* **1995**, *11*, 161−173.

(4) Soga, S.; Kozawa, T.; Narumi, H.; Akinaga, S.; Irie, K.; Matsumoto, K.; Sharma, S. V.; Nakano, H.; Mizukami, T.; Hara, M. Radicicol leads to selective depletion of Raf kinase and disrupts K-Ras-activated aberrant signaling pathway. *J. Biol. Chem.* **1998**, *273*, 822−828.

(5) Kwon, H. J.; Yoshida, M.; Muroya, K.; Hattori, S.; Nishida, E.; Fukui, Y.; Beppu, T.; Horinouchi, S. Morphology of Ras-Transformed Cells Becomes Apparently Normal Again With Tyrosine Kinase Inhibitors Without a Decrease in the Ras-Gtp Complex. *J. Biochem.* **1995**, *118*, 221−228.

(6) Schulte, T. W.; Blagosklonny, M. V.; Romanova, L.; Mushinski, J. F.; Monia, B. P.; Johnston, J. F.; Nguyen, P.; Trepel, J.; Neckers, L. M. Destabilization of Raf-1 By Geldanamycin Leads to Disruption of the Raf-1-Mek-Mitogen-Activated Protein-Kinase Signaling Pathway. *Mol. Cell. Biol.* **1996**, *16*, 5839−5845.

(7) Stancato, L. F.; Silverstein, A. M.; OwensGrillo, J. K.; Chow, Y. H.; Jove, R.; Pratt, W. B. The hsp90-binding antibiotic geldanamycin decreases Raf levels and epidermal growth factor signaling without disrupting formation of signaling complexes or reducing the specific enzymatic activity of raf kinase. *J. Biol. Chem.* **1997**, *272*, 4013−4020.

(8) Schulte, T. W.; An, W. G.; Neckers, L. M. Geldanamycin-induced destabilization of Raf-1 involves the proteasome. *Biochem. Biophys. Res. Commun.* **1997**, *239*, 655−659.

(9) Fukazawa, H.; Li, P.-M.; Yamamoto, C.; Murakami, Y.; Mizuno, S.; Uehara, Y. Specific inhibition of cytoplasmic protein tyrosine kinases by herbimycin A *in vitro*. *Biochem. Pharmacol.* **1991**, *42*, 1661−1671.
(10) Whitesell, L.; Mimnaugh, E. G.; Decosta, B.; Myers, C. E.; Neckers, L. M. Inhibition of Heat-Shock Protein Hsp90-Pp60(V-Src) Heteroprotein Complex-Formation By Benzoquinone Ansamycins − Essential Role For Stress Proteins in Oncogenic Transformation. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8324−8328.
(11) Smith, D. F.; Whitesell, L.; Nair, S. C.; Chen, S.; Prapapnich, V.; Rimerman, R. A. Progesterone receptor structure and function altered by geldanamycin, an Hsp90 binding agent. *Mol. Cell. Biol.* **1995**, *15*, 6804−6812.
(12) Whitesell, L.; Cook, P. Stable and Specific Binding of Heat-Shock-Protein-90 By Geldanamycin Disrupts Glucocorticoid Receptor Function in Intact-Cells *Mol. Endocrinology* **1996**, *10*, 705−712.
(13) Hartson, S. D.; Barrett, D. J.; Burn, P.; Matts, R. L. Hsp-90-Mediated Folding of the Lymphoid-Cell Kinase P56(Lck1). *Biochemistry* **1996**, *35*, 13451−13459.
(14) Garcia-Cardena, G.; Fan, R.; Shah, V.; Sorrentino, R.; Cirino, G.; Papapetropoulos, A.; Sessa, W. C. Dynamic activation of endothelial nitric oxide synthase by Hsp90. *Nature* **1998**, *392*, 821−824.
(15) Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. Identification and structural characterisation of the ATP/ADP binding site in the Hsp90 molecular chaperone. *Cell* **1997**, *90*, 65−75.
(16) Grenert, J. P.; Sullivan, W. P.; Fadden, P.; Haystead, T. A. J.; Clark, J.; Mimnaugh, E.; Krutzsch, H.; Ochel, H.-J.; Schulte, T. W.; Sausville, E.; Neckers, L. M.; Toft, D. O. The amino-terminal domain of heat shock protein 90 (hsp90) that binds geldanamycin is an ATP/ADP switch domain that regulates hsp90 conformation. *J. Biol. Chem.* **1997**, *272*, 23843−23850.
(17) Panaretou, B.; Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone in vivo. *EMBO J.* **1998**, *17*, 4829−4836.
(18) Ali, J. A.; Jackson, A. P.; Howells, A. J.; Maxwell, A. The 43-kilodalton N-terminal fragment of the DNA gyrase B protein hydrolyses ATP and binds coumarin drugs. *Biochemistry* **1993**, *32*, 2717−2724.
(19) Prodromou, C.; Roe, S. M.; Piper, P. W.; Pearl, L. H. A molecular clamp in the crystal structure of the N-terminal domain of the yeast Hsp90 chaperone. *Nature Struct. Biol.* **1997**, *4*, 477−482.
(20) Stebbins, C. E.; Russo, A. A.; Schneider, C.; Rosen, N.; Hartl, F. U.; Pavletich, N. P. Crystal structure of an Hsp90-geldanamycin complex: Targetting of a protein chaperone by an antitumor agent. *Cell* **1997**, *89*, 239−250.
(21) Schnur, R. C.; Corman, M. L. Tandem [3,3]-Sigmatropic Rearrangements in an Ansamycin − Stereospecific Conversion of an (S)-Allylic Alcohol to an (S)-Allylic Amine Derivative. *J. Org. Chem.* **1994**, *59*, 2581−2584.
(22) Cutler, H. G.; Arrendale, R. F.; Springer, J. P.; Cole, P. D.; Roberts, R. G.; Hanlin, R. T. Monorden from a novel source, *Neocosmospora tenuicristata*: stereochemistry and plant growth regulatory properties. *Agric. Biol. Chem.* **1987**, *51*, 3331−3338.
(23) Schnur, R. C.; Corman, M. L.; Gallaschun, R. J.; Cooper, B. A.; Dee, M. F.; Doty, J. L.; Muzzi, M. L.; Diorio, C. I.; Barbacci, E. G.; Miller, P. E.; Pollack, V. A.; Savage, D. M.; Sloan, D. E.; Pustilnik, L. R.; Moyer, J. D.; Moyer, M. P. Erbb-2 Oncogene Inhibition By Geldanamycin Derivatives − Synthesis, Mechanism of Action, and Structure−Activity-Relationships. *J. Med. Chem.* **1995**, *38*, 3813−3820.
(24) Schnur, R. C.; Corman, M. L.; Gallaschun, R. J.; Cooper, B. A.; Dee, M. F.; Doty, J. L.; Muzzi, M. L.; Moyer, J. D.; Diorio, C. I.; Barbacci, E. G.; Miller, P. E.; Obrien, A. T.; Morin, M. J.; Foster, B. A.; Pollack, V. A.; Savage, D. M.; Sloan, D. E.; Pustilnik, L. R.; Moyer, M. P. Inhibition of the Oncogene Product P185(Erbb-2) in-Vitro and in-Vivo By Geldanamycin and Dihydrogeldanamycin Derivatives. *J. Med. Chem.* **1995**, *38*, 3806−3812.
(25) Joab, I.; Radanyi, C.; Renoir, M.; Buchou, T.; Catelli, M.-G.; Binart, N.; Mester, J. Common nonhormone binding component in nontransformed chick oviduct receptors of four natural steroids. *Nature* **1984**, *308*, 850−853.
(26) Opperman, H.; Levinson, W.; Bishop, J. M. A cellular protein that associates with the transforming protein of Rous Sarcoma Virus is also a heat-shock protein. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 1067−1071.
(27) Hartmann, F.; Horak, E. M.; Cho, C.; Lupu, R.; Bolen, J. B.; StetlerStevenson, M. A.; Pfreundschuh, M.; Waldmann, T. A.; Horak, I. D. Effects of the tyrosine-kinase inhibitor geldanamycin on ligand-induced Her-2/neu activation, receptor expression and proliferation of Her-2-positive malignant cell lines. *Int. J. Cancer* **1997**, *70*, 221−229.
(28) Chavany, C.; Mimnaugh, E.; Miller, P.; Bitton, R.; Nguyen, P.; Trepel, J.; Whitesell, L.; Schnur, R.; Moyer, J. D.; Neckers, L. P185(Erbb2) Binds to Grp94 in-Vivo − Dissociation of the P185(Erbb2)/Grp94 Heterocomplex By Benzoauinone Ansamycins Precedes Depletion of P185(Erbb2). *J. Biol. Chem.* **1996**, *271*, 4974−4977.
(29) Dai, K.; Kobayashi, R.; Beach, D. Physical interaction of mammalian CDC37 with CDK4. *J. Biol. Chem.* **1996**, *271*, 22030−22034.
(30) Stancato, L. F.; Chow, Y.-H.; Hutchinson, K. A.; Perdew, G. H.; Jove, R.; Pratt, W. B. Raf exists in a native heterocomplex with Hsp90 and p50 that can be reconstituted in a cell-free system. *J. Biol. Chem.* **1993**, *268*, 21711−21716.
(31) Sepehrnia, B.; Paz, I. B.; Dasgupta, G.; Momand, J. Heat-shock protein 84 forms a complex with mutant p53 protein predominantly within a cytoplasmic compartment of the cell. *J. Biol. Chem.* **1996**, *271*, 15084−15090.
(32) Whitesell, L.; Shifrin, S. D.; Schwab, G.; Neckers, L. M. Benzoquinonoid Ansamycins Possess Selective Tumoricidal Activity Unrelated to Src Kinase Inhibition. *Cancer Res.* **1992**, *52*, 1721−1728.
(33) An, W. G.; Schnur, R. C.; Neckers, L.; Blagosklonny, M. V. Depletion of p185(erbB2), Raf-1 and mutant p53 proteins by geldanamycin derivatives correlates with antiproliferative activity. *Cancer Chemother. Pharmacol.* **1997**, *40*, 60−64.
(34) Supko, J. G.; Hickman, R. L.; Grever, M. R.; Malspeis, L. Preclinical Pharmacological Evaluation of Geldanamycin As an Antitumor Agent. *Cancer Chemother. Pharmacol.* **1995**, *36*, 305−315.
(35) Prodromou, C.; Piper, P. W.; Pearl, L. H. Expression and crystallisation of the yeast Hsp82 chaperone, and preliminary X-ray diffraction studies of the amino-terminal domain. *PROTEINS: Struct. Funct. Genet.* **1996**, *25*, 517−522.
(36) Kraulis, P. J. MOLSCRIPT − A program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallogr.* **1991**, *24*, 946−950.
(37) Merrit, E. A.; Murphy, M. E. P. Raster3D version 2.0 − a program for photorealistic molecular graphics. *Acta Crystallogr.* **1994**, *50*, 869−873.
(38) Wallace, A. C.; Laskowski, R. A.; Thornton, J. M. Ligplot − A program to generate schematic diagrams of protein interactions. *Protein Eng.* **1995**, *8*, 127−134.

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