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Letter to the Editor: Backbone resonance assignments of the 25kD N-terminal ATPase domain from the Hsp90 chaperone

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Biological context

The eukaryotic Hsp90 chaperones are essential and ubiquitous proteins with key roles in the folding, assembly and activation of a range of specifically-targeted client proteins involved in signal transduction, cell-cycle control or transcription regulation (Caplan, 1999). Hsp90s contain two highly conserved domains connected by a variable charged linker sequence. Electron microscopy images show that Hsp90 forms a dimer, with a persistent link via the C-terminal domains, and that upon addition of ATP the N-terminal domains also transiently dimerize. Studies of C-terminal truncation mutants have shown that dimerization of the N-termini is necessary and sufficient for recruitment of the p23/Sba1 co-chaperone to the complex (Prodromou et al., 2000). Consequently, Hsp90 can be though of as an ATP driven 'molecular clamp', with the closing of the clamp enabling it to hold its co-chaperones. A structure of the N-terminal 25kD domain of yeast Hsp90 has been determined by X-ray crystallography (Prodromou et al., 1997). The antitumour agents geldanamycin and radicicol are targeted to the ATP binding site on this domain (Pearl and Prodromou, 2000). The inhibition of the Hsp90 ATPase activity disrupts the activation of its client proteins, many of which are known antitumour targets. Consequently, Hsp90 is an extremely attractive target for the development of wide-spectrum antitumour drugs (Piper, 2001). This first assignment of an ATPase domain from Hsp90 provides a basis for future mechanistic studies, investigations of protein-protein association and screening for potential inhibitor molecules.

Methods and experiments

^{15}N-labelled and ^{2}H,^{13}C,^{15}N-labelled protein were expressed in *E. Coli* BL21(DE3)pLysS cells that had been transformed using a plasmid encoding for residues 1-207 of the *S. Cerevisiae* Hsp90 sequence (SwissProt accession: P02829) plus a N-terminal hexahistidine tag. A standard two-step purification consisting of immobilized nickel affinity and then size exclusion chromatography was used. The final concentration of the protein used in NMR studies was 0.5 mM in 20 mM Tris buffer in 90%H_2O/10%D_2O at pH 8.0.

Assignments of ^{1}H^{15}N, ^{13}CO, ^{13}Cα and ^{13}Cβ resonances were determined from the following experiments: HNCO, HN(CA)CO, HNCA, HN(CO)CA, HNCACB and HN(CO)CACB (Yamazaki et al., 1994) recorded on a Varian UNITYplus 500. ^{1}H-^{15}N HSQC and ^{15}N-edited 3D NOESY-HSQC spectra (200 ms mixing time) were recorded from ^{2}H,^{13}C,^{15}N-protein on a Varian INOVA 800. NOESY-HSQC and TOCSY-HSQC spectra were recorded for the ^{15}N sample on a UNITYplus 600. Spectra were processed using NMRPipe and NMRDraw version 2.1 (Delaglio et al., 1995) and analysed using ANSIG version 3.3 (Kraulis et al., 1994). ^{1}H chemical shifts were directly, and ^{15}N and ^{13}C indirectly, referenced to DSS in the standard manner. All spectra were recorded at 298 K. Sequence

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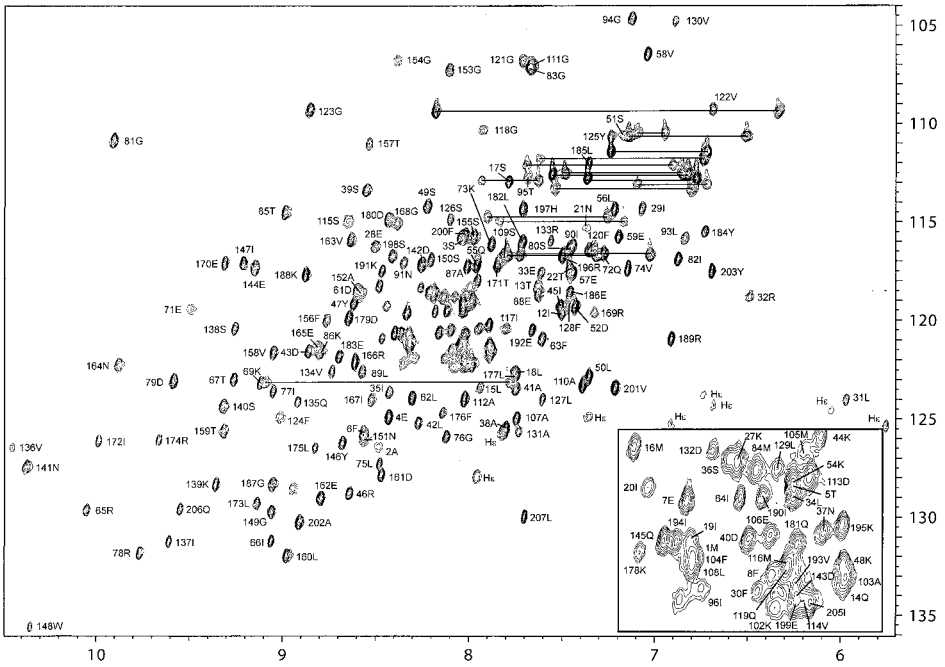

Figure 1. Assigned ^{1}H-^{15}N HSQC spectrum of the N-terminal ATPase domain of yeast HSP90 obtained at 800 MHz and 298 K.

specific resonance assignments were found by correlating intra- and inter-residue resonances of ^{13}CO, $^{13}C\alpha$ and $^{13}C\beta$ shifts, which were then assigned using the probabilistic method of Grzesiek and Bax (1993). The backbone assignments were confirmed through the observation of the inter-residue NOEs expected from the sequential connectivity and the known X-ray structure.

Extent of assignments and data deposition

The recombinant protein consists of 207 residues of Hsp90 (including 5 prolines) plus the hexahistidine tag. We observe only 200 backbone amide peaks in the ^{1}H-^{15}N HSQC and have been able to assign 192 of these. From the Hsp90 sequence proper, unique assignments have been obtained for the HN, N, Cα and Cβ resonances (and partially for Hα) of all non-proline residues, except for three short segments; residues 9–11, 23–26 and 97–101. Two of these segments (9–11, 97–101) form neighbouring solvent exposed strands in the X-ray structure, and the third is a surface loop. Eight resonances remain unassigned in the HSQC as they have very weak and absent signals in the triple resonance and NOESY spectra. The assignments have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under accession code 5355.

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