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Letter to the Editor: Complete backbone resonance assignments of p47: The 41kDa adaptor protein of the AAA ATPase p97

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Biological context

p47 was first identified in complex with the ATPase, p97 (Kondo et al., 1997), which belongs to the family of *A*TPases *A*ssociated with different cellular *A*ctivities (AAA) (Patel and Latterich, 1998). p47 is required for the p97-regulated membrane reassembly of the endoplasmic reticulum (ER), the nuclear envelope and the Golgi apparatus (Kondo et al., 1997). It is thought to assist p97 in the disassociation of post-fusion Golgi membrane surface receptor complexes (specifically syntaxin 5) using the energy from p97 ATP hydrolysis or binding and prepare them for further rounds of membrane fusion (Rabouille et al., 1998). p47 can exist as a trimer and contains three independent domains (Kondo et al., 1997). The N-terminal UBA domain spans residues 1-45, and has been reported to interact with mono-ubiquitin (Meyer et al., 2002). The central domain for which no structure is reported encompasses residues 179-247 and was originally named FAF, after its believed discovery within the Fas-associated factor 1 (FAF1) sequence. However, this domain is not present in FAF1 and this annotation appears to be erroneous. The domain does exist in all p47 sequences, its homologue shp1 from *Saccharomyces cerevisiae* (Zhang et al., 1995) and the eyes closed gene (eyc) from *Drosophila melanogaster* (Sang and Ready, 2002). This represents the best characterised family of proteins containing this domain and we have therefore renamed it the SEP domain, after **s**hp1, **e**yc and **p**47 (new SMART accession number SM00553 (Schultz et al., 2000)). The three dimensional structure of the C-terminal UBX domain is known and adopts a ubiquitin-like fold (Yuan et al., 2001). In this communication, we present the complete backbone assignments for the three domains within p47 (UBA, SEP and UBX) and intervening linker regions.

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Methods

p47 was dissected into two constructs comprising amino acids 1–174 and 171–370. p47(1–174) and p47(171–370) constructs were cloned and expressed as recombinant proteins within *E. Coli*. The expressed fragments contain a N-terminal hexahistidine tag (MRGSHHHHHHGS) for purification and were readily concentrated to mM concentrations. ^{15}N, ^{13}C double-labelled samples of p47(1–174) and p47(171–370) were produced in minimal media, containing 0.07% $^{15}NH_4Cl$ and 0.2% ^{13}C-glucose, supplemented with 50 μg ml^{-1} ampicillin. Protein expression was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside. Clarified cell lysate was purified essentially to homogeneity in a single step on Ni^{2+}-chelating HiTrap Column (Pharmacia). Purified proteins were dialysed into 20mM sodium acetate buffer at pH 5.3 and concentrated to approximately 0.3–0.5 mM for NMR.

The majority of NMR spectra were recorded at 298 K on a 500 MHz four-channel Bruker DRX500 spectrometer equipped with a z-shielded gradient triple resonance cryoprobe. Sequence-specific backbone ^{1}HN, ^{15}N, ^{13}Cα and ^{13}Cβ were determined using

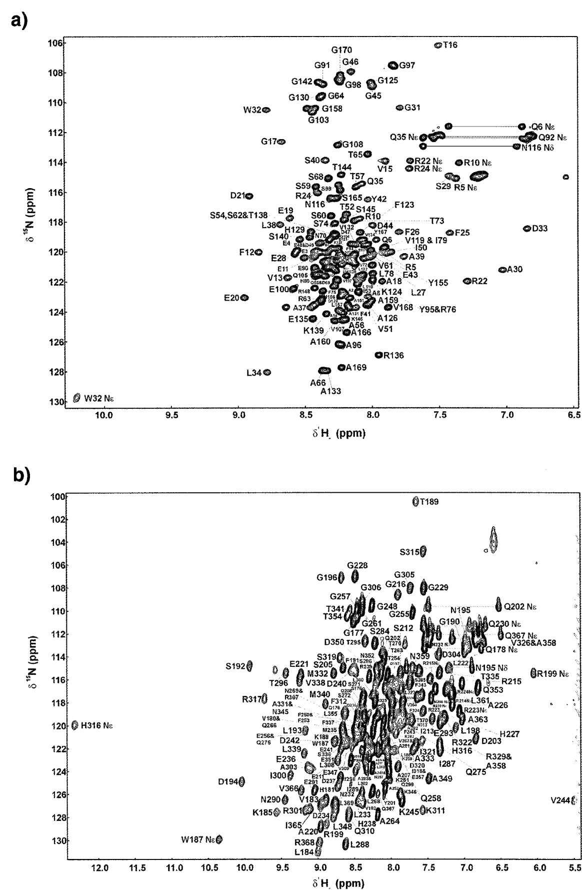

Figure 1. Assigned ^{1}H-^{15}N HSQC NMR spectra of (a) p47(1–174) construct containing the UBA domain and (b) p47(171–370) containing SEP and UBX domains. Sequential assignments of the amides are indicated.

standard triple resonance methods (for review see Bax, 1994). H_α and H_β assignments were obtained using HBHA(CBCACO)NH (Bax, 1994). All triple resonance experiments employed constant-time evolution in the ^{15}N dimension, whereas in the CBCA(CO)NH and HBHA(CBCACO)NH experiments both indirect dimensions were recorded in constant-time mode. The side chain assignments were achieved using HCCH-total correlation (TOCSY) spectroscopy (Bax et al., 1990).

Extent of assignments

The ^{1}H-^{15}N HSQC spectra of the ^{15}N,^{13}C-labelled p47(1–174) and p47(171–370) are shown in Figure 1 with indicated assignments. Using the standard triple-resonance assignment methodology, backbone assignments could be made for 100% of the residues from the three structured domains of p47. The side chain assignments were assessed to be essentially complete with the exception of overlapping resonances in regions containing the aromatic, asparagine and glutamine residues. Assignments could also be made for the majority of the two inter-domain linker regions 46–178 and 248–281. Chemical shift and line-width data (data not shown) also suggest that these regions are highly flexible and are likely to be unstructured in solution. Chemical shift data were used to identify secondary structure elements and clearly show the presence of three domains. The UBA domain possesses three helices that is characteristic of the UBA domain family. The C-terminal UBX domain exhibits the mixed α/β topology of the ubiquitin fold. The central SEP domain, for which no sequence homology with proteins of known structure can be identified, adopts a novel $\beta\beta\beta\alpha\alpha\beta$ secondary structure arrangement.

A table of the assignments (including ^{1}HN, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$ and ^{13}C′) is available as supplementary material and has been deposited in the BioMagResBank in Madison, WI, U.S.A. (accession codes 5874 and 5876).

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