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Letter to the Editor: Assignment of the ^{1}H, ^{15}N, ^{13}C resonances of the N-terminal domain of the human TFIIH P62 subunit

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Biological context

TFIIH is a multiprotein complex involved in transcription initiation of class II genes by RNA polymerase II. It is also required in Nucleotide Excision Repair of damaged DNA (NER) (Frit et al., 1999) and thus appears to be a key component that links two major cellular processes. This 500 kDa complex is composed of nine subunits, two of which are DNA helicases (XPD/Rad3 and XPB/Ssl2). Recently, the electron microscopy structure of the whole complex TFIIH from human (Schultz et al., 2000) and the electron crystal structure of the five-subunit core TFIIH complex from yeast (Chang and Kornberg, 2000) were reported. The structures, in addition to biochemical data, allow to localize most of the TFIIH subunits within a ring-shaped envelope. However, very few high resolution data are available on individual subunits. The human cyclinH structure was solved by X-ray crystallography (Andersen et al., 1996) and the solution structures of both the cysteine-rich domain of p44 subunit (Fribourg et al., 2000) and the RING finger domain of MAT1 subunit (Gervais et al., 2000) were recently obtained by NMR.

The p62 subunit (Tfb1 in yeast) belongs to the core TFIIH, and is involved in both basal transcription and in DNA repair (Wang et al., 1995). The p62 subunit was found to interact with the p44 (Ssl1) subunit and with the XPB (Ssl2) and XPD (Rad3) helicases. Moreover, several interactions between p62 and transcriptional activators such as p53, VP16, the EBNA2 transactivator and the transcriptional activators E2F-1 and AF-1 were reported. Finally, recent studies have shown that the protein could be a target of phosphorylation by the cdc2/cyclinB kinase and may be involved in repression of transcription at mitosis (Long et al., 1998).

No structural data are yet available on the p62 subunit and no structural homology could be deduced from its primary sequence analysis. We report here the ^{1}H, ^{13}C and ^{15}N chemical shift assignments of the N-terminal domain (residues 1–108) of human p62, which encompasses the potential phosphorylation site.

Methods and experiments

Proteolysis experiments carried out on the different domains of the human p62 protein revealed the presence of a resistant and stable domain in its N-terminal region (1–108). The construct of this domain was expressed as a GST-fusion protein. $^{13}C/^{15}N$-labeled samples were produced by growing *E. coli* in enriched medium CELTONE® -CN (Martek). The purification procedure of this domain requires three steps and allows production of 4 mg of protein per liter of culture (details on the expression and purification of the protein will be published elsewhere). $^{13}C/^{15}N$-labeled sample contained 1.5 mM p62 dissolved in 20 mM deuterated Tris, pH 7.4 in H_2O/D_2O (9:1).

All heteronuclear experiments were recorded at 293 K on a Bruker DRX600 spectrometer equipped with a z-gradient triple resonance probe. ^{1}H spectra were acquired at 293 K on a Bruker DRX-800 spectrometer. Data were processed with Felix 2.10 (Molecular Simulations Inc.) and analysed with XEASY (Bartels et al., 1995).

Backbone and side-chain resonances were obtained from the combination of the ^{15}N-TOCSY-HSQC and ^{15}N-NOESY-HSQC spectra with HNCA,

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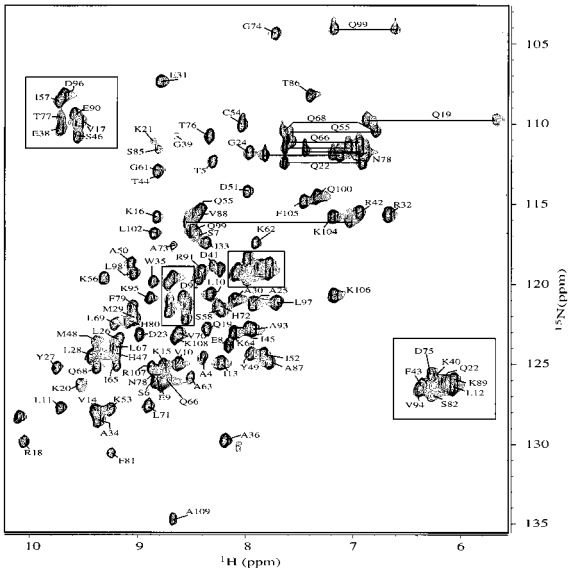

Figure 1. 2D [^{1}H,^{15}N]-HSQC NMR spectrum of the N-terminal domain of human p62 subunit (1–108). NH_2 side chain resonances of Gln and Asn are connected by lines.

HN(CO)CA, CBCA(CO)NH and HNCACB spectra. ^{1}H,^{13}C side-chain assignments were performed with HBHA(CO)NH, H(C)(CO)NH-TOCSY, C(CO)NH-TOCSY and HCCH-TOCSY spectra (for review, see Sattler et al., 1999). The aromatic resonances were assigned using 2D [^{1}H,^{1}H] TOCSY and NOESY recorded on an unlabeled sample.

Extent of assignments and data deposition

Figure 1 shows the 2D [^{1}H,^{15}N] HSQC spectrum of the uniformly ^{13}C/^{15}N-labeled human p62 fragment (1–110). Analysis of triple resonance experiments allowed us to sequentially assign resonances for amino acid residues 4 through 109 (except residues N83-E84). The two residues P59, E60 located in the vicinity of the potential phosphorylation site at S58 could not be assigned. A full list of the ^{1}H, ^{15}N, and ^{13}C chemical shift assignments of the N-terminal domain of human p62 has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4901.

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