



## Letter to the Editor: $^1\text{H}$ , $^{13}\text{C}$ and $^{15}\text{N}$ resonance assignments of the ERK2 binding domain of the MAPK phosphatase MKP-3

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### Biological context

Mitogen-activated protein kinases (MAPKs) play a pivotal role in numerous cellular processes, including neuronal differentiation, mitogenesis, oncogenic transformation and apoptotic cell death (Cobb and Goldsmith, 1995). The biological importance of MAPK regulation is manifested by the tight control of their activity through dual phosphorylation of threonine and tyrosine in the consensus motif TXY within the activation loop. While a considerable amount is known about the activation of MAPKs, the molecular mechanisms of their specific down-regulation are much less understood (Keyse, 1994). Recently, a group of dual-specificity MAPK phosphatases (MKPs) have been shown to exhibit distinct substrate specificity towards MAPKs. All MKPs consist of an N-terminal domain and a C-terminal phosphatase domain (Keyse, 1994; Denu and Dixon, 1995). The divergent non-catalytic N-terminal domains have recently been suggested to be important for substrate specificity towards MAPKs. Arkininstall and co-workers recently demonstrated that inactivation of the MAPK ERK2 by MKP-3 is highly specific and substrate recognition is achieved via direct binding of the N-terminal domain of MKP-3 to ERK2 (Camps et al., 1998). Interestingly, this interaction also results in enhancement of MKP-3 phosphatase activity by ~30-fold. In efforts to understand the detailed molecular mechanism of ERK2 inactivation by MKP-3, we have undertaken NMR structural analysis of MKP-3. Here we report the nearly complete sequence-specific backbone and side-chain  $^1\text{H}$ ,  $^{15}\text{N}$ , and  $^{13}\text{C}$  resonance

assignments of the N-terminal ERK2 binding (EB) domain of MKP-3.

### Methods and experiments

The N-terminal EB domain of MKP-3 (residues 1–154) was subcloned into a pET15b vector and expressed in *E. coli* BL21(DE3) cells. Uniformly  $^{15}\text{N}$ -labeled or  $^{13}\text{C}/^{15}\text{N}$ -labeled protein samples were prepared by growing the bacteria in minimal media containing  $^{15}\text{NH}_4\text{Cl}$  with or without  $^{13}\text{C}_6$ -glucose. Uniformly  $^{13}\text{C}/^{15}\text{N}$ -labeled and fractionally deuterated proteins were prepared in a similar fashion by using 75%  $^2\text{H}_2\text{O}$ . The N-terminal domain of MKP-3 was purified by affinity chromatography on a nickel-IDA column (Invitrogen) followed by the removal of the hexa-histidine tag by thrombin cleavage. The cleaved protein was further purified by ion-exchange chromatography. NMR samples of the protein (~0.5 mM) were prepared in 50 mM imidazole buffer of pH 6.0 containing 100 mM NaCl, 200 mM urea, 0.5 mM EDTA and 5 mM DTT- $d_{10}$  in  $\text{H}_2\text{O}/^2\text{H}_2\text{O}$  (9/1) or  $^2\text{H}_2\text{O}$ . The low concentration of urea, which did not affect the protein structure as supported by the NMR spectra, was used to stabilize the protein for the NMR structural study. All NMR experiments were carried out at 25 °C on Bruker DRX500 and DRX600 spectrometers. The NMR data were processed and analyzed using the NMRPipe and NMRView programs. Deuterium-decoupled triple-resonance experiments HNCA, HN(CO)CA, HN(CA)CB and HN(COCA)CB with sensitivity enhancement (Yamazaki et al., 1994) recorded with a uniformly  $^{13}\text{C}/^{15}\text{N}$ -labeled and fractionally (75%) deuterated sample, were used to obtain the backbone resonance assignments. The backbone assignments

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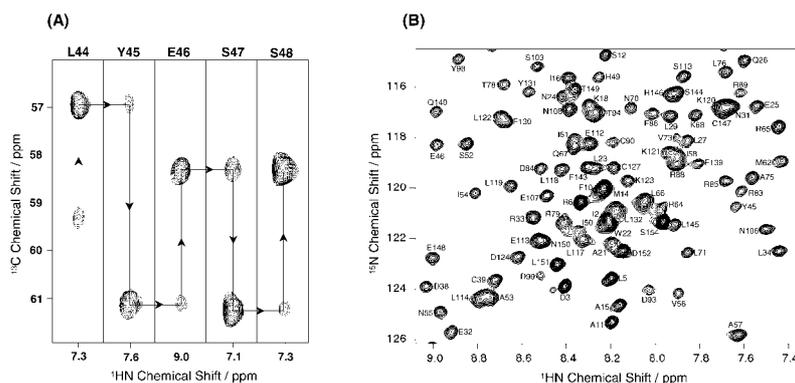


Figure 1. MKP-3 N-terminal EB domain (residues 1–154). (A) Representative strips at distinct  $^{15}\text{N}$  planes of the HNCA experiment showing connectivities for the residues Leu 44 to Ser 48. (B) Central region of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum collected at pH 6.0 and  $25^\circ\text{C}$ . The assignments are annotated by the resonance peaks.

were confirmed through sequential NH-NH and NH- $\text{H}\alpha$  NOEs identified in the  $^{15}\text{N}$ -edited 3D NOESY-HSQC spectrum (100 ms mixing time). The side chain  $^1\text{H}$  and  $^{13}\text{C}$  atoms were assigned using a 3D (H)C(CO)NH-TOCSY (Sattler et al., 1999) recorded on the  $^2\text{H}(75\%)/^{13}\text{C}/^{15}\text{N}$ -labeled sample. Side chain  $^1\text{H}$  resonances were assigned using a 3D HCCH-TOCSY spectrum of 18 ms mixing time (Clare and Gronenborn, 1994) using a fully protonated  $^{13}\text{C}/^{15}\text{N}$ -labeled sample in  $^2\text{H}_2\text{O}$ , and confirmed with a 3D  $^{15}\text{N}$ -dispersed TOCSY-HSQC (80 ms mixing time), in which the intra-residue correlations of nearly all non-proline residues were observed. The side chain  $^1\text{H}$  and  $^{13}\text{C}$  resonances for aromatic residues (8 Tyr, 6 Phe and 1 Trp) were assigned using a combination of experiments, including 2D  $^1\text{H}$  NOESY and TOCSY in addition to  $^{13}\text{C}$  HSQC and 3D HCCH-TOCSY recorded in the aromatic carbon region.

#### Extent of assignments and data deposition

The high quality of spectra from the 3D triple-resonance experiments allowed us to obtain nearly complete backbone assignments of  $^1\text{H}^{\text{N}}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}\alpha$  and  $^{13}\text{C}\beta$  atoms for all residues. Representative strips from distinct  $^{15}\text{N}$  planes of the HNCA experiment are depicted in Figure 1A. Figure 1B displays the central region of the 2D  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum for the EB domain of MKP-3. The side chain  $^1\text{H}$  and  $^{13}\text{C}$  resonance assignments were obtained for about 95% of the residues. A total of 40 slowly exchanging amide protons have been identified with a series of  $^{15}\text{N}$ -HSQC spectra recorded on a uniformly  $^{15}\text{N}$ -labeled sample after the  $\text{H}_2\text{O}$  buffer was changed to  $^2\text{H}_2\text{O}$  buffer. A total of 30  $^3J_{\text{NH}-\text{H}\alpha}$  coupling constants were

measured with a 3D HNHA spectrum (Vuister and Bax, 1993). Deviations of the  $^{13}\text{C}\alpha$  and  $^1\text{H}\alpha$  chemical shifts from random coil values (Wishart et al., 1995), characteristic sequential and medium range NOEs and  $^3J_{\text{NH}-\text{H}\alpha}$  coupling constants indicate that the EB domain of MKP-3 consists mainly of alternating  $\beta$ -strands and  $\alpha$ -helices. A table of the  $^1\text{H}$ ,  $^{15}\text{N}$  and  $^{13}\text{C}$  chemical shift assignments of the MKP-3 EB domain has been deposited in the BioMagResBank database (<http://www.bmrb.wisc.edu>) under accession number 4818.

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