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Letter to the Editor: Sequence-specific resonance assignment of the second Ran-binding domain of human RanBP2

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Biological context

Active import and export through the pores of the nuclear envelope of eukaryotic cells is maintained via the GTPase cycle of the Ras-related protein Ran (Görlich and Kutay, 1999). Human Ran-binding protein 2 (RanBP2) is a 358 kDa nucleoporin situated on fibrils surrounding the nuclear pore on the cytoplasmic side (Wu et al., 1995; Yokoyama et al., 1995). Taking part in the GTPase cycle it is involved especially in nuclear export processes and also is considered to work as a chaperone for opsins. The four Ran-binding domains (RanBD1-RanBD4) are homologous to that of Ran-binding protein 1. They have a length of about 150 amino acid residues each, a mean pairwise sequence identity of 49% and turned out to be both essential and sufficient for RanBP2 acting as a cofactor for the RanGTPase-activating protein RanGAP1 (Beddow et al., 1995; Novoa et al., 1999; Villa Braslavsky et al., 2000). The crystal structure of RanBP2$^{\mathrm{RanBD1}}$ in complex with RanGppNHp has been solved previously (Vetter et al., 1999), however, the solution structure of the uncomplexed Ran-binding domain is still unknown. The resonance assignment of the folded part of RanBP2$^{\mathrm{RanBD2}}$ (RanBP2$^{\mathrm{RanBD2\Delta N}}$) should faciliate interesting binding studies which will, e.g., allow to find out where the C-terminal residues of Ran contact the domain. Our data support the presumption of the fold on the whole being the same, in the complexed case of RanBD1 and for RanBD2 alone. However, there are hints that the solution structure of the non-complexed domain may show an additional 5th β-strand common to other known PH-domains (Figure 1b).

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Methods and experiments

Cloning, expression and isotope labeling of RanBP2$^{\mathrm{RanBD2}}$

The original clone was a gift of T. Nishimoto (Kyushu University, Fukuoka, Japan), where the RanBP2$^{\mathrm{RanBD2}}$ construct included residues 1996–2154 of RanBP2 and was embedded in a pGEX 2T vector (Pharmacia) with a N-terminal GST moiety. This complete domain could easily be expressed and purified (including a thrombin cleavage to separate the GST domain) but featured an increased tendency to aggregate under conditions necessary for NMR measurements. Therefore we cloned a N-terminal truncated construct comprising the RanBP2 residues 2028–2154 in a pGEX 2T vector (RanBP2$^{\mathrm{RanBD2\Delta N}}$). The deleted residues of the sequence probably adopt an unstructured conformation. Expression of the protein was performed in *E. coli* BL21(DE3). The culture was grown up to $OD_{600} = 0.5$, supplemented with 0.2 mM IPTG and incubated at 30 °C overnight. The cells were sedimentated, resuspended in phosphate buffered saline (pH 7.4) and lysed by sonification. The lysate was purified by affinity chromatography over a glutathione sepharose (GSH) column (XK26, Pharmacia Biotech). The protein was precipitated with 3 M ammonium sulfate and dissolved in buffer containing 20 mM Tris/Cl, pH 7.6, 100 mM NaCl, 100 mM KCl, 1 μM β-mercaptoethanol and 1 mM ATP. The fusion protein was cleaved by incubation with 20 U thrombin per mg GST-fusion protein for 17 h at 25 °C. GST was removed by application of the digestion products over a second GSH column. RanBP2$^{\mathrm{RanBD2\Delta N}}$ was concentrated up to 30 g l^{-1} by ultrafiltration.

a)

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GSGEEDEKVL YSQRVKLFRF DAEVSQWKER
GLGNLKILKN EVNGKLRMLM RREQVLKVCA
NHWITTTMNL KPLSGSDRAW MWLASDFSDG
DAKLEQLAAK FKTPELAEEF KQKFEECQRL
LLDIPLQTPK
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b)

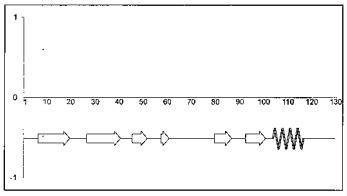

Figure 1. (a) Sequence of the RanBD2ΔN construct analysed by NMR spectroscopy. Shadowed residues origin from the pGEX 2T vector. The sequence GEE....QTP corresponds to residues 2028-2154 in RanBP2. (b) Chemical shift indices of $RanBP2^{RanBD2\Delta N}$ in solution versus the secondary-structure elements as known from the crystalline complex of $RanBP2^{RanBD1}$ with RanGppNHp. Correponding to numbers 2028–2154 in full length RanBP2 with their terminal attachments the residues are numbered from 1 to 130. Residues 10–19, 30–13, 33–39, 46–51, 61–73, 77–85, 88–90 and 94–100 have positive CSI, whereas those of residues 105 to 120 are negative.

Beside residues 2028–2154 from RanBP2 the RanBD2ΔN protein included a GlySer dipeptide at the N-terminus originating from the thrombin cleavage site of the fusion protein and a C-terminal lysine from the pGEX vector (Figure 1a). The complete peptide chain consists of 130 amino acid residues. It maintains the ability to bind specifically with RanGTP but shows a considerable reduction in binding affinity (Zhao et al.; in preparation). For the introduction of ^{15}N mono- and $^{15}N/^{13}C$ double isotope labels *E. coli* were grown in minimal medium containing 1 g l^{-1} ^{15}N NH_4Cl or 1 g l^{-1} ^{15}N NH_4Cl plus 2 g l^{-1} ^{13}C-glucose respectively. Successful incorporation of the isotopes into the RanBD2ΔN construct was verified by ESI mass spectroscopy.

NMR spectroscopy

All NMR samples contained 150 mM Na_2SO_4, 10 mM DTE, 0.5 mM EDTA, 1 mM NaN_3, and 0.1 mM DSS in 10 mM potassium phosphate buffer at pH 6.5. The protein concentrations varied between 0.7 mM and 1.2 mM. Measurements were done in a mixture of 92% ^1H_2O/8% ^2H_2O as solvent or in 99% ^2H_2O.

The spectra were recorded on Bruker DRX800 and DRX600 spectrometers at 298 K. ^1H chemical shifts were referenced directly to internal DSS, ^{15}N and ^{13}C shifts were indirectly referenced to DSS with shift ratios of 1, 0.251449530, 0.101329118 for ^1H, ^{13}C and ^{15}N respective (Markley et al., 1998). Sequence-specific resonance assignments were obtained on the basis of HNCA, HN(CO)CA, CBCA(CO)NH, CBCANH, HBHA(CBCACO)NH, HNCO, ^1H-^{15}N-HSQC, ^1H-^{15}N-TOCSY-HSQC and ^1H-^{15}N-NOESY-HSQC experiments. Chemical shift indices (CSI) were calculated with the program CSI v1.1 (Wishart and Sykes, 1994; courtesy of Sykes et al., http://www.pence.ualberta.ca/ftp). Data processing and analysis were carried out on a Silicon Graphics O2 workstation with the software packages WINNMR and AURELIA from Bruker (Neidig et al., 1995).

Extent of assignments and data deposition

With the combined information from all heteronuclear experiments 98% of the backbone amide protons and ^{15}N nuclei in the dominant conformation of the protein could be assigned, prolines not counted. 99% of the CA, 94% of the C, 98% of the HA and 99% of the CB shifts were obtained. Figure 1 shows a CSI plot versus the corresponding secondary structure elements expected from Vetter et al. (1999).

A list of ^1H, ^{15}N and ^{13}C shifts was deposited in the BioMagResBank under accession number 5159.

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