Journal of Biomolecular NMR **30:** 221–222, 2004.

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the C-terminal BRCT domain from human BRCA1

Olaf J. Gaiser[a,b], Hartmut Oschkinat[a], Udo Heinemann[b] & Linda J. Ball[a,*]
[a]*Forschungsinstitut für Molekulare Pharmakologie and* [b]*Max Delbrück Center for Molecular Medicine, Robert-Rössle-Str. 10, D-13125 Berlin, Germany*

Received 17 March 2004; Accepted 2 June 2004

Key words: BRCA1, BRCT domain, breast cancer, NMR, resonance assignments

Abbreviations: BRCA1 – breast and ovarian cancer susceptibility gene product 1; BRCT – BRCA1 C-terminal domain; IPTG – isopropyl β-D-thiogalactopyranoside; GST – glutathione S-transferase.

Biological context

BRCT domains are found in a large number of proteins which display a broad range of biological activities (Bork et al., 1997; Callebaut and Mornon, 1997). The tumor suppressor protein BRCA1, associated with breast and ovarian cancer, contains two successive BRCT domains in its C-terminal region. These tandem repeats, referred to as BRCT-n and BRCT-c for N- and C-terminal BRCT domains, respectively, mediate essential biological functions including DNA damage signalling, cell cycle regulation, apoptosis, chromatin remodelling and regulation of transcription (Chapman and Verma, 1996; Somasundaram et al., 1997).

The structure of the BRCT repeat $(BRCT)_2$ from human BRCA1 has been solved by X-ray crystallography (Williams et al., 2001). We have recently shown by NMR spectroscopy that the isolated BRCT-c domain is also fully folded in solution at physiological pH in the absence of BRCT-n adopting a structure similar to that of BRCT-c in $(BRCT)_2$ but with small differences at the BRCT-n/c interface and in three flexible loop regions. Despite evidence of dimerization reported for other BRCT domains (Krishnan et al., 2001, Zhang et al., 1998), analytical ultracentrifugation has shown that the human BRCT-c domain exists predominantly as monomer in solution (O.J. Gaiser et al., submitted).

Binding studies to date have suggested direct interactions of BRCT-c with both the universal tumor suppressor protein p53 and double-stranded DNA (Chai et al., 1999; Yamane et al., 2000), but so far no structural information on either of these interactions is available. NMR provides an excellent method for screening the BRCT-c domain against these ligands and others, by measurement of ^{15}N and $^{1}H_N$ chemical shift perturbations (CSPs). In addition, the solution structure obtained from NMR will allow detailed studies of the domains' dynamics and association behaviour under near physiological solution conditions. To this end, we have expressed, isolated and assigned the 110-residue C-terminal BRCT domain of human BRCA1.

Methods and experiments

Expression and purification of BRCT-c

The DNA sequence encoding BRCT-c (residues 1755-1863 of human BRCA1, Swiss-Prot accession code: P38398) was cloned into the BamHI and SalI sites of the pGEX-4T-1 vector (Amersham Biosciences). BRCT-c was co-expressed as a GST-fusion protein in *Escherichia coli* BL21[$_p$REP$_p$4-groESL/CodonPlusRIL] cells with the chaperonins GroEL and GroES encoded on the plasmid pREP4-groESL (Roche) to improve BRCT-c solubility. Cells were grown at 32 °C in M9 minimal medium containing 100 mg/l carbenicillin, 30 mg/l kanamycin and 34 mg/l chloramphenicol and supplemented with 1.3% (w/v) D-glucose. Expression was induced by addition of 0.5 mM IPTG and cells were grown for additional 5–8 h at 25 °C before lysing with a French Press (2 × 1000 bar). GST-BRCT-c was then purified by affinity chromatography on a glutathione Sepharose 4B column. The GST moiety was cleaved with thrombin protease (Roche) and separated by size exclusion chromatography on a Superdex 75 column (Amersham

*To whom correspondence should be addressed. E-mail: linda@fmp-berlin.de

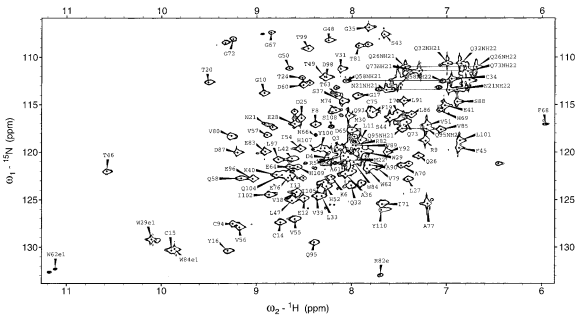

Figure 1. Assigned ^{1}H-^{15}N-HSQC spectrum of the C-terminal BRCT domain from human BRCA1 (residues 1755-1863) recorded at 296 K on a Bruker DMX 750 spectrometer.

Biosciences) to yield the BRCT-c domain with an additional N-terminal glycine. This was then purified to homogeneity on a MonoQ anion-exchange column (Amersham Biosciences). Samples for NMR spectroscopy were de-salted on a FastDesalting column (Amersham Biosciences) and concentrated using Ultrafree concentrators (Millipore). All chromatography steps were performed at 4 °C. Uniformly ^{15}N- and ^{13}C,^{15}N-labeled BRCT-c were grown in *Escherichia coli* BL21 cells in M9 minimal medium containing 0.5 g/l $^{15}NH_4Cl$ and either 1.3% (w/v) $^{12}C_6$-glucose or 0.5% (w/v) $^{13}C_6$-glucose, respectively, as the sole nitrogen and carbon sources and purified as above. Protein molecular masses were determined by mass spectrometry.

NMR spectroscopy

All NMR experiments were acquired at 296 K on BRCT-c dissolved in 10 mM KH_2PO_4, 10 mM K_2HPO_4, 5 mM KCl and 10 mM deuterated DTT, at pH 6.8. A 1.3 mM ^{15}N-labeled sample in 93% H_2O (7% D_2O) was used for 3D HNHA, HNHB and ^{15}N-edited NOESY-HSQC and TOCSY-HSQC experiments. 2D-NOESY, TOCSY and DQF-COSY spectra were acquired in both 93% H_2O/7% D_2O and in 99.98% D_2O. A 0.7 mM ^{13}C,^{15}N-labeled sample was used for 3D CBCA(CO)NNH, CBCANNH, HBHA(CO)NNH and HBHANNH experiments and then transferred into 99.98% D_2O for 3D HCCH-TOCSY and ^{13}C-edited NOESY-HMQC experiments. Spectra were acquired using Bruker DRX600 and DMX750 spectrometers with triple resonance probes equipped with self-shielded triple axis gradient coils. Data were processed using the XWIN-NMR program (version 1.3) of Bruker BioSpin GmbH (Rheinstetten, Germany) and the AZARA program (version 2.1) of W. Boucher (unpublished). Assignment was carried out on Silicon Graphics O2 workstations using the interactive program ANSIG (v.3.3) (Kraulis, 1989).

Extent of assignment and data deposition

With the exception of the N-terminal residues Gly 1 (from the vector) and Ser 2 (position 1755 in BRCA1), which were not detected, all non-proline, backbone $^{13}C_\alpha$, ^{15}N and ^{1}H resonances were assigned, as were 101 of 110 backbone ^{13}CO resonances. The assigned ^{15}N HSQC spectrum of the isolated BRCT-c domain is shown in Figure 1. More than 90% of the sidechain aliphatic and aromatic ^{13}C and ^{1}H resonances were assigned. 1 of 3 Asn, and 5 of 8 Gln sidechain NH_2 groups were also assigned, as were the $^{15}N\varepsilon/^1H\varepsilon$ atoms of the Arg 82 sidechain. The $^{15}N\varepsilon/^1H\varepsilon$ atoms of the remaining Arg 5 and Arg 9 sidechains were not detected due to rapid exchange. The assignments are deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) under accession code BMRB-6114.

Acknowledgements

The authors gratefully acknowledge financial support from the German Ministry for Education and Research (BMBF) through the Leitprojekteverbund Proteinstrukturfabrik and the Fonds der Chemischen Industrie.

References

Bork, P., Hofmann, K., Bucher, P., Neuwald, A.F., Altschul, S.F. and Koonin, E.V. (1997) *FASEB J.*, **11**, 68–76.

Callebaut, I. and Mornon, J.P. (1997) *FEBS Lett.*, **400**, 25–30.

Chai, Y.L., Cui, J., Shao, N., Shyam, E., Reddy, P. and Rao, V.N. (1999) *Oncogene*, **18**, 263–268.

Chapman, M.S. and Verma, I.M. (1996) *Nature*, **382**, 678–679.

Kraulis, P. (1989) *J. Magn. Reson.*, **24**, 627–633.

Krishnan, V.V., Thornton, K.H., Thelen, M.P. and Cosman, M. (2001) *Biochemistry*, **40**, 13158–13166.

Somasundaram, K., Zhang, H., Zeng, Y.X., Houvras, Y., Peng, Y., Wu, G.S., Licht, J.D., Weber, B.L. and El-Deiry, W.S. (1997) *Nature*, **389**, 187–190.

Williams, R.S., Green, R. and Glover, J.N. (2001) *Nat. Struct. Biol.*, **8**, 838–842.

Yamane, K., Katayama, E. and Tsuruo, T. (2000) *Biochem. Biophys. Res. Commun.*, **279**, 678–684.

Zhang, X., Morera, S., Bates, P.A., Whitehead, P.C., Coffer, A.I., Hainbucher, K., Nash, R.A., Sternberg, M.J., Lindahl, T. and Freemont, P.S. (1998) *EMBO J.*, **17**, 6404–6411.