Journal of Biomolecular NMR, **24:** 169–170, 2002.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the human phosphatase PRL-3

Guennadi Kozlov[a,c], Jing Cheng[b,d], Carine Lievre[b,c], Denis Banville[b], Kalle Gehring[a,c] & Irena Ekiel[b,c,d,*]

[a]*Department of Biochemistry, McGill University, 3655 Promenade Sir William Osler, Montreal, Quebec H3G 1Y6, Canada;* [b]*Health Sector, Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montreal, Quebec H4P 2R2, Canada;* [c]*Montreal Joint Centre for Structural Biology and* [d]*Department of Chemistry and Biochemistry, Concordia University, Montreal, Quebec H3G 1M8, Canada*

Received 20 August 2002; Accepted 11 September 2002

Key words: dual-specificity phosphatase, NMR resonance assignments, PRL-3

Biological content

The phosphatases are a family of signal transduction enzymes which together with protein kinases control cellular protein phosphorylation. The PRL class of phosphatases constitutes a novel class of small (19.5 kDa) prenylated PTPases (Zeng et al., 2000) with oncogenic activity (Cates et al., 1996). They contain the consensus PTPase active site sequence VHCXAGXXR. PRL-1 and PRL-2 are ubiquitously expressed (Zeng et al., 1998); PRL-3 is prominently expressed in cardiac and skeletal muscle (Matter et al., 2001). Recent SAGE (serial analysis of gene expression) experiments showed that PRL-3 is highly overexpressed in liver metastases of colorectal cancer, but not in nonmetastatic tumors and in normal colorectal epithelium (Saha et al., 2001). Further support for the involvement of PRL-3 in metastasis was provided by the finding of gene amplification in a significant fraction of metastatic lesions from different patients (Saha et al., 2001). Because of its high levels of expression, PRL-3 may constitute a useful marker for metastasis and possibly also a new therapeutic target. More studies at both the physiological and biochemical levels are needed to evaluate the involvement and possible causative role of PRL-3 in metastasis. Toward these general objectives, we have initiated structure-function studies to gain a better understanding of the molecular function and substrate specificity of PRL-3.

*To whom correspondence should be addressed. E-mail: Irena.Ekiel@bri.nrc.ca

Methods and experiments

PRL-3 sequence was amplified by RT-PCR subcloned into pET15b vector (Novagen Inc., Madison, WI) and over-expressed in *E. coli* BL21(DE3) as a His-tagged fusion protein. The protein was purified by immobilized metal affinity chromatography on Ni^{2+}-loaded chelating Sepharose (Amersham Pharmacia Biotech, Piscatqway, NJ). Isotopically enriched PRL-3 was prepared from cells grown on minimal M9 media containing ^{15}N-ammonium chloride with or without $^{13}C_6$-glucose (Cambridge Isotopes Laboratory, Andover, MA). The N-terminal His-tag was cleaved from PRL-3 by treatment overnight with thrombin (Haematologic Technologies Inc., Essex Junction, VT) at 6 units per mg of fusion protein at room temperature. Benzamidine sepharose and Ni^{2+}-loaded chelating Sepharose were used to remove thrombin and the His-tag from PRL-3. The resulting protein contains three residues (Gly-Ser-His) from the vector's cleavage site. The expressed protein does not include the C-terminal prenylation site, and comprises amino acids 1 to 169 of PRL-3. NMR samples were 3 mM in 50 mM phosphate buffer, 100 mM NaCl, 10 to 12 mM DTT and 0.1 mM sodium azide at pH 6.8. NMR experiments (Bax and Grzesiek, 1993) were performed at 308 K on Bruker DRX500 and 800 MHz Varian UNITYplus spectrometers.

Main-chain C^{α}, H^{α}, N, NH, CO and side-chain C^{β} resonances were assigned using HNCACB, CBCA(CO)NH, HNCO and HNHA experiments. The side-chain signal assignments were obtained from ^{1}H-

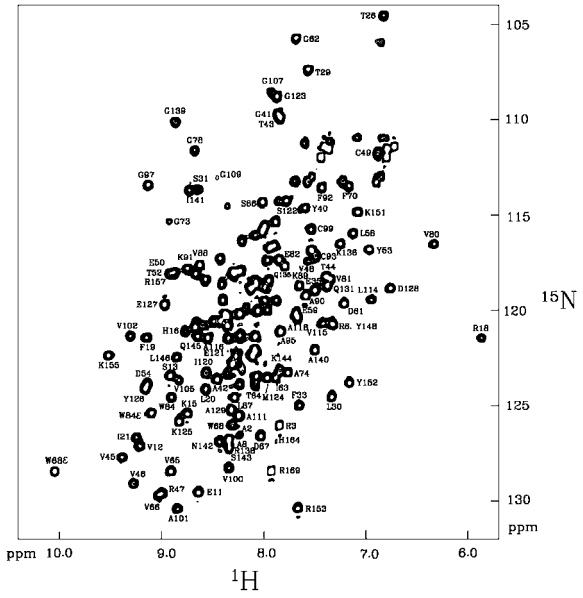

Figure 1. 500 MHz ^{1}H-^{15}N HSQC spectrum of [ul-^{15}N] PRL-3. The PRL-3 sample conditions: 3 mM, 5% D_2O, 50 mM sodium phosphate, 100 mM NaCl, 12 mM DTT, pH 6.8, 308 K. Amino acid labels were omitted from the middle of HSQC for clarity.

^{13}C HSQC, 3D HCCH-TOCSY, CC(CO)NH-TOCSY, ^{1}H-^{15}N-HSQC TOCSY and ^{1}H-^{15}N-HSQC NOESY. Chemical shifts were measured relative to internal DSS for ^{1}H and calculated for ^{13}C and ^{15}N assuming $\gamma^{15}N/\gamma^{1}H = 0.101329118$ and $\gamma^{13}C/\gamma^{1}H = 0.251449530$ (Wishart et al., 1995). NMR spectra were processed using Bruker XWINNMR and GIFA (Pons et al., 1996) software and analysed with XEASY (Bartels et al., 1995).

Extent of assignments and data deposition

All the ^{1}H and ^{15}N backbone resonances were assigned except for the 3 amino acids from the his-tag and residues Met1, Met4, Asn5, Asn27, His103, His166 and Lys167, for which signals could not be detected on HSQC spectra. The H^{α}, H^{β}, C^{α}, C^{β} and CO resonances were assigned for all PRL-3 residues except Met4, Pro75, Pro76 and His166. Nearly complete ^{1}H side-chain assignments, including aromatic rings, were obtained for non-proline residues. Figure 1 shows the ^{1}H-^{15}N HSQC spectrum of uniformly ^{15}N-enriched PRL-3. The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 5455.

Acknowledgements

We would like to thank the Canadian National High Field NMR Centre (NANUC) for their assistance and use of the facilities. Operation of NANUC is funded by the Canadian Institutes of Health Research, the Natural Science and Engineering Research Council of Canada and the University of Alberta. This work was supported by the Canadian Institutes of Health Research grant 43954 to I.E. NRC publication No. 44854.

References

Bartels, C., Xia, T.-H., Billeter, M., Guntert, P. and Wüthrich, K. (1995) *J. Biomol. NMR*, **5**, 1–10.

Bax, A. and Grzesiek, S. (1993) *Acc. Chem. Res.*, **26**, 131–138.

Cates, C.A., Michael, R.L., Stayrook, K.R., Harvey, K.A., Burke, Y.D., Randall, S.K., Crowell, P.L. and Crowell, D.N. (1996) *Cancer Lett.*, **110**, 49–55.

Matter, W.F., Estridge, T., Zhang, C., Belagaje, R., Stancato, L., Dixon, J., Johnson, B., Bloem, L., Pickard, T., Donaghue, M., Acton, S., Jeyaseelan, R., Kadambi, V. and Vlahos, C.J. (2001) *Biochem. Biophys. Res. Commun.*, **283**, 1061–1068.

Pons, J.-L., Malliavin, T.E. and Delsuc, M.A. (1996) *J. Biomol. NMR*, **8**, 445–452.

Saha, S., Bardelli, A., Buckhaults, P., Velculescu, V.E., Rago, C., St Croix, B., Romans, K.E., Choti, M.A., Lengauer, C., Kinzler, K.W. and Vogelstein, B. (2001) *Science*, **294**, 1343–1346.

Wishart, D.S., Bigam, C.G., Yao, J., Abildgaard, F., Dyson, H.J., Oldfield, E., Markley, J.L. and Sykes, B.D. (1995) *J. Biomol. NMR*, **6**, 135–140.

Zeng, Q., Hong, W. and Tan, Y.H. (1998) *Biochem. Biophys. Res. Commun.*, **244**, 421-427.

Zeng, Q., Si, X., Horstmann, H., Xu, Y., Hong, W. and Pallen, C.J. (2000) *J. Biol. Chem.*, **275**, 21444-21452.