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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C assignments of FLIN4, an intramolecular LMO4:ldb1 complex

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Biological context

LMO4 (LIM-only protein 4) was originally deposited in the GenBank database as a breast tumor autoantigen (Racevskis et al., 1999). It has recently been shown to be overexpressed in over 50% of primary invasive breast carcinomas and deregulation of the *lmo4* gene is thought to contribute to breast tumorigenesis (Visvader et al., 2001). Two other members of the small family of LMO proteins to which LMO4 belongs are known oncogenes. LMO1 and -2 (otherwise known as rhombotin-1 and -2) were originally discovered in patients with acute T-cell lymphoblastic leukemia (T-ALL) and are oncogenic in transgenic mice. Further, LMO2 is essential for haematopoiesis. All members of the LMO family bind with high affinity to ldb1 (LIM domain binding protein 1; also known as NLI and CLIM2), a nuclear protein that can also bind to homeodomain proteins that contain LIM domains. LMO-ldb1 complexes take part in higher order transcriptional complexes and are thought to be important for LMO function in both normal and oncogenic development. The LMO4:ldb1 complex appears to be important for regulating proliferation of breast epithelium and overexpression of this gene is associated with breast cancer. LMO4 and ldb1 form a multimeric protein complex with the breast cancer associated protein BRCA1 and CtIP; both BRCA1 and CtIP interact directly with LMO4 (Sum et al., 2002). Similarly, the LMO2:ldb1 complex promotes proliferation of immature T-cells, and directly contributes to leukemogenesis. Moreover, LMO4 (but not LMO2) is expressed in *mature* T-cells and it is thought to play a role in normal T-cell development. Current models for T-ALL suggest that inappropriately expressed LMO2 displaces LMO4 as the normal binding partner for ldb1. We are interested in solving the structures of both LMO2:ldb1 and LMO4:ldb1 complexes in order to better understand normal and disease state processes and to use this information to design specific LMO4 and LMO2 inhibitors.

The LMO proteins are composed almost entirely of two LIM domains. These domains consist of two sequential zinc-ligating modules and function as mediators of specific protein:protein interactions (Dawid et al., 1998). Three-dimensional structures of a number of LIM domains have been determined (e.g., Perez-Alvarado et al., 1994; Velyvis et al., 2001), revealing a highly conserved fold, but different surface features suggest that modes of interaction of LIM domains with partner proteins may have marked dissimilarities. To date no structures of a LIM:partner complex have been published, although the interaction of PINCH LIM1 and the ankyrin repeat domain of ILK has been studied using NMR (Velyvis et al., 2001).

The interaction of LMO4 with ldb1 is mediated mainly through the N-terminal LIM domain of LMO4 and a 38-residue region towards the C-terminus of ldb1 known as LID (LIM interaction domain) (Jurata et al., 1998). While it was straightforward to produce recombinant forms of ldb1, the production of domains from LMO4 proved problematic, due to limited solubility and stability. Fortunately ldb1(LID) peptides stabilize LMOs, and we could produce milligram quantities of an LMO4:ldb1(LID) complex by engineering an intramolecular complex, whereby the N-terminal LIM domain of LMO2 or LMO4 were fused, via an eleven-residue linker, to ldb1(LID)

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(Deane et al., 2001). We have recently reported the assignments of the LMO2:ldb1(LID) fusion (Matthews et al., 2001). We report here the ^{1}H, ^{13}C and ^{15}N chemical shift assignments of the LMO4:ldb1(LID) fusion, FLIN4 (fusion of the LIM interacting domain of ldb1 and the N-terminal LIM domain of LMO4).

Methods and experiments

FLIN4 is a 122-amino-acid protein with a molecular weight of 13.0 kDa. The design and construction of the gene encoding this fusion protein, and the purification of the protein have been described previously (Deane et al., 2001). Uniformly labeled ^{15}N- and ^{13}C,^{15}N-FLIN4 were prepared. FLIN4, in a buffer containing sodium phosphate (20 mM, pH 7.0), sodium chloride (50 mM) and dithiothreitol (1 mM), was concentrated in Centricon YM-3 centrifugal filter devices (Millipore) to concentrations of ~500 μM and supplemented with 5 % v/v D_2O and 20 μM d_4-TSP. Experiments were performed at 298 K on a Bruker DXR600 spectrometer. Data were processed using XWINNMR and analyzed using XEASY (Bartels et al., 1995).

Backbone and side-chain assignments were made using a combination of HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO, CC(CO)NH-TOCSY, HCC(CO)NH-TOCSY, HCCH-TOCSY, HNHA, and ^{15}N-NOESY-HSQC spectra. Aromatic resonances were assigned using 2D [^{1}H,^{1}H] TOCSY, DQFCOSY and NOESY spectra recorded on an unlabeled sample.

Extent of assignments and data deposition

Figure 1 shows the 2D [^{1}H,^{15}N] HSQC spectrum of uniformly ^{15}N labeled FLIN4. Full backbone, 93% side-chain hydrogen and 80% side-chain carbon assignments have been made for FLIN4 with the exception of residues 1–3, 73, 76, 77 and 81. Residues 73, 76, 77 and 81 lie in the unstructured linker region of FLIN4 and their signals are absent in heteronuclear experiments. Most of these residues can be assigned in 2D homonuclear experiments where those signals tend to be broad. This is consistent with the existence of a chemical exchange process on the μs–ms timescale. Other minor peaks, which we have not been able to assign, also appear in the [^{1}H,^{15}N] HSQC. These peaks are likely to have arisen from the same or another conformational exchange process.

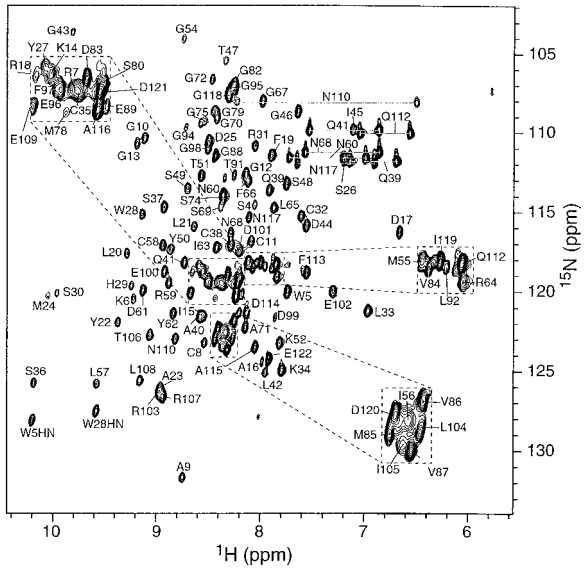

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum and assignments of FLIN4. NH_2 side-chain resonances of Gln and Asn are connected by lines.

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References

Bartels, C., Xia, T.H., Billeter, P., Guntert, P. and Wüthrich, K. (1995) *J. Biomol. NMR,* **6**, 1–10.

Dawid, I.B., Breen, J.J. and Toyama, R. (1998) *Trends Genet.,* **14**, 156–162.

Deane, J.E., Sum, E., Mackay, J.P., Lindeman, G.J., Visvader, J.E. and Matthews, J.M. (2001) *Prot. Engin.*, **14**, 493–494.

Jurata, L.W., Pfaff, S.L. and Gill, G.N. (1998) *J. Biol. Chem.,* **273**, 3152–3157.

Matthews, J., Visvader, J. and Mackay, J. (2001) *J. Biomol. NMR,* **21**, 385-386.

Perez-Alvarado, G.C., Miles, C., Michelsen, J.W., Louis, H.A., Winge, D.R., Beckerle, M.C. and Summers, M.F. (1994) *Nat. Struct. Biol.,* **1**, 388–398.

Racevskis, J., Dill, A., Sparano, J.A. and Ruan, H. (1999) *Biochim. Biophys. Acta,* **1445**, 148–153.

Sum, E.Y., Peng, B., Yu, X., Chen, J., Byrne, J., Lindeman, G.J. and Visvader, J.E. (2002) *J. Biol. Chem.,* **277**, 7849–7856.

Velyvis, A., Yang, Y., Wu, C. and Qin, J. (2001) *J. Biol. Chem.,* **276**, 4932–4939.

Visvader, J.E., Venter, D., Hahm, K., Santamaria, M., Sum, E.Y., O'Reilly, L., White, D., Williams, R., Armes, J. and Lindeman, G.J. (2001) *Proc. Natl Acad. Sci. USA,* **98**, 14452–14457.