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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C assignments of FLIN2, an intramolecular LMO2:ldb1 complex

Jacqueline M. Matthews[a,*], Jane E. Visvader[b] & Joel P. Mackay[a]
[a]*Department of Biochemistry, University of Sydney, NSW 2006, Australia;* [b]*Walter and Eliza Hall Institute for Medical Research, Parkville, Victoria 3050, Australia*

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Biological context

LMO2 (LIM-only protein 2/rhombotin) is an essential hematopoietic regulatory factor. Mice in which the gene for LMO2 is disrupted die in utero with a complete absence of blood cells. Further, the aberrant overexpression of LMO2, as a result of the chromosomal translocation t(11;14)(p13;q11), results in acute T-cell lymphoblastic leukemia (T-ALL) in children. LMO2 is composed almost entirely of two LIM domains. LIM domains comprise two sequential zinc-ligating modules and function as mediators of specific protein:protein interactions (Dawid et al., 1998). Indeed, LMO2 forms multiprotein complexes and specifically interacts with many different proteins. In particular, it binds to the widely expressed nuclear protein ldb1 (LIM domain binding protein 1 NLI/CLIM2). Ldb1 can bind to many other nuclear LIM proteins (Agulnick et al., 1996) and can also homodimerize. Higher order ldb1-LIM complexes may modulate gene expression by forming DNA-binding transcription factor complexes. In T-ALL, it has been proposed that overexpressed LMO2 displaces the related protein LMO4 as the binding partner for ldb1 in adult T-cells (Rabbitts et al., 1997) preventing normal T-cell development. The LMO2:ldb1 interaction is specifically mediated through the N-terminal LIM domain of LMO2 and a 38-residue region towards the C-terminus of ldb1 known as LID (LIM interaction domain) (Jurata et al., 1998).

LIM-containing proteins are functionally diverse and have been divided into three groups according to sequence similarities (Dawid et al., 1998); LMO2 belongs to Group 1. Three-dimensional structures have been determined for members of Group 2 (e.g., Konrat et al., 1998) and Group 3 (Velyvis et al., 2001), revealing a highly conserved fold. However, different surface features differ between groups, suggesting that their modes of interaction with partner proteins may have marked dissimilarities. To date no structures of a LIM:partner complex have been solved, although the interaction of PINCH LIM1 and the ankyrin repeat domain of ILK has been studied using NMR (Velyvis et al., 2001).

The structure of an LMO2:ldb1 complex should (a) provide insight into the mode of interaction of a Group 1 LIM domain with its partner protein; and (b) provide a template from which to develop a reagent that could specifically inhibit the LMO2:ldb1 interaction in the T-cells of patients with T-ALLs. While it was straightforward to produce recombinant forms of ldb1, the production of domains from LMO2 proved problematic, due to limited solubility and stability. Fortunately ldb1(LID) peptides stabilize LMO2, and we could produce milligram quantities of an LMO2:ldb1(LID) complex by engineering an intramolecular complex, whereby the N-terminal LIM domain of LMO2 was fused, via an eleven residue linker, to ldb1(LID) (Deane et al., 2001). This fusion protein is termed FLIN2 (fusion of the LIM interacting domain of ldb1 and the N-terminal LIM domain of LMO2). We report here the ^{1}H, ^{13}C and ^{15}N chemical shift assignments of FLIN2.

Methods and experiments

FLIN2 is a 114-amino-acid protein with a molecular weight of 12.6 kDa. The design and construction of the

*To whom correspondence should be addressed. E-mail: j.matthews@biochem.usyd.edu.au

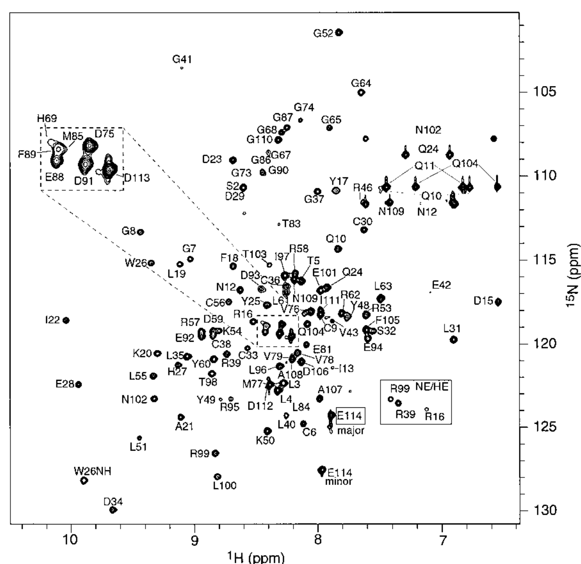

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum and assignments of FLIN2. NH_2 side-chain resonances of Gln and Asn are connected by lines. Arg side-chain resonances are boxed.

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-FLIN2 were prepared using the strategy developed by Cai et al. (1998). FLIN2, 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 HNHA, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO, CC(CO)NH-TOCSY, HCC(CO)NH-TOCSY, ^{15}N-TOCSY-HSQC 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, while histidine imidazole resonances were assigned using the approach described by Pelton et al. (1993).

Extent of assignments and data deposition

Figure 1 shows the 2D [^{1}H,^{15}N] HSQC spectrum of uniformly ^{15}N labeled FLIN2. Full backbone, ~90% side-chain hydrogen and ~72% of side-chain carbon assignments have been made for FLIN2 with the exception of residues 1, 42–49 and 69–72. No N assignment could be made for G14. From comparisons with existing structures of LIM domains, residues 42–49 are predicted to form a loop in the second zinc(II)-binding module of LMO2(LIM1), and residues 69–72 form part of the linker region between LMO2(LIM1) and ldb1(LID). Their signals are absent in heteronuclear experiments, but most of these residues can be assigned in 2D homonuclear experiments at pH 5.6 where those signals tend to be broad. This is consistent with the existence of some chemical exchange process on the μs–ms time scale. The HN and N atoms of the C-terminal residue (E114) also display conformational heterogeneity, appearing in most spectra as a major and minor form (~7:3). Other minor peaks, which we have not been able to assign, also appear in the [^{1}H,^{15}N] HSQC. It is likely that these peaks are also a result of conformational averaging.

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