Backbone and side-chain assignments of a tethered complex between LMO4 and DEAF-1

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Abstract The transcriptional regulator LMO4 and the transcription factor DEAF-1 are both essential for brain and skeletal development. They are also implicated in human breast cancers; overexpression of LMO4 is an indicator of poor prognosis, and overexpression of DEAF-1 promotes epithelial breast cell proliferation. We have generated a stable LMO4–DEAF-1 complex comprising the C-terminal LIM domain of LMO4 and an intrinsically disordered LMO4-interaction domain from DEAF-1 tethered by a glycine/serine linker. Here we report the ^1H , ^{15}N and ^{13}C assignments of this construct. Analysis of the assignments indicates the presence of structure in the DEAF-1 part of the complex supporting the presence of a physical interaction between the two proteins.

Keywords LMO4 · DEAF-1 · Transcriptional complex · Embryonic development · Breast cancer

Biological context

The LIM-domain only protein, LMO4, and the transcription factor DEAF-1 (Deformed epidermal autoregulatory

factor-1/NUDR/Suppressin) are co-expressed in a range of cell types (Huggenvik et al. 1998; Kenny et al. 1998), exhibit similar knock-in or knock-out phenotypes (Barker et al. 2008), and physically interact (Cubeddu et al. 2012; Sugihara et al. 1998). Therefore, it is likely that LMO4 and DEAF-1 perform at least some of their cellular functions as a complex. Both LMO4 and DEAF-1 are implicated in mouse embryonic development. In particular, both proteins have important roles in neural tube closure and skeletal patterning (Hahm et al. 2004). LMO4 is overexpressed in human breast cancers, where it is indicative of poor prognosis (Sum et al. 2005). Overexpression of DEAF-1 enhances the proliferation of human breast epithelial cells in vitro and mouse epithelial cells in vivo (Barker et al. 2008). LMO4 consists of two LIM (LIN-11/ISL1/MEC-3) domains, which are non-classical zinc fingers that mediate protein–protein interactions, and is thought to nucleate transcriptional complexes. In contrast, DEAF-1 contains multiple domains including a DNA-binding SAND (Sp100/AIRE1/NucP41/75/DEAF-1) domain, a nuclear localisation signal, a protein-interacting MYND (Myeloid translocation protein 8/Nervy/DEAF-1) domain and a coiled-coil domain that contains a nuclear export signal (Jensik et al. 2004).

Recombinant LMO4, expressed in isolation, is mostly insoluble and tends to aggregate. These problems can be overcome by engineering ‘tethered complexes’ with LMO4 binding partners, which form native-like interactions. We produced a stable tethered complex between the C-terminal LIM domain of LMO4 (LMO4_{77–147}) and DEAF-1_{404–418}, which is part of a region in DEAF-1 that was shown to mediate interactions with LMO4 (Cubeddu et al. 2012). We have assigned the backbone and side-chain resonances of LMO4_{77–147}–DEAF-1_{404–418}. The structure of the LMO4_{77–147}–DEAF-1_{404–418} complex will reveal the molecular details of this interaction and provide insight

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into the formation of higher order transcriptional complexes.

Methods and experiments

Protein production

LMO4₇₇₋₁₄₇-DEAF-1₄₀₄₋₄₁₈ was generated using overlap extension PCR and cloned into pGEX-2T to produce a tethered construct tagged with glutathione *S*-transferase (GST). The construct comprises residues 77–147 of mouse LMO4 (NCBI accession: NP_001155242), an 8-residue linker (GGSGGSGS) followed by residues 404–418 of canonical DEAF-1 from mouse (NM_016874). Isotopically labelled protein was produced as described previously (Marley et al. 2001). In brief, transformed Rosetta 2 *Escherichia coli* cells were cultured at 37 °C in LB media containing carbenicillin (100 µg mL⁻¹), chloramphenicol (34 µg mL⁻¹) and 0.5 % glucose to an OD_{600nm} of ~0.7. The cells were transferred to minimal media containing isotopes of interest, carbenicillin and chloramphenicol. They were allowed to recover for 1 h at 20 °C and protein expression was induced with 0.4 mM isopropyl β-D-thiogalactopyranoside for 16 h at 20 °C.

Purification

The cells were resuspended in 20 mM Tris-base; pH 8.0, 150 mM NaCl and 1 mM DTT (buffer A) containing 10 % glycerol and EDTA-free protease inhibitor cocktail (1 tablet/50 mL), lysed with the addition of lysozyme (100 µg mL⁻¹), freeze-thawing (3×), addition of DNaseI (10 µg mL⁻¹) and sonication. The cleared lysate was applied to glutathione resin equilibrated in buffer A and incubated for 1 h at 4 °C. The resin was washed with 20 column volumes (CVs) of buffer A, 40 CVs of buffer A containing 300 mM NaCl and then 2 CVs of 20 mM HEPES sodium salt; pH 7.0, 150 mM NaCl, 1 mM DTT and 2 mM CaCl₂ (buffer B). GST-LMO4₇₇₋₁₄₇-DEAF-1₄₀₄₋₄₁₈ was eluted from the resin in buffer B containing 50 mM reduced glutathione and cleaved by treatment with thrombin (20 U, 16 h, 25 °C). LMO4₇₇₋₁₄₇-DEAF-1₄₀₄₋₄₁₈ was separated from GST by size exclusion chromatography (preparative grade Superdex 75; GE Healthcare) in 20 mM sodium acetate; pH 5.0, 50–150 mM NaCl and 1 mM Tris (2-chloroethyl) phosphate hydrochloride (TCEP·HCl). Fractions containing LMO4₇₇₋₁₄₇-DEAF-1₄₀₄₋₄₁₈ were pooled and concentrated, with the addition of chloramphenicol and EDTA-free protease inhibitor (Roche) to ~0.5 mM, using a stirred-cell concentrator and then freeze-dried. The freeze-dried protein was stored at -20 °C until required.

NMR experiments

Freeze-dried LMO4₇₇₋₁₄₇-DEAF-1₄₀₄₋₄₁₈ was resuspended in either H₂O (90 %) and D₂O (10 %), or D₂O alone, such that the sample contained 20 mM sodium acetate pH 5.0, 35 mM NaCl, 0.5 mM TCEP·HCl, 34 µg mL⁻¹ chloramphenicol and EDTA-free protease inhibitor cocktail (1 tablet/50 mL). NMR experiments were conducted at a protein concentration (*c*) of ~0.5 mM as estimated by the equation $A_{215\text{nm}} - A_{225\text{nm}} \times 144 = c$ (µg mL⁻¹), where $A_{215\text{nm}}$ and $A_{225\text{nm}}$ are the absorbance at 215 and 225 nm, respectively. Proton chemical shifts were referenced to 4,4-dimethyl-4-silapentanesulfonic acid (DSS) at 0.00 ppm. ¹³C and ¹⁵N chemical shifts were referenced indirectly to the same signal. NMR experiments were recorded at 298 K on either a 600 or 800 MHz spectrometer (Bruker Avance III) equipped with a 5-mm TCI CryoProbe (Bruker). The spectra recorded included ¹⁵N-HSQC, ¹³C-HSQC, CBCA(CO)NH, HNCACB, HCC(CO)NH, HNCO, HN(CA)CO, (H)CCH-TOCSY, HBCBCGCDCEHE, HBCBCGCDHD (Yamazaki et al. 1993), histidine-HSQC (Pelton et al. 1993), ¹⁵N-NOESY, ¹³C-aliphatic-NOESY, ¹H-COSY, ¹H-TOCSY and ¹H-NOESY.

Data processing

Data were processed using Topspin (Bruker Biospin) and assignments were made using Sparky (T. D. Goddard and D. G. Kneller, University of California at San Francisco). TALOS+ (Shen et al. 2009) was used to predict secondary structure based on C_α, C_β, H_N, N and C' shifts.

Assignments and data deposition

The tethered intra-molecular complex between LMO4₇₇₋₁₄₇-DEAF-1₄₀₄₋₄₁₈ has 88 residues derived from the native proteins (Fig. 1a). The first two residues (glycine-1 and serine-2) remain from thrombin cleavage of the GST affinity tag. The artificial eight-residue glycine/serine linker (901–908) tethers the C-terminus of LMO4₇₇₋₁₄₇ to the N-terminus of DEAF-1₄₀₄₋₄₁₈. The assigned amide groups are shown on the ¹⁵N-HSQC (Fig. 2); residues corresponding to LMO4 and DEAF-1 are numbered as they appear in the native, full-length proteins. All assigned C_α and C_β shifts were within twice the standard deviation of the average chemical shift reported in the BMRB. Assignments could not be made for glycine-1. Backbone assignments for DEAF-1₄₀₄₋₄₁₈ are complete apart from A405-C' and 92 % of the side-chain atoms have been assigned. Assignments for the backbone of LMO4₇₇₋₁₄₇ are near complete (98 %) and 85 % of the side-chain atoms have been assigned. Carbon atoms on aromatic rings were

a

77 121 147 1901 908 404 418

LMO4 DEAF1

TALOS+ 1RUT

b

$\delta C_{\alpha} - \delta C_{\beta}$ chemical shift (ppm)

77 121 147 1901 908 404 418

YIRLFGNSGACSGQSIPASELVMAQQGVNHYLKCFTCTCRNRLVPGDRFHYINGSLSFCEHDRPTALINggsgsgsgsIAPFPEAALPTSHPK

[illegible]

18898). Note that in early preparations, a second, weaker set of signals was observed in the ^{15}N -HSQC spectrum. These signals were clustered between 7.8 and 8.5 ppm in the ^1H dimension (Figure S1). Freeze-drying of the purified protein eliminated these additional signals.

Secondary structure predictions based on chemical shift data were carried out using TALOS+ (Fig. 1a). The predicted secondary structure of LMO4₇₇₋₁₄₇ is similar to the high resolution crystal structure of LMO4 in complex with Ldb1 (1RUT). Even though a helical structure is predicted for three consecutive residues, H109–K111, at least four consecutive residues are required to make a complete helical turn. With the exception of S908, the chemical shift differences ($\delta C_\alpha - \delta C_\beta$) calculated for residues in the glycine/serine linker (901–908) are close to values expected in random coils (Fig. 1b), indicating that this sequence is most likely disordered, as intended. ¹⁵N-HSQC spectra were also collected for constructs containing either a longer (11-residue) synthetic glycine/serine linker, or a 5-residue N-terminal extension of DEAF-1 (LMO4₇₇₋₁₄₇–DEAF-1₃₉₉₋₄₁₈). These spectra are very similar to the original LMO4₇₇₋₁₄₇–DEAF-1₄₀₄₋₄₁₈ construct (Figure S2 and S3) suggesting that the structures of the constructs are also similar. In particular, resonances that correspond to DEAF-1₄₀₄₋₄₁₈ are maintained, suggesting that the original construct allows native-like complex formation between LMO4 and DEAF-1.

In contrast to most of the residues in the glycine/serine linker, although DEAF-1₄₀₄₋₄₁₈ is predicted to be disordered by PONDR[®], some of backbone chemical shifts observed for the DEAF-1 peptide in the intra-molecular complex differ significantly from those expected of residues in random coils, suggesting that this region is structured in the context of LMO4₇₇₋₁₄₇–DEAF-1₄₀₄₋₄₁₈ (Fig. 1b). To further support the argument for complex formation, a preliminary analysis of the NOE data from ¹⁵N-NOESY and ¹³C-NOESY spectra was carried out. NOEs could be unambiguously assigned as being between DEAF-1₄₀₄₋₄₁₁ and LMO4₇₇₋₁₄₇ protons, which suggests that an interaction occurs between those regions in this construct.

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Conflict of interest The authors declare they have no conflicts of interest.

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