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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N backbone resonance assignments of the SAND domains from glucocorticoid modulatory element binding proteins-1 and -2

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Abbreviations: GME – Glucocorticoid Modulatory Element; GMEB – GME Binding protein; SAND – Sp100, AIRE1, NucP41/75, DEAF1; Sp100 – Speckled protein 100 kDa; AIRE1 – autoimmune regulator-1; DEAF1 – deformed epidermal autoregulatory factor-1; NUDR – nuclear DEAF1-related; Pu – purine; Py – pyrimidine.

Biological context

The glucocorticoid modulatory element binding proteins, GMEB-1 and GMEB-2, are ubiquitous, multifunctional DNA-binding proteins with important roles in the modulation of transcription upon steroid hormone activation (Chen et al., 2002). The GMEBs were independently identified both through their binding to the glucocorticoid modulatory element (GME) (Oshima et al., 1995) and via their involvement in *Parvovirus* replication (Christensen et al., 1997). GMEB dimers bind target DNA containing tandem PuCGPy motifs. Upon binding to GME DNA, the GMEBs enhance glucocorticoid signalling (Chen et al., 2002). The GMEBs also control steroid responses via binding to the glucocorticoid receptor and the CREB-binding protein (Kaul et al., 2000). Since GMEB binding sites are postulated to exist in the majority of promoters (Burnett et al., 2001), these proteins may regulate many different genes.

Despite their many roles, the molecular basis of GMEB function is unclear. GMEBs are large (> 55 kDa) but have little homology with other proteins. However, they have 40% overall sequence identity with each other, rising to 80% in their SAND domains. The SAND domain, a novel DNA-binding fold, occurs in proteins implicated in transcriptional regulation (Bottomley et al., 2001). The SAND domain family includes Sp100 proteins, AIRE1, nuclear phosphoprotein NucP41/75 and DEAF1 (the *Drosophila* ortholog of NUDR), and these proteins are linked to many diseases, including acute promyelocytic leukemia and acute polyglandular syndrome-1.

We performed NMR studies of extended GMEB SAND domains and here report their ^{1}H, ^{13}C and ^{15}N backbone resonance assignments, enabling functional studies of both proteins. A collaborative x-ray crystallographic study has also yielded the GMEB-1 SAND domain structure at 1.55 Å resolution (Lo Surdo et al., submitted). In concert, this structural data aids an understanding of the involvement of these proteins in the glucocorticoid response and in *Parvovirus* replication. Moreover, this work lays the foundation for determining the structural basis of the interactions of GMEB SAND domains with their numerous reported ligands.

Methods and experiments

The SAND domains of GMEB-1 (GenBank NM_006582, residues E89-K182) and GMEB-2 (GenBank AF059273, residues E81-K178) were cloned into a modified pET-24d vector (Novagen). N-terminally His-tagged SAND domains were produced in *E. coli* upon induction by IPTG for 16 h at 23 °C. Uniformly ^{15}N- and ^{13}C/^{15}N-labelled samples were purified via Ni^{2+} affinity chromatography as described previously (Bottomley et al., 2001). The His-tags were removed at 23 °C with tobacco etch virus protease. Samples were further purified on Mono-Q

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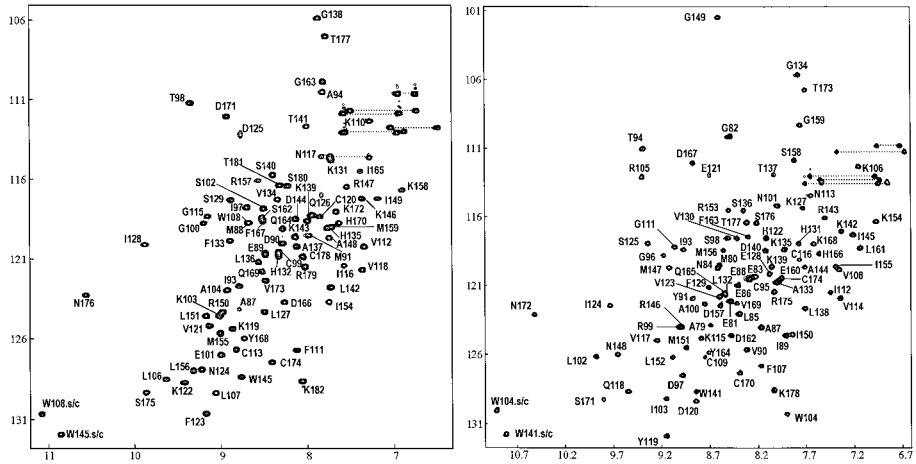

Figure 1. Assigned ^{1}H-^{15}N HSQC NMR spectra of GMEB-1 SAND domain (left) and GMEB-2 SAND domain (right), recorded at 27 °C. Unassigned side-chain resonance pairs are connected by dotted lines.

Sepharose and Superdex-75 resins (AP Biotech). The protein molecular weights were confirmed by mass spectroscopy (GMEB-1 SAND: 11022 Da, GMEB-2 SAND: 11536 Da). 1 mM NMR samples were dissolved in 20 mM sodium phosphate buffer pH 6.4, 0.1 M NaCl, 3 mM DTT and 0.02% NaN_3 in 90% H_2O, 10% D_2O.

NMR spectra were acquired at 27 °C on Bruker DRX-500 and DRX-600 spectrometers. The ^{1}H, ^{13}C and ^{15}N chemical shifts were assigned by standard methods (Sattler et al., 1999), using the following experiments: ^{1}H-^{15}N HSQC, HNCO, HNCA, CBCA(CO)NH, HNCACB, ^{15}N-separated NOESY and an HNHA experiment on GMEB-1 SAND, yielding $^{3}J_{\mathrm{HNH}\alpha}$ coupling constants. Spectra were processed with NMRPipe (Delaglio et al., 1995) and analyzed using NMRView (Johnson and Blevins, 1994). ^{1}H chemical shifts were directly referenced to TSP at 25 °C pH 6.0; ^{13}C and ^{15}N referencing was performed assuming $\gamma^{13}\mathrm{C}/\gamma^{1}\mathrm{H} = 0.251449530$ and $\gamma^{15}\mathrm{N}/\gamma^{1}\mathrm{H} = 0.101329118$ (Wishart et al., 1995).

Extent of assignments and data deposition

All backbone resonances in the ^{1}H-^{15}N HSQC spectra have been assigned, as have Trp side chain N/H^{N} resonances. For GMEB-1, assignments are >96% complete for N, H^{N}, CO, C$^{\alpha}$, C$^{\beta}$ and H$^{\alpha}$ resonances. Backbone N/H^{N} resonances were absent for the exposed residues E92, Y95, G152 and G153. For GMEB-2, assignments are 100% complete for all N, H^{N}, C$^{\alpha}$ and C$^{\beta}$ resonances excluding the N-terminal residue. The assignments for both proteins, plus the coupling constants for GMEB-1, have been deposited in the BMRB (accession numbers 5592 and 5593).

The $^{3}J_{\mathrm{HNH}\alpha}$ coupling constants and the secondary chemical shifts suggest that the solution and crystal structures of GMEB-1 are the same. The similarity of the chemical shifts of GMEB-1 and GMEB-2 SAND domains suggest that these structures are also very similar. These data enable comparative analysis of the relationship between chemical shifts, structure and function.

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