Journal of Biomolecular NMR, **24:** 71–72, 2002.
KLUWER/ESCOM

Letter to the Editor: Chemical shift and secondary structure conservation of the PNT/SAM domains from the Ets family of transcription factors

Cameron D. Mackereth[a], Manuela Schärpf[a], Lisa N. Gentile[b] & Lawrence P. McIntosh[a,*]
[a]*Department of Biochemistry and Molecular Biology, Department of Chemistry, and The Biotechnology Laboratory, The University of British Columbia, Vancouver, British Columbia, Canada, V6T 1Z3;* [b]*Department of Chemistry, Western Washington University, Bellingham, WA 98225, U.S.A.*

Received 20 June 2002; Accepted 15 July 2002

Key words: Erg, Ets-1, GABPα, PNT domain, SAM domain, secondary chemical shift

Biological context

The DNA-binding ETS domain defines the Ets family of transcription factors. A subset of this family, including Ets-1, Ets-2, GABPα, Erg, Fli-1 and Tel, also share a conserved Pointed (PNT) or SAM domain that may function in oligomerization, MAP kinase docking or association with other regulatory proteins. The solution structure of the PNT/SAM domain from Ets-1 (PDB: 1qbv; Slupsky et al., 1998a) has an architecture of a core four α-helical bundle interfaced with an additional N-terminal helix. In contrast, the crystal structure of the homologous domain from Tel (1ji7; Kim et al., 2001) shows only the four core helices, as also seen in the structures of SAM domains from non-Ets family members such as p73 (1cok), polyhomeotic (1kw4) and the Ephrin receptors (1b4f, 1sgg, 1b0x). The additional N-terminal helix present in Ets-1 is an integral part of the structured PNT/SAM domain as evident by ^{15}N relaxation measurements and the requirement of these residues for proper protein expression and folding (Slupsky et al., 1998a). However, the extent to which this helix exists in PNT/SAM domains from other Ets proteins remains to be established.

Methods and experiments

The genes encoding the PNT/SAM domains of murine GABPα (residues 168–254) and human Erg (residues 108–201 of isoform p55Erg) were cloned into pET22b and pET28a, respectively, using PCR-generated restriction enzyme sites. Proteins were expressed in BL21(λDE3) grown in Luria Broth or M9 minimal media supplemented with $^{15}NH_4Cl$ (1 g l^{-1}) or $^{13}C_6$-glucose (3 g l^{-1}). Purification of GABPα$^{(168-254)}$ was achieved with Q-sepharose anion-exchange chromatography, followed by dialysis into 20 mM sodium phosphate (pH 7.2), 20 mM NaCl and 10 mM DTT. His_6-tagged Erg$^{(108-201)}$ was purified using Ni^{2+}-affinity chromatography, with final dialysis into 20 mM potassium phosphate (pH 7.0), 50 mM NaCl and 2 mM DTT. Following thrombin removal of the His_6-tag, there remained three N-terminal residues (Gly-Ser-His) preceding Met108 in Erg$^{(108-201)}$.

Samples contained 0.5 to 2.5 mM protein with 10% or 99% D_2O added for the lock. All spectra were recorded at 30 °C using a 500 MHz Varian Unity or a 600 MHz Varian Inova NMR spectrometer, both equipped with a triple resonance gradient probe. The spectra were analyzed using Felix 95.0/2000 (Accelrys, Inc.) and Sparky 3 (T.D. Goddard and D.G. Kneller, University of California, San Francisco). Backbone and aliphatic sidechain assignment were obtained from ^{13}C-and ^{15}N-HSQC, HNCACB, CBCA(CO)NH, HNCO, HACAN, (H)CC(CO)NH-, H(CC)(CO)NH- and HCCH-TOCSY spectra (Sattler et al., 1999; Kanelis et al., 2000). Stereospecific assignments were achieved using the method of Senn et al. (1989) for Val and Leu methyl groups and McIntosh et al. (1997) for the sidechain amides of Gln and Asn. Resonances of aromatic sidechains were assigned as described previously (Slupsky et al., 1998b).

We have determined the ^{1}H, ^{13}C and ^{15}N resonance assignments for the PNT/SAM domains of GABPα and Erg. With the exception of the 12 N-terminal residues of Erg$^{(108-201)}$, a striking level of

*To whom correspondence should be addressed. E-mail: mcintosh@otter.biochem.ubc.ca

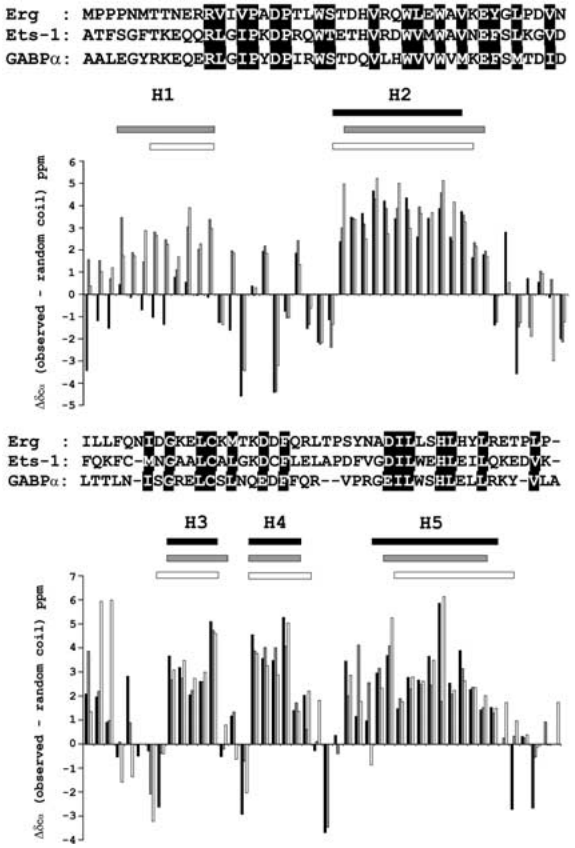

Figure 1. $^{13}C^{\alpha}$ secondary chemical shifts for Erg$^{(108-201)}$ (black), Ets-1$^{(29-138)}$ (gray) and GABPα$^{(168-254)}$ (white) calculated using random coil reference values from Wishart and Sykes (1994). Also illustrated is a sequence alignment (with highly conserved residues boxed), along with horizontal bars showing the locations of helical regions defined by chemical shift, $^{3}J_{HN-H\alpha}$ coupling, and in the case of Ets-1$^{(29-138)}$, tertiary structural analysis. Erg shares the core four α-helices (H2–H5) common to all PNT/SAM structures, yet clearly lacks the N-terminal helix (H1) found in Ets-1 and GABPα.

similarity exists between the $^{13}C^{\alpha}$ secondary chemical shifts ($\delta_{obs} - \delta_{random\ coil}$) observed for corresponding residues from the sequence alignment of Ets-1, GABPα and Erg (Figure 1). The secondary chemical shifts of mainchain $^{1}H^{\alpha}$, $^{13}C'$, $^{1}H^{N}$, and ^{15}N exhibit similar conservation (data not shown). These results confirm the potential of programs that exploit reference chemical shifts from one protein to predict the chemical shifts of a homologous protein (e.g., SHIFTY; Wishart et al., 1997). This observed conservation in secondary shifts should allow for facile NMR spectral assignment of additional PNT/SAM domains.

Deduction of secondary structure from chemical shifts and $^{3}J_{HN-H\alpha}$ values (not shown) also demonstrates that GABPα, but not Erg, contains the additional N-terminal helix found in Ets-1. This result confirms a surprising diversity in the architecture of the Ets family PNT/SAM domains that may extend to variation in function.

Extent of assignments and data deposition

Complete backbone ^{15}N, $^{1}H^{N}$, $^{13}C'$ and $^{13}C^{\alpha}$ assignments of GABPα$^{(168-254)}$ and Erg$^{(108-201)}$ have been determined with the exception of proline ^{15}N chemical shifts in GABPα$^{(168-254)}$, $^{13}C'$ chemical shifts in Erg$^{(108-201)}$ for residues preceding a proline, and the $^{13}C^{\alpha}$ resonance for the C-terminal Pro201. Assignment of resonances from sidechain carbon, nitrogen and non-labile hydrogen nuclei are nearly complete. Methyl groups of Leu and Val have been stereospecifically assigned, as have the sidechain amides of Asn and Gln. The ^{1}H, ^{13}C and ^{15}N chemical shifts for GABPα$^{(168-254)}$ and Erg$^{(108-201)}$ have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under BMRB accession numbers 5401 and 5399, respectively.

Acknowledgements

This work was supported by the National Cancer Institute of Canada with funds from the Canadian Cancer Society and instrumentation from PENCE. L.P.M. is a CIHR Scientist.

References

Kanelis, V., Donaldson, L., Muhandiram, D.R, Rotin, D., Forman-Kay, J.D. and Kay, L.E. (2000) *J. Biomol. NMR*, **16**, 253–259.

Kim, C.A., Phillips, M.A., Kim, W., Gingery, M. Tran, H.H., Robinson, M.A., Faham, S. and Bowie, J.U. (2001) *EMBO J.*, **20**, 4173–4182.

McIntosh, L.P., Brun, E. and Kay, L.E. (1997) *J. Biomol. NMR*, **9**, 306–312.

Sattler, M., Schleucher, J. and Griesinger, C. (1999) *Prog. NMR Spectrosc.*, **34**, 93–158.

Senn, H., Werner, B., Messerle, B.A., Weber, C., Traber, R. and Wüthrich, K. (1989) *FEBS Lett.*, **249**, 113–118.

Slupsky, C.M., Gentile, L.N., Donaldson, L.W., Mackereth, C.D., Seidel, J.J., Graves, B.J. and McIntosh, L.P. (1998a) *Proc. Natl. Acad. Sci. USA*, **96**, 12129–12134.

Slupsky, C.M., Gentile, L.N. and McIntosh, L.P. (1998b) *Biochem. Cell Biol.*, **76**, 379–390.

Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.

Wishart, D.S., Watson, M.S., Boyko, R.F. and Sykes, B.C. (1997) *J. Biomol. NMR*, **10**, 329–336.