Journal of Biomolecular NMR (2005) 31: 175–176

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments and secondary structure of murine angiogenin 4

Yun-Ru Pan, Kuen-Phon Wu, Yuan-Chao Lou, You-Di Liao & Chinpan Chen*
Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan, R.O.C.

Received 4 August 2004; Accepted 18 October 2004

Key words: angiogenesis, angiogenin, microbicidal protein, NMR, ribonuclease

Biological context

Angiogenin (Ang) is a member of the pancreatic-like ribonucleases (RNase) superfamily isolated from mammals, reptiles, and the amphibians. Human angiogenin (hAng) is best known for its role in neovascularization/angiogenesis because of the function in inducing the formation of new blood vessels (Fett et al., 1985), and has emerged as a promising anticancer target (Eberle et al., 2000). hAng is composed of 123 amino acid residues cross-linked with three disulfide bridges (Strydom et al., 1985) and shares 33% sequence identity with RNase A. The ribonucleolytic activity of hAng was reported to be 10^4 to 10^6-fold lower than that of RNase A. Nevertheless the ribonucleolytic activity is essential for its angiogenic activity (Shapiro et al., 1989). Similar to hAng, angiogenins from other species all contain the essential catalytic residues, and display weak catalytic activity toward RNase A substrates. It was found that the murine hosts the largest multiplicity of different sequences that are clustered together on chromosome 14 and encode proteins with 72–81% sequence identity (Strydom, 1998). Recently, an interesting observation reported on murine angiogenin 4 (mAng4; 121 residues) is that mAng4 is an abundant antimicrobial constituent of murine Paneth cell granules, and plays an important role in antimicrobial host defense (Ganz, 2003; Hooper et al., 2003). The identification of a microbicidal RNase in Paneth cells should prompt us not only to reexamine the specific role of these enzymes in intestinal immunity, but also to study how the catalytic and noncatalytic functions of these and other antimicrobial enzymes contribute to host defense. There is a lack of knowledge of the inherent structure–function relationships of mAng4, and of its relationship to the other members of this RNase family. Therefore, a high-resolution structural analysis on mAng4 that shares 64% sequence identity with hAng is very attractive since it might provide further insight into the structure–function relationships that govern the antimicrobial response. To date, structural studies on angiogenins are all related to human and bovine angiogenins, and were mostly carried out using X-ray crystallography. Here we report the ^{1}H, ^{13}C, and ^{15}N resonance assignments and secondary structure of the recombinant mAng4 in which the N-terminal Ala residue (Ala^{1}) is replaced with Met.

Methods and experiments

The gene encoding mAng4 was tagged with a *Nde*-containing sequence CATATG inserted into the pET22b expression vector (Novagen, Germany) through *Nde*I and *Bam*HI site. The gene was expressed in BL21 (DE3) strain at 37 °C overnight in the presence of 0.5 mM IPTG. After refolding, the uniformly ^{15}N- and/or ^{13}C-labeled proteins were purified by CM52 (Whatman, USA) and Mono S (Pharmacia, Taiwan branch) column chromatographies. The authenticity of mAng 4 sample was verified by SDS-PAGE and ES/MS analyze. Approximately 2.5 mg of the recombinant $^{13}C/^{15}N$-labeled mAng4 was purified from one liter of culture. All NMR experiments were performed on a Bruker AVANCE 600 spectrometer equipped with a triple (^{1}H, ^{13}C and ^{15}N) resonance probe

*To whom correspondence should be addressed. E-mail: bmchinp@ibms.sinica.edu.tw

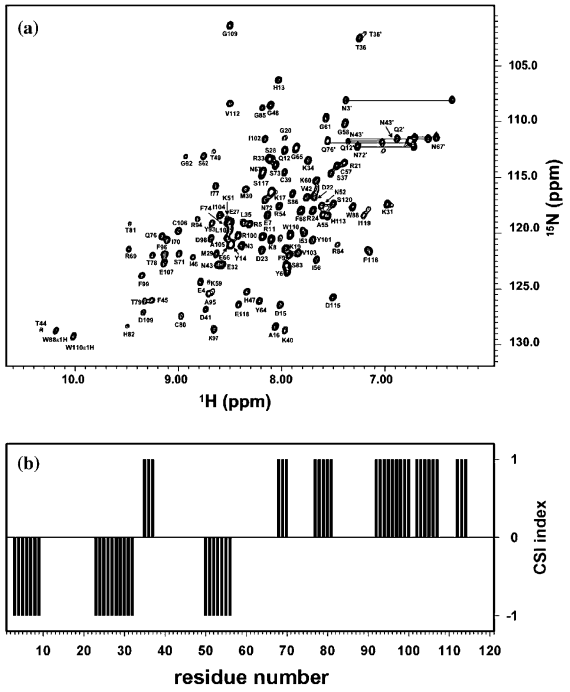

Figure 1. (a) A 600 MHz 2D ^{1}H–^{15}N HSQC spectrum of recombinant mAng4 acquired at 310 K and pH 5.0. The assignments are indicated with one-letter amino acid code and residue number. Side-chain NH_2 peaks of Asn and Gln are marked with asterisk and linked by horizontal lines. Due to weak intensity, cross peaks of Lys[49] and Cys[90] are not seen in the Figure (b). The CSI consensus plot generated on the basis of $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and $^{13}C'$ chemical shifts clearly displays that mAng4 contains 3α-helices and 6β-strands secondary structures.

including shielded z-gradient at 310 K. NMR samples were prepared in 20 mM phosphate buffer in 90% H_2O/10% D_2O at pH 5.0 and contained 0.3 ml of 1.0 mM protein in a Shigemi NMR tube (Allison Park, PA, USA). All heteronuclear NMR experiments were carried out as described in review articles (Kay, 1995). All spectra were processed using XWIN-NMR and analyzed using AURELIA on SGI O_2 workstations. Linear prediction was used in the ^{13}C and ^{15}N dimensions to improve the digital resolution. Sequence-specific assignment of the backbone atoms was achieved by the independent connectivity analysis of CBCA (CO)NH, HNCACB, HNCO, HN(CA)CO and C(CO)NH. The ^{1}H resonances were assigned using TOCSY–HSQC, HAHB(CO)NH, HCCH–TOCSY and HC(CO)NH. Combined information from 2D ^{1}H–^{15}N HSQC and 3D NOESY–HSQC experiments yielded assignments for side-chain amide resonances of the Asn and Gln residues.

Extent of assignments and data deposition

Figure 1a shows the 2D ^{1}H–^{15}N HSQC spectrum of mAng4 acquired at 310 K and pH 5.0. We have assigned all resonances of the backbone nuclei ($^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, $^{1}H^{\alpha}$ and $^{13}C'$), with the exception of the H^{N} of Gln[2], Tyr[25] and Phe[114]. In contrast, the amide protons of the corresponding residues in hAng could be assigned under similar condition (313 K and pH 5.0) by using uniformly ^{15}N-enriched sample (Lequin et al., 1998). In addition, over 95% of the side-chain resonances were unambiguously assigned, and the unassigned side-chain resonances that are severely overlapped are mostly from Pro and aromatic residues. The consensus chemical shift index (CSI) plot shown in Figure 1b (Wishart and Sykes, 1994) reveals that mAng4 consists of 3α-helices and 6β-strands, similar to hAng. The ^{1}H, ^{15}N and ^{13}C chemical shifts at 310 K have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 6279.

Acknowledgements

This work was supported by Academia Sinica and the National Science Council (NSC 93-2311-B-001-071), Taipei, Taiwan, ROC. The NMR spectra were obtained at the High-field Biomacromolecular NMR Core Facility at Academia Sinica, supported by the National Science and Technology Program for Medical Genomics.

References

Eberle, K., Oberpichler, A., Trantakis, C., Krupp, W., Knupfer, M., Tschesche, H. and Seifert, V. (2000) *Anticancer Res.*, **20**, 1679–1684.

Fett, J.W., Strydom, D.J., Lobb, R.R., Alderman, E.M., Bethune, J.L., Riordan, J.F. and Vallee, B.L. (1985) *Biochemistry*, **24**, 5480–5486.

Ganz, T. (2003) *Nat. Immunol.*, **4**, 213–214.

Hooper, L.V., Stappenbeck, T.S., Hong, C.V. and Gordon, J.I. (2003) *Nat. Immunol.*, **4**, 269–273.

Kay, L.E. (1995) *Prog. Biophys. Mol. Biol.*, **63**, 277–299.

Lequin, O., Thuring, H., Robin, M. and Lallemand, J.Y. (1997) *Eur. J. Biochem.*, **250**, 712–726.

Shapiro, R., Fox, E.A. and Riordan, J.F. (1989) *Biochemistry*, **28**, 1726–1732.

Strydom, D.J. (1998) *Cell Mol. Life Sci.*, **54**, 811–824.

Strydom, D.J., Fett, J.W., Lobb, R.R., Alderman, E.M., Bethune, J.L., Riordan, J.F. and Vallee, B.L. (1985) *Biochemistry*, **24**, 5486–5494.

Wishart, D.S. and Sykes, B.D. (1994) *Meth. Enzymol.* **239**, 363–392.