Journal of Biomolecular NMR, **17:** 87–88, 2000.
KLUWER/ESCOM

Letter to the Editor: Sequence-specific ^{1}H, ^{13}C, and ^{15}N assignment of the human melanoma inhibitory activity (MIA) protein

Raphael Stoll[a], Christian Renner[a], Dorothee Ambrosius[b], Michaela Golob[d], Wolfgang Voelter[c], Reinhard Buettner[d], Anja-Katrin Bosserhoff[d,*] & Tad A. Holak[a,*]
[a]*Max Planck Institute of Biochemistry, D-82152 Martinsried, Germany;* [b]*Roche Diagnostics GmbH, Pharmaceutical Research, D-82377 Penzberg, Germany;* [c]*Tübingen University, Institute of Physiological Chemistry, D-72076 Tübingen, Germany;* [d]*University Hospital of RWTH Aachen, D-52074 Aachen, Germany*

Received 11 February 2000; Accepted 9 March 2000

Key words: human melanoma inhibitory activity, MIA, NMR assignments

Biological context

MIA is translated as a 131 amino acid precursor molecule that is processed into a mature 107 amino acid protein after cleavage of a putative secretion signal (Blesch et al., 1994). MIA provides a clinically useful parameter in patients with malignant melanoma, as enhanced values were measured in the sera of all patients with metastatic melanoma stage III and IV (Bosserhoff et al., 1999a). Expression of the wild-type MIA protein gene was not detected in normal skin and melanocytes but was associated with progression of melanocytic tumours (Bosserhoff et al., 1997). Recently, it was shown that the MIA protein specifically inhibits attachment of melanoma cells to fibronectin and laminin and thereby promotes invasion and metastasis in vivo (Bosserhoff et al., 1999b). These studies revealed that MIA binds to fibronectin, laminin, and tenascin and thereby masks the binding site of integrins to these extracellular matrix (ECM) components (Bosserhoff et al., 1999b). Thus, the growth-inhibitory activity in vitro reflects the ability of the protein to interfere with the attachment of cell lines to culture dishes in vitro (Bosserhoff et al., 1999b).

In the present paper we report the ^{1}H, ^{13}C, and ^{15}N assignments of human MIA.

Methods and results

The recombinant human MIA protein was obtained from an *E. coli* BL21(DE3) expression system for 108 residues comprising the open reading frame of human MIA from amino acid G25 to Q131 plus an additional N-terminal methionine cloned in a modified pQE-40 vector (Qiagen). This resulted in a primary structure of M1 to Q108 of the polypeptide used in this study.

Media contained ampicillin (100 μg l^{-1}) and kanamycin (25 μg l^{-1}) as antibiotics for selection. Expression was performed at 30 °C in shaking cultures at 280 rpm and was induced by 1 mM IPTG (Biomol) at a cell density of OD_{600nm} of 0.7. Cells were harvested after 4 h (LB medium) or 8 h (minimal medium) by centrifugation. LB medium was used for the preparation of protonated protein samples (Sambrook et al., 1989). The uniformly ^{13}C/^{15}N and ^{15}N isotopically enriched protein samples were prepared by growing the bacteria in minimal media containing $^{15}NH_4Cl$ either with or without $^{13}C_6$-glucose. For selectively enriched samples (^{15}N-Val, ^{15}N-Leu, ^{15}N-Phe, and ^{15}N-Gly), the minimal medium consisted of 300 to 1000 mg l^{-1} of the isotopically enriched amino acids and all other amino acids. The protein was renatured from *E. coli* inclusion bodies as previously published (Jaenicke and Rudolph, 1986). Refolded human MIA was applied to hydrophobic interaction chromatography using Toyopearl Butyl-650S (Tosohaas) and was further purified on a S-Sepharose Fast Flow column (Sigma). Finally, a gel filtration was performed on a Superdex 200 Prep Grade (Sigma). Fractions containing human MIA were pooled and concentrated. Typically, NMR samples contained up to 1 mM of protein in 100 mM potassium phosphate and 150 mM NaCl at pH 7.0, including 0.02% NaN_3 and protease inhibitors (Roche Molecular Biochemicals). All pro-

*To whom correspondence should be addressed.

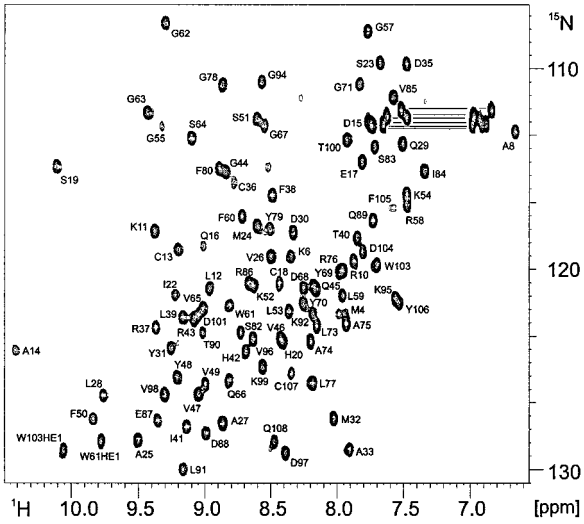

Figure 1. ^{1}H-^{15}N-HSQC spectrum of uniformly ^{15}N-enriched human MIA at 300 K and pH 7.0. The assignment of each resonance is indicated next to the corresponding signal. Signals connected by horizontal lines correspond to side chain amide groups of glutamine residues.

tein samples used for NMR spectroscopy contained 10% or 100% D_2O.

NMR experiments were carried out at 300 K on Bruker AMX 500, DRX 500, DRX 600 and DMX 750 spectrometers. All spectrometers were equipped with triple resonance (^{1}H, ^{13}C, ^{15}N) probeheads and pulsed-field gradient (PFG) accessories. For backbone assignment triple resonance experiments CBCA(CO)NH, CT-HNCA and CT-HNCO were recorded. Triple resonance experiments, 2D TOCSY (τ_m = 53 ms), 3D ^{1}H-^{15}N TOCSY-HSQC (τ_m = 50 ms), long mixing time 2D NOESY (τ_m = 120 ms), and long mixing time 3D ^{1}H-^{15}N NOESY-HSQC (τ_m = 120 ms) experiments in H_2O were recorded in a water flip-back version, suppressing the water signal with the WATERGATE scheme as described previously (Rosinke et al., 1997). Isotropic mixing was performed with DIPSI-2 or MLEV-17 spin-lock sequences. Side-chain resonances were assigned using 2D NOESY (τ_m = 120 ms) in D_2O, 3D ^{1}H-^{15}N TOCSY-HSQC, and 3D ^{1}H-^{15}N NOESY-HSQC experiments. Residual water in experiments measured on samples in D_2O was removed by application of a weak presaturation field. 2D ^{1}H-^{15}NHSQC spectra with reduced signal loss due to fastchemical exchange were recorded using procedures described by Mori et al. (1995). Resolution in the indirect dimensions was increased by linear prediction and zero-filling. Assignment was performed using our software NMRXplorer, which is based on CC-NMR (Kalus et al., 1998).

Extent of assignments and data deposition

Figure 1 shows the ^{1}H-^{15}N-HSQC spectrum of human MIA. Resonances of all backbone atoms were assigned with the exception of the amide protons of G2, L7, D9, R56, D72, and K102, which were not observed in the NMR spectra. Resonances of most aliphatic and aromatic side chain protons could also be assigned unambiguously.

A table of the ^{1}H, ^{15}N, and ^{13}C chemical shift assignments of human MIA has been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number 2411.

Acknowledgements

R.S. gratefully recognizes support from the Studienstiftung and the Fonds der Chemischen Industrie. This work was supported by DFG grants (SFB 413 and BO 1573/1-1) and the Deutsche Krebshilfe (10-1532-Bo).

References

Blesch, U., Bosserhof, A.K., Apfel, R., Behl, C., Hessdörfer, B., Schmitt, A., Jachimczak, P., Lottspeich, F., Buettner, R. and Bogdahn, U. (1994) *Cancer Res.*, **54**, 5695–5701.

Bosserhoff, A.K., Kondo, S., Moser, M., Dietz, U., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Buettner, R. and Sandell, L.J. (1997) *Dev. Dyn.*, **208**, 516–525.

Bosserhoff, A.K., Lederer, M., Kaufmann, M., Hein, R., Stolz, W., Apfel, R., Bogdahn, U. and Buettner, R. (1999a) *Anticancer Res.*, **19**, 2691–2694.

Bosserhoff, A.K., Golob, M., Hein, R. and Buettner, R. (1999b) *J. Invest. Dermatol.*, **113**, 74.

Jaenicke, R. and Rudolph, R. (1986) *Methods Enzymol.*, **131**, 218–250.

Kalus, W., Zweckstetter, M., Renner, C., Sanchez, Y., Georgescu, J., Grol, M., Demuth, D., Schumacher, R., Dony, C., Lang, K. and Holak, T.A. (1998) *EMBO J.*, **17**, 6558–6572.

Rosinke, B., Renner, C., Mayr, E.M., Jaenicke, R. and Holak, T.A. (1997) *J. Mol. Biol.*, **271**, 645–655.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.