Journal of Biomolecular NMR **29:** 547–548, 2004.
© 2004 Kluwer Academic Publishers. Printed in the Netherlands.


# Letter to the Editor: $^{1}$H, $^{13}$C and $^{15}$N resonance assignments of human 5,10-methenyltetrahydrofolate synthetase

Evelyne H. Copeland[a], Irena Ekiel[b,c] & Miroslaw Cygler[a,c,*]
[a]*Department of Biochemistry, McGill University, Montréal, Québec H3G 1Y6, Canada*
[b]*Department of Chemistry and Biochemistry, Concordia University, Montréal, Québec, H3G 1M8, Canada*
[c]*Biotechnology Research Institute, NRC, 6100 Royalmount Avenue, Montréal, Québec H4P 2R2, Canada*

Received 4 March 2004; Accepted 9 April 2004

*Key words:* folic acid, methenyltetrahydrofolate synthetase, triple resonance experiments,

## Biological context

Folate-mediated one-carbon metabolism plays a crucial role in several major cellular processes. The thermodynamically stable 5-formyltetrahydrofolate (5-FTHF, leucovorin), which is not utilized as a one-carbon donor is transferred back to the folate pool by the 5,10-methenyltetrahydrofolate synthetase (MTHFS), the only enzyme that catalyzes its conversion to other reduced folates. The role of human MTHFS, a 202 amino acid protein of 23 kDa is potentially important in the treatment of certain cancers. 5-FTHF has been administered in chemotherapy to rescue normal cells from high dose levels of the antifolate methotrexate (Jolivet et al., 1983). 5-FTHF was also used to potentiate the antitumor activity of fluorouracil, a potent inhibitor of thymidylate synthase, the enzyme involved in deoxyuridylate methylation (Machover et al., 1982).

Endogenously, 5,10 methenyl-THF can be converted to 5-FTHF by a 'side' reaction catalyzed by serine hydroxymethyltransferase (SHMT). SHMT is involved in the generation of glycine and one-carbon units. The polyglutamated form of 5-FTHF is an inhibitor of SHMT and may regulate SHMT activity (Stover and Schirch, 1990). Through modulation of intracellular levels of 5-FTHF polyglutamates, which are potent inhibitors of many folate-dependent enzymes, MTHFS may regulate folic acid-dependent reactions of amino acid, purine and pyrimidine synthesis. This enzyme is a potential target for pharmaceutical applications in the manipulation of cell growth and development, which are significant in cancer prevention and treatment. We have undertaken structural studies of human MTHFS in solution using NMR spectroscopy in order to gain insight into the reaction mechanism, cofactor binding and role of $Mg^{2+}$. Here we report the first assignment of $^{1}$H, $^{13}$C and $^{15}$N chemical shifts for hMTHFS as a basis for the determination of its three-dimensional structure and characterization of dynamic processes within these protein-ligand complexes.

A truncated 197-residue construct of MTHFS, which retains full enzymatic activity, was used in this NMR study. Sequence alignment with homologous proteins showed that the mammalian protein contains a hydrophobic pentapeptide on the N-terminus. Solubility conditions were optimized for the deletion mutant, $MTHFS_{6-202}$, in which these five amino acids were removed.

## Methods and experiments

Human $MTHFS_{6-202}$ was cloned into the *Nde*I/*Bam*HI restriction sites of the pET 11c vector (Stratagene) and expressed in *E. coli* BL21 (DE3) cells carrying plasmid pLysS. Uniformly $^{15}$N- as well as $^{15}$N-, $^{13}$C-labeled protein was grown in $^{15}$N-$NH_4Cl$ and $^{13}$C-glucose enriched M9 minimal media supplemented with thiamine and trace metals. The expressed protein was purified by affinity chromatography and eluted by substrate competition. Column preparation included coupling of substrate carboxyl groups to primary amines on Poros NH support. Samples for NMR measurements contained 1 mM to 1.5 mM MTHFS in 20 mM pipes buffer pH 6.5, 100 mM KCl, 15 mM 2-mercaptoethanol and 1.5 mM leucov-

*To whom correspondence should be addressed. Biotechnology Research Institute, NRC, 6100 Royalmount Avenue, Montréal, Québec H4P 2R2, Canada. E-mail: mirek@bri.nrc.ca

*Figure 1.* $^{1}H$-$^{15}N$ HSQC spectrum of the 23 kDa human MTHFS collected at 30 °C, pH 6.5. For clarity, only a portion of residue-specific assignments of backbone amide groups is indicated. Side-chain amide cross peaks are connected by lines and other side-chain correlations are annotated (sc).

orin with the addition of $^{2}H_2O$ to 10% v/v. NMR experiments were carried out at 30 °C either on the Bruker DRX500, Bruker DRX800 or Varian UNITY plus 800 MHz spectrometers. NMR data were processed with GIFA (Pons et al., 1996) and NMRPipe software (Delagio et al., 1995) and analyzed using a combination of XEASY (Bartels et al., 1995) and NMRView (Johnson and Blevins, 1994). Backbone and side-chainresonances were assigned using 3D-HNCACB, CBCA(CO)NH, HN(CA)CO, HNCO, HNCA, HNHA, HCCH-COSY, NOESY-HSQC (mixing time 90 ms), and HCCH-TOCSY (mixing time 18 ms) experiments. DSS was used as a chemical-shift reference standard at 0 ppm to calibrate proton chemical shifts (in ppm). $^{15}N$ and $^{13}C$ shifts were referenced indirectly assuming the gyromagnetic ratios of $\gamma^{15}N/\gamma^{1}H = 0.101329118$ and $\gamma^{13}C/\gamma^{1}H = 0.251449530$ (Wishart et al., 1995).

## Extent of assignments and data deposition

Analysis from triple resonance experiments provided the resonance assignments for MTHFS. Degenerate resonances were confirmed by sequential NOE data. 99% completeness was obtained for amide $^{1}H$ and $^{15}N$ backbone resonance assignments missing only for residues S2 and Y147. In total, 98% of $^{1}H^{\alpha}$, 95% of $^{1}H^{\beta}$, 98% of $^{13}C^{\alpha}$ and $^{13}C^{\beta}$, and, 96% of $^{13}CO$ resonances were assigned. Figure 1 shows the $^{1}H$-$^{15}N$ HSQC spectrum of uniformly $^{15}N$ labeled MTHFS (197 residues). Mostly complete assignment of side-chain $^{1}H$ resonances was obtained with over 85% assignment of nonlabile $^{1}H$ resonances. Main chain and side-chain $^{1}H$, $^{13}C$ and $^{15}N$ resonances of hMTHFS have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5983.

## Acknowledgements

We would like to thank the Biotechnology Research Institute, NRC and Canadian National High Field NMR Centre (NANUC) for use of their facilities, Dr K. Gehring and the members of his lab for their assistance and Dr R.E. MacKenzie for critical comments. This work was supported by an NSERC grant to MC.

## References

Bartels, C., Xia, T-H., Billeter, M., Güntert, P. and Wüthrich K. (1995) *J. Biomol. NMR*, **5**, 1–10.

Delagio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Johnson, B.A. and Blevins, R.A. (1994) *J. Biomol. NMR*, **4**, 603–614.

Jolivet, J., Cowan, K.H., Curt, G.A., Clendamin, N.J. and Chabner, B.A. (1983) *N. Eng. J. Med.*, **309**, 1094–1104.

Machover, D., Schwarzenberg, L., Goldschmidt, E., Tourani, J.M., Michalski, B., Hayat, M., Dorval, T., Misset, J.L., Jasmin, C., Maral, R. and Mathe, G. (1982) *Cancer Treatment Rep.*, **66**, 1803–1807.

Pons, J.L., Malliavin T.E. and Delsuc M.A. (1996) *J. Biomol. NMR*, **8**, 445–452.

Stover, P. and Schirch, V. (1990) *J. Biol. Chem.*, **265**, 14227–14233.

Wishart, D.S., Bigam, C.G., Yao, J., Abildgaard, F., Dyson, J., Oldfield, E., Markley, J.L. and Sykes, B.D. (1995) *J. Biomol. NMR*, **6**, 135–140.