Journal of Biomolecular NMR, **14:** 189–190, 1999.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of *Escherichia coli* 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase and its complex with MgAMPPCP

Genbin Shi, Jinhai Gao & Honggao Yan*
Department of Biochemistry, Michigan State University, East Lansing, MI 48824, U.S.A.

Received 5 February 1999; Accepted 3 March 1999

Key words: antibiotic resistance, 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase, resonance assignment

Biological context

6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) catalyzes the transfer of pyrophosphate from ATP to 6-hydroxymethyl-7,8-dihydropterin, the first reaction in the folate biosynthetic pathway. Folate cofactors are essential for life (Blakley and Bekovic, 1984). Mammals are unable to synthesize folates but have an active transport system for deriving folates from diets. In contrast, most microorganisms must synthesize folates de novo because they lack the active transport system. Thus, the folate biosynthetic pathway is an ideal target for development of antimicrobial agents. Inhibitors of dihydropteroate synthase and dihydrofolate reductase, both of which are enzymes in the folate biosynthetic pathway, are currently used as antibiotics for treating many infectious diseases (O'Grady et al., 1997). However, the rates of resistance to these antibiotics have increased dramatically during the past two decades (Huovinen, 1997). The rapid increase in resistance to these and other antimicrobial agents has caused a worldwide health care crisis (Murray, 1997).

HPPK was first identified in *E. coli* in 1964 (Weisman and Brown, 1964). The gene for *E. coli* HPPK was cloned in 1992 (Talarico et al., 1992). The *E. coli* enzyme is highly homologous to HPPKs from many pathogenic microbial organisms. For example, it shares ∼56% identity with *H. influenzae* HPPK, ∼40% identity with *P. carinii* HPPK/DHPS and ∼33% identity with *M. tuberculosis* HPPK. A small (158 residues, ∼18 kDa), stable, monomeric protein, *E. coli* HPPK is not only a new target for development of antimicrobial agents but also an excellent model system for studying the mechanisms of pyrophosphoryl transfer. In this report, we describe the nearly complete sequential resonance assignments of unliganded HPPK and its complex with the inhibitor MgAMPPCP obtained by multinuclear multidimensional NMR spectroscopy.

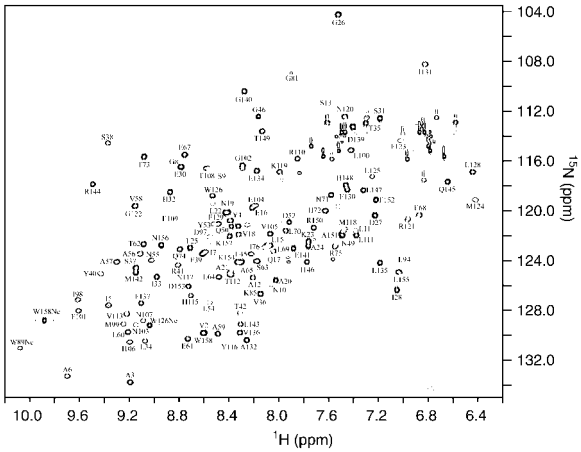

Figure 1. The ^{1}H-^{15}N HSQC spectrum of unliganded HPPK recorded at 25 °C and a ^{1}H frequency of 600 MHz with coherence selection by pulsed field gradients and sensitivity enhancement. The NMR sample contained ∼1.5 mM HPPK in 20 mM sodium phosphate buffer, pH 7.4. Sequential assignments are indicated with the one-letter amino acid code and residue number.

Methods and results

Uniformly ^{15}N-labeled and ^{15}N/^{13}N C-labeled HPPK proteins were produced by growing the *E. coli* strain BL21(DE3) containing the overexpression construct pET-17b/HPPK in M9 media containing $^{15}NH_4Cl$ and

*To whom correspondence should be addressed. E-mail: yanh@pilot.msu.edu.

$^{15}NH_4Cl/[^{13}C_6]$-D-glucose, respectively. The *E. coli* strain DL49HPS (kindly provided by Dr. D.M. LeMaster), containing the plasmid pLysS and the overexpression construct pET-17b/HPPK, was used for the selective ^{15}N labeling. The *E. coli* strain was grown in a rich medium containing a ^{15}N-amino acid (Muchmore et al., 1989). Selective ^{15}N labeling was performed for the following amino acids: Ala, Gly, Ile, Leu, Phe and Val. Production of HPPK was induced in all cases by addition of isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 0.33 mM when the OD_{600} of the culture reached 0.8. The proteins were purified and lyophilized as described elsewhere (G.S. and H.Y., in preparation).

NMR samples were prepared by dissolving lyophilized HPPK in 20 mM sodium phosphate, pH 7.4 (pH meter reading without correction for isotope effects), in H_2O/D_2O (9/1). The protein concentration was ∼1.5 mM. The complex samples contained 40 mM $MgCl_2$ and 8 mM AMPPCP. All NMR data were acquired at 25 °C on a Varian Inova 600 spectrometer with a triple-resonance probe head. The NMR experiments performed have been described in recent excellent reviews (Bax and Grzesiek, 1993; Kay, 1995), including sensitivity-enhanced 2D ^1H-^{15}N HSQC, 3D ^{15}N-edited NOESY-HSQC (150 ms mixing time), HNCA, HNCACB, CBCA(CO)NH, HNCO and HCCH-TOCSY. All the NMR data were processed and analyzed on a Sun Ultra 1 or an SGI Indigo 2 workstation as previously described (Wang and Yan, 1998). The sequential resonance assignments were established by the combined analysis of the double- and triple-resonance NMR data of uniformly labeled HPPKs. The assignments were also facilitated and confirmed by six selective ^{15}N labeling experiments. The sequential resonance assignment of unliganded HPPK was assisted by the resonance assignment of the HPPK·MgAMPPCP complex because many weak peaks of the unliganded enzyme became much stronger upon binding of MgAMPPCP. Figure 1 shows the ^1H-^{15}N HSQC spectrum of unliganded HPPK.

Extent of assignments and data deposition

For unliganded HPPK, eight residues have not been assigned. These unassigned residues are T1, P14, P43, Q79, Q80, A86, R92 and L96. Seven ^1H-^{15}N HSQC cross peaks involving main-chain amides have not been assigned. Twelve main-chain amides do not show cross peaks in the HSQC spectrum. Of the 19 unassigned or missing amides, 18 are located in the segment E77-L96, a long loop as revealed by a 1.5 Å resolution crystal structure (Xiao et al., manuscript submitted). For the HPPK·MgAMPPCP complex, five residues have not been assigned. These residues are T1, P14, P43, Q80 and R82. Four main-chain amide HSQC cross peaks have not been assigned. One main-chain amide does not show up in the ^1H-^{15}N HSQC spectrum. The unassigned or missing main-chain amides are those of Q48, Q80, R82, W9, T112 and Y116. More than 90% of the side-chains have been assigned for both forms of the enzyme. The assignments have been deposited in the BioMagResBank database under the code numbers 4299 and 4300.

Acknowledgements

This work was supported in part by an NIH grant (GM51901). This study made use of a Varian INOVA-600 NMR spectrometer at Michigan State University funded in part by NSF Grant BIR9512253.

References

Bax, A. and Grzesiek, S. (1993) *Acc. Chem. Res.*, **26**, 131–138.
Blakley, R.L. and Bekovic, S.J. (Eds.) (1984) *Chemistry and Biochemistry of Folates*, Vol. 1: *Folates and Pterins*, Wiley, New York, NY.
Huovinen, P. (1997) *Clin. Infect. Dis.*, **24**, S63–66.
Kay, L.E. (1995) *Prog. Biophys. Mol. Biol.*, **63**, 277–299.
Muchmore, D.C., McIntosh, L.P., Russell, C.B., Anderson, D.E. and Dahlquist, F.W. (1989) *Methods Enzymol.*, **177**, 44–73.
Murray, B.E. (1997) *Ad. Intern. Med.*, **42**, 339–367.
O'Grady, F., Lambert, H.P., Finch, R.G. and Greenwood, D. (Eds.) (1997) *Antibiotic and Chemotherapy: Anti-Infective Agents and Their Use in Therapy*, 7th ed., Churchill Livingstone, New York, NY.
Talarico, T.L., Ray, P.H., Dev, I.K., Merrill, B.M. and Dallas, W.S. (1992) *J. Bacteriol.*, **174**, 5971–5977.
Wang, L. and Yan, H. (1998) *Biochemistry*, **37**, 13021–13032.
Weisman, R.A. and Brown, G.M. (1964) *J. Biol. Chem.*, **239**, 326–331.