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Letter to the Editor: Assignment of the ^{1}H, ^{13}C and ^{15}N resonances of the LpxC deacetylase from *Aquifex aeolicus* in complex with the substrate-analog inhibitor TU-514

Brian E. Coggins[a], Xuechen Li[b], Ole Hindsgaul[b], Christian R.H. Raetz[a] & Pei Zhou[a,*]
[a]*Department of Biochemistry, Duke University Medical Center, Durham, NC 27710, U.S.A.;* [b]*Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2*

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Biological context

Lipopolysaccharides are anchored into the outer monolayer of the outer membrane of Gram-negative bacteria via the hydrophobic moiety lipid A (endotoxin), which is produced by a nine step biosynthetic pathway that is conserved across a variety of Gram-negative organisms, and is required for their survival and growth. The second step in the biosynthesis of lipid A is catalyzed by LpxC, which deacetylates UDP-3-*O*-acyl-*N*-acetylglucosamine. Because LpxC activity is crucial for the survival of Gram-negative bacteria, and because LpxC shows no sequence homology to known mammalian enzymes, it has been identified as an excellent target for the development of novel antibiotics (Raetz and Whitfield, 2002). A variety of LpxC inhibitors have been described which bind to LpxC's catalytic Zn^{2+} through a hydroxamate group, and a number of these have bactericidal activity. Although several 'broad-spectrum' inhibitors have been reported for LpxC *in vitro*, those that have significant bactericidal activity show strong species specificity, presumably on account of subtle differences in active site structures across the LpxC family (Onishi et al., 1996; Jackman et al., 2000).

The LpxC enzyme from the hyperthermophilic bacterium *Aquifex aeolicus* is particularly well suited for structural study by nuclear magnetic resonance (NMR) spectroscopy as it is the smallest member of the LpxC family, at 32 kDa or 282 residues, with by far the greatest thermal stability. The substrate-analog inhibitor TU-514 (3,6-anhydro-3-deoxy-*N*-hydroxy-3-*C*-hydroxymethyl-4-*O*-myristoyl-D-gluco-heptonamide) is the most potent against the *A. aeolicus* enzyme of those reported and is a broad spectrum substrate analog inhibitor (Jackman et al., 2000).

As a first step towards the development of broad-spectrum LpxC inhibitors, we report the ^{1}H, ^{13}C and ^{15}N assignments of the *A. aeolicus* LpxC in complex with the inhibitor TU-514.

Methods and experiments

The recombinant vector pAaLpxC containing the *A. aeolicus* LpxC gene on a pET21 plasmid (Jackman et al., 2000) was expressed in *E. coli* BL21(DE3)STAR cells (Invitrogen) grown in M9 minimal medium. Samples were prepared with [*U*-^{15}N], [*U*-$^{13}C/^{15}N$], [*U*-$^{2}H/^{13}C/^{15}N$], [50%-^{2}H, *U*-$^{13}C/^{15}N$] and [10%-^{13}C] labeling, using ^{13}C-glucose and $^{15}NH_4Cl$ as the sole carbon and nitrogen sources, respectively, with D_2O and deuterated ^{13}C-glucose used in deuterated samples (isotopically-labeled compounds were obtained from Cambridge Isotopes Laboratory). Samples were also prepared in which only Val, Ile, Leu, Lys, Phe or Tyr were labeled with ^{15}N, following the labeling protocol of LeMaster and Richards (1988). LpxC was purified by anion-exchange chromatography (Q-Sepharose Fast Flow, Amersham Biosciences) followed by size-exclusion chromatography (Sephacryl S-200 HR, Amersham Biosciences). An estimated equal amount of TU-514 was added to purified AaLpxC in dilute solution, and

*To whom correspondence should be addressed. E-mail: peizhou@biochem.duke.edu

the mixture was left for one hour to allow the complex to form. All samples were buffer-exchanged into 25 mM sodium phosphate, pH 6.5, with 150 mM KCl, 4 mM dithiothreitol, 5% D_2O and 5% DMSO. Additional TU-514 (∼10% excess) was then added to ensure that all LpxC remained bound. Samples contained from 0.3 to 0.5 mM LpxC in volumes ranging from 300 to 500 µl.

NMR experiments were conducted at 50 °C using Varian INOVA 600 and 800 MHz spectrometers. The following experiments were recorded for backbone assignment: ^{1}H-^{15}N HSQC, HNCA, HN(CO)CA, HN(CA)CB, HN(COCA)CB and HNCO (Ferentz and Wagner, 2000). A series of ^{1}H-^{15}N HSQC experiments were also recorded using the samples labeled by residue type. Sidechain resonances were assigned using HN(COCA)HA, 3D ^{15}N-TOCSY-HSQC, H(CCO)NH-TOCSY, ^{15}N-NOESY-HSQC, ^{13}C-NOESY-HSQC and HCCH-TOCSY experiments (Clore and Gronenborn, 1998). Aromatic resonances were assigned using 2D-homonuclear NOESY, 2D-homonuclear TOCSY and ^{13}C-aromatic NOESY-HSQC experiments (Clore and Gronenborn, 1998). A ^{1}H-^{13}C HSQC of the 10%-^{13}C sample was used for stereospecific assignment of Val and Leu methyl groups (Szyperski et al., 1992). ^{1}H chemical shifts were referenced to DSS, and ^{15}N and ^{13}C chemical shifts were referenced indirectly by multiplying the ^{1}H carrier frequency by 0.101329118 and 0.25144953, respectively. Spectral data were processed using FELIX (Accelrys) and analyzed in XEASY (Bartels et al., 1995). Sequential assignments were carried out by using the PACES program (Coggins and Zhou, 2003), and were confirmed by manual analysis and by reference to NOESY data. F1/F2 ^{15}N/^{13}C-filtered 2D NOESY experiments were used to assign TU-514 while bound to ^{2}H/^{13}C/^{15}N-labeled protein (Clore and Gronenborn, 1998).

Extent of assignment and data deposition

The ^{1}H-^{15}N HSQC spectrum is shown in Figure 1. Backbone resonances have been assigned for the entire protein except M1, G2, S59, R68, S102, L200 and K208. C^{β} resonance assignments could not be made for 24 out of 255 non-glycine residues. Aliphatic sidechain ^{1}H and ^{13}C resonances assignments were obtained for over 90% of the residues. Partial (Hδ) or complete aromatic proton resonance assignments have been determined for all the aromatic sidechains

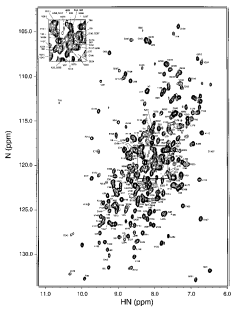

Figure 1. ^{1}H-^{15}N HSQC spectrum of *A. aeolicus* LpxC complexed with TU-514 at 50 °C. The central region, denoted by the box, has been enlarged in the top left corner. The resonances of G43 and G251 are folded in the nitrogen dimension.

including 16 Phe, 9 Tyr, and 2 Trp. The ^{1}H, ^{13}C and ^{15}N resonance assignments of *A. aeolicus* LpxC and the ^{1}H assignments of TU-514 have been deposited in the BioMagResBank with accession number 5627. All carbon chemical shifts have been corrected for deuterium isotope effects.

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