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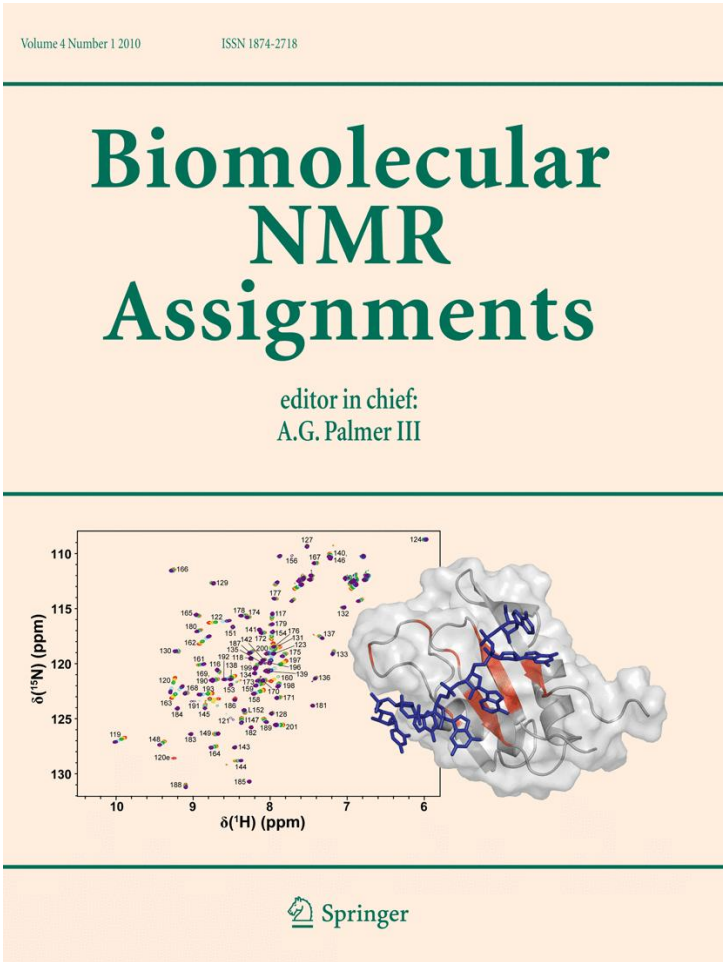

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ARTICLE

Assignment of ^{1}H, ^{13}C and ^{15}N backbone resonances of *Escherichia coli* LpxC bound to L-161,240

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Abstract The UDP-3-*O*-(*R*-3-hydroxymyristoyl)-*N*-acetylglucosamine deacetylase LpxC catalyzes the committed reaction of lipid A biosynthesis, an essential pathway in Gram-negative bacteria. We report the backbone resonance assignments of the 34 kDa LpxC from *Escherichia coli* in complex with the antibiotic L-161,240 using multidimensional, multinuclear NMR experiments. The ^{1}H chemical shifts of complexed L-161,240 are also determined.

Keywords *Escherichia coli* · Antibiotic · Lipid A · Inhibitor · Deacetylase

Biological context

Lipid A, the membrane anchor of lipopolysaccharide, is an essential molecule synthesized by nine enzymes in the cytosol and on the inner surface of the inner membrane of Gram-negative bacteria (Raetz and Whitfield 2002). The committed reaction of lipid A biosynthesis in *Escherichia coli* is catalyzed by the cytosolic enzyme LpxC, a UDP-3-*O*-(*R*-3-hydroxymyristoyl)-*N*-acetylglucosamine deacetylase that consists of 305 amino acids. Recent structural studies of LpxC from the thermophilic bacterium *Aquifex aeolicus* revealed that LpxC is a unique enzyme with no obvious structural similarity to other proteins (Coggins et al. 2003; Whittington et al. 2003; Barb and Zhou 2008).

LpxC is a target for drug design, and many potent LpxC inhibitors display good antibiotic activity. L-161,240, the first potent inhibitor of *E. coli* LpxC (K_i = 50 nM) reported in the literature, has antibacterial activity comparable to that of ampicillin (Onishi et al. 1996). Unfortunately, L-161,240 has a relatively narrow spectrum of inhibition with regard to diverse LpxC orthologues, and it does not inhibit the growth of *Pseudomonas aeruginosa,* the primary cause of fatality in cystic fibrosis patients. Understanding the molecular details of the *E. coli* LpxC–L-161,240 interaction should facilitate the further optimization of L-161,240 and the design of more potent derivatives to expand the spectrum of inhibition (Barb et al. 2007).

Methods and experiments

L-161,240 was prepared as previously described (Jackman et al. 2000). Stable isotopes were purchased from Cambridge Isotope Laboratory (Andover, MA).

LpxC protein was expressed and purified as previously described (Coggins et al. 2003) from a pET-21a plasmid encoding the full-length, wild-type *E. coli* LpxC. Following purification, perdeuterated LpxC was incubated in 25 mM sodium phosphate, 150 mM KCl, pH 7.0 at 37°C for at least 2 days to facilitate amide back-exchange. The

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LpxC–L-161,240 complex was prepared by incubating purified LpxC with a 1.2-fold molar excess of L-161,240 (dissolved in DMSO) in 25 mM sodium phosphate, 150 mM KCl, 10% DMSO, pH 7.0 overnight at room temperature. This complex was purified using a Sephacryl S-200 HR column (GE Healthcare) pre-equilibrated with 20 mM sodium phosphate pH 6.25, 100 mM KCl, 2 mM dithiothreitol. NMR samples were prepared in this buffer with 10% or 100% D_2O and contained 0.8–1.0 mM LpxC or LpxC–L-161,240 complex.

NMR data were collected on 600 and 800 MHz Varian (Palo Alto, CA) Inova spectrometers equipped with triple-resonance, cryogenically-cooled probes at 37°C. FIDs were processed using NMRPIPE (Delaglio et al. 1995) and datasets analyzed using XEASY (Bartels et al. 1995).

Assignments and data deposition

The ^{15}N-HSQC-TROSY spectrum of free *E. coli* LpxC was of poor quality (data not shown), but was markedly improved by the addition of L-161,240 (Fig. 1a). A titration of LpxC with L-161,240 indicated that this complex was in the slow exchange regime on the NMR time scale (data not shown), which is consistent with the ~50 nM K_d for the L-161,240–LpxC complex (Onishi et al. 1996).

Most backbone amide resonances for the *E. coli* LpxC–L-161,240 complex could be observed using uniformly ^{2}H/^{13}C/^{15}N-labeled LpxC with standard TROSY-based triple-resonance experiments, including HNCA (Fig. 1b), HN(CO)CA, HN(CA)CB, HN(COCA)CB, HNCO (Salzmann et al. 1999) and 'just-in-time' HN(CA)CO (Werner-Allen et al. 2006). Amide resonances of specific amino acid types were identified in a ^{15}N-HSQC-TROSY spectrum using LpxC containing ^{15}N-labeled Lys (15 in *E. coli* LpxC), Leu (29), Val (22) or Ile (19). The PACES algorithm was used to identify resonance connectivity and predict the backbone dihedral angles based on the chemical shift values (Coggins and Zhou 2003).

Because the slow back-exchange of amide protons in a uniformly ^{2}H/^{13}C/^{15}N-labeled protein limited the number of observable resonances within the core of the protein, we

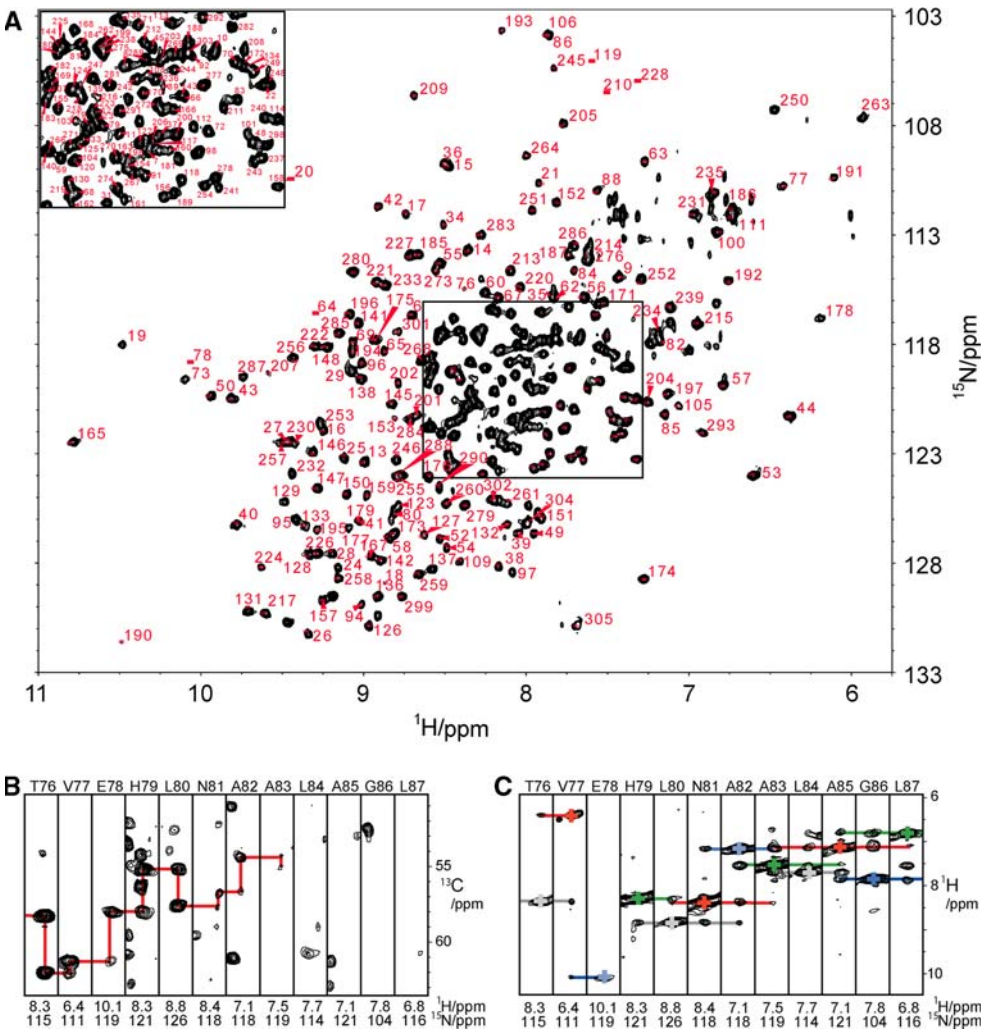

Fig. 1 ^{15}N-TROSY-HSQC spectrum of **a** the *E. coli* LpxC—L-161,240 complex at 800 MHz. Many backbone assignments were obtained from TROSY-based triple-resonance experiments including a TROSY-HNCA experiment (**b**). Remaining resonances were identified using a ^{15}N-separated NOESY-TROSY-HSQC experiment (**c**). Connectivity in **b** is displayed with a *thick red line*. Amide self peaks (*diagonals*) in **c** are shown with a *colored "+" symbol*, and related cross peaks in adjacent strips are connected with a *thick line of the same color* as the marker. The amino acid identities are shown at the *top* of the figure, and the corresponding ^{1}H and ^{15}N chemical shifts are at the *bottom* of each strip

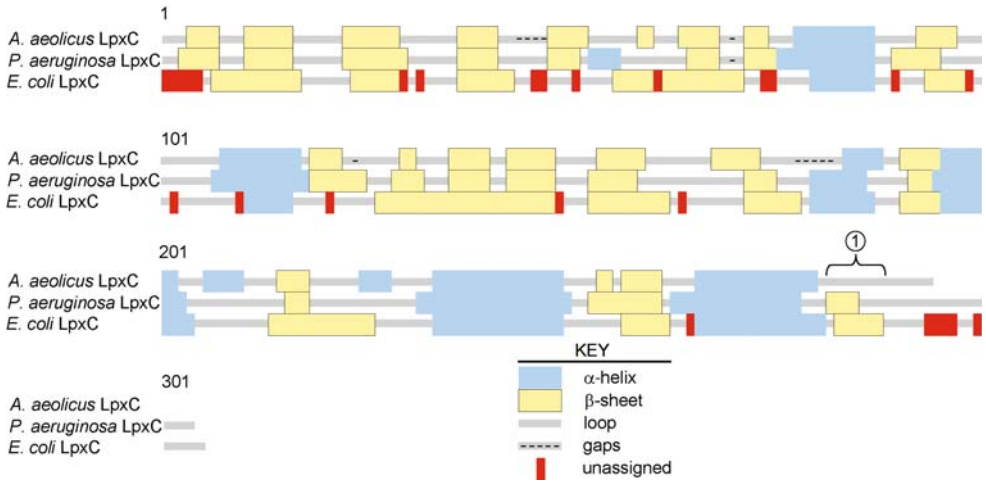

Fig. 2 Secondary structure from the reported *A. aeolicus* LpxC (Coggins et al. 2003) and *P. aeruginosa* LpxC (Mochalkin et al. 2008) structures are compared with the predicted secondary structure of *E. coli* LpxC. An additional β-strand observed in *P. aeruginosa* LpxC and predicted for *E. coli* LpxC is marked at position 1. Gaps in the structural alignment are indicated with a "-". Unobservable (Pro, *N*-terminal Met) or unassigned amide resonances of *E. coli* LpxC are indicated with *red rectangles*. The numbers correspond to the *E. coli* LpxC amino acid sequence

collected a second set of TROSY-HNCA and TROSY-HN(CO)CA experiments using uniformly $^{13}C/^{15}N$-labeled LpxC in complex with L-161,240. Hα resonances were assigned using a TROSY-HN(CA)HA experiment (Hu et al. 2003). Additional assignments were made using a three dimensional ^{15}N-separated NOESY-TROSY-HSQC experiment (Fig. 1c).

As a result of the backbone resonance assignment, 282 of the 292 amide resonances (96.6%) were assigned. Additionally, 90.2% of the Cα, 78.0% of the Hα, 86.4% of the Cβ and 85.9% of the CO resonances have been assigned. L-161,240 resonances in the LpxC-bound complex were assigned using a two dimensional ^{1}H–^{1}H homonuclear NOE experiment recorded with a sample containing ^{2}H-labeled LpxC and unlabeled L-161,240 in a 100% D_2O NMR buffer (Figure S1). These chemical shifts have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under BMRB accession number 16475.

The secondary structure prediction based on the backbone chemical shifts of *E. coli* LpxC shows considerable similarity to the observed secondary structure of *A. aeolicus* LpxC and *P. aeruginosa* LpxC, though many β-strands are predicted to be longer than observed homologous counterparts (Fig. 2). A β-strand from residues 283 to 288 (as indicated in Fig. 2) is predicted that is not present in *A. aeolicus* LpxC, but is present in *P. aeruginosa* LpxC. The role of this additional β-strand remains to be investigated.

Protein stability and mutation analyses indicate *E. coli* LpxC has a disordered C-terminal tail essential for regulation of LpxC through FtsH-mediated degradation (Fuhrer et al. 2006). Consistent with these observations, the five C-terminal residues appear highly dynamic, as judged by nitrogen and carbon linewidths that are considerably narrower than the majority of the LpxC resonances (data not shown), and likely form no stable secondary structural elements. *A. aeolicus* LpxC and *P. aeruginosa* LpxC accordingly have disordered C-termini (Whittington et al. 2003; Coggins et al. 2005; Mochalkin et al. 2008).

Unlike the C-terminus, the majority of the L-161,240 resonances are characterized by linewidths similar to what is expected for a $\sim$34 kDa complex, suggesting L-161,240 binds with one dominant conformation (Figure S1); however, the methyl-esters at positions 19 and 21 have signals that are considerably broader (45 Hz linewidths) than the propyl methyl signal at position 17 (25 Hz linewidth), which may be indicative of conformational heterogeneity of L-161,240 at these positions or interaction with LpxC side-chains in this region.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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