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¹H, ¹³C and ¹⁵N assignments of the holo-acyl carrier protein of *Pseudomonas aeruginosa*

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Abstract

Acyl carrier proteins (ACPs) are a group of highly conserved and abundant proteins in bacteria. ACPs play a central role as the acyl group carriers in bacterial fatty acid biosynthesis, providing building blocks for membrane biogenesis and the production of secondary metabolites. In the versatile human pathogen *Pseudomonas aeruginosa*, three ACP homologs have been identified. One homolog, AcpP, exhibits the strongest sequence homology to the canonical *Escherichia coli* ACP. Here we report the ¹H, ¹³C and ¹⁵N assignments of the holo-AcpP of *P. aeruginosa*.

Keywords

Acyl carrier protein; *Pseudomonas aeruginosa*; Nuclear magnetic resonance; Fatty acid synthesis

Biological context

Fatty acids are produced by iterative cycles of a series of well characterized condensation, dehydration, and reduction reactions that elongate acyl chains (White et al. 2005). In eukaryotes the fatty acid synthase machinery consists of a single polypeptide chain encoding multiple functionalities (Maier et al. 2010). In prokaryotes, however, the machinery is assembled from multiple peptides and this difference has led to efforts to develop antibiotics that target components of the prokaryotic fatty acid synthase system (Zhang et al. 2006). The acyl carrier protein (ACP) is a central component of the fatty acid synthase system that transports acyl chain intermediates between enzymes (White et al. 2005). ACPs are a group of highly conserved and abundant proteins in bacteria (Chan and Vogel 2010). Acyl chains are covalently attached to ACP via a sulfhydryl group at the end of a 4'-phosphopantetheinate prosthetic group that is covalently attached to a conserved serine residue of ACP. The prosthetic group is thought to act like a swinging arm, transferring the fatty acid intermediates between reactive centers.

Pseudomonas aeruginosa is a ubiquitous gram-negative bacterium that can survive in a wide variety of environments due to its low nutritional requirements. It has become one of the most common causes of infections, particularly in immunocompromised individuals and patients with cystic fibrosis (Li et al. 2005). Due to both intrinsic and acquired mechanisms, *P. aeruginosa* exhibits resistance to many current antibiotics, causing difficult-to-treat and untreatable infections (Breidenstein et al. 2011).

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There are three ACP paralogs identified in *P. aeruginosa*, designated as AcpP, Acp1, and Acp3 (Raychaudhuri et al. 2005). The *acpP* gene is located within a cluster of genes encoding essential enzymes of fatty acid synthesis, strongly suggesting AcpP is involved in membrane biogenesis and thus required for survival. Indeed, consistent with AcpP being essential for growth, no AcpP knockout is available from two genome wide transposon-based mutant libraries of *P. aeruginosa* (Jacobs et al. 2003; Liberati et al. 2006). As a first step in the study of AcpP interaction with fatty acid biosynthetic enzymes, we report backbone and sidechain resonance assignments for all residues of the holo-AcpP of *P. aeruginosa*.

Methods and experiments

Protein expression and purification

The expression vector was kindly provided by Dr. Peter Tipton at University of Missouri-Columbia. The coding sequence of AcpP was inserted between the *Nde*I and *Bam*HI restriction sites of pET-14b vector containing an N-terminal polyhistidine tag (Raychaudhuri et al. 2005). The expression vector was transformed into *E. coli* strain BL21(DE3) for overexpression of His-AcpP induced by 0.4 mM IPTG. The uniformly ^{13}C - and ^{15}N -labeled protein was expressed in MOPS minimal medium (Neidhardt et al. 1974) containing 0.1% $^{15}\text{NH}_4\text{Cl}$ and 0.2% ^{13}C -U-glucose. Nickel-NTA agarose was used to purify the His-tagged AcpP with a step gradient of imidazole. The His-tag was cleaved with thrombin leaving a non-native amino terminal extension of three residues, GSH. The residue numbering used here is based on the wild type sequence with the three non-native residues numbered -2, -1 and 0, leaving the initial native methionine residue to be called M1. The cleaved AcpP was further purified by size-exclusion chromatography in buffer containing 40 mM potassium phosphate (pH 6.5) and 1 mM EDTA. Fractions containing the most pure protein were pooled and concentrated.

As more than 90% of the overexpressed AcpP was in the apo form, conversion of apo-AcpP to the holo form was carried out using *E. coli* holo-ACP synthase (AcpS) as described previously (Wu et al. 2009). Briefly, the reaction mixture contained 2 mM apo-AcpP, 10 mM CoA, 2.5 mM DTT, 10 mM MgCl_2 , and 4 μM AcpS in 50 mM Tris (pH 8.5). After incubation at 37°C for 30 min, the His-tagged AcpS was removed by Ni-NTA agarose and the holo-AcpP was purified by size-exclusion chromatography in buffer containing 40 mM potassium phosphate (pH 6.5), 1 mM DTT and 1 mM EDTA. The concentration of the purified holo-AcpP was determined by Bradford method. For NMR spectroscopy a 2 mM solution of the protein was prepared initially in the above buffer with the addition of 5% D_2O . After collection of all the HN based experiments the sample was lyophilized then dissolved in 100% D_2O for the collection of the ^1H - ^{13}C HSQC, (HB)CB(CGCD)HD and HMBC-HSQC spectra.

NMR spectroscopy

NMR spectra were acquired at 298K using a Bruker Avance III 600 MHz spectrometer fitted with a room temperature 5 mm triple resonance (H-C/N/D) probe equipped with z-axis gradients. Topspin 2.1 software (Bruker, Billerica MA) was used to acquire the data, NMRpipe was used for processing, and CCPN Analysis 2.5 for data analysis. Sequential backbone assignments were obtained using ^1H - ^{15}N HSQC, HNCACB, CBCA(CO)NH, (H)CC(CO)NH and H(CCCO)NH experiments. The latter two experiments in conjunction with a ^1H - ^{13}C HSQC were used to extend assignments out the sidechains. Assignments for backbone carbonyl resonances were obtained from a HNCB spectrum, for glutamine and asparagine sidechain amides carbonyls from the (H)CC(CO)NH and H(CCCO)NH spectra, for aromatic protons from (HB)CB(CGCD)HD and ^{15}N edited NOESY-HSQC spectra, and

for methionine methyls from a HMBC-HSQC spectrum. Assignments were verified by analysis of ^{15}N and ^{13}C edited NOESY-HSQC spectra, as well as HNHA and HNHB experiments.

Assignment and data deposition

Figure 1 shows the excellent dispersion of the AcpP backbone amide resonances, typical of a folded protein. Assignments of the amide resonances are indicated. Excluding the three non-native amino terminal residues, assignments were obtained for 76 of 78 backbone N resonances, 76 of 77 backbone HN resonances, 78 of 78 C α resonances, 80 of 80 H α resonances, 75 of 78 C' resonances, and 77 of 77 C β resonances. For E49 the backbone amide proton and nitrogen resonances could not be identified. The missing E49 amide resonance precluded identification of the E48 C' resonance. The C' resonance of I55 was not found because it precedes a proline, and the C' resonance of the carboxyl terminal residue I78 was not identified. The backbone N resonance of P56 was not identified. The ^1H , ^{13}C and ^{15}N assignments have been deposited in the Biological Magnetic Resonance Data bank under accession number 18331.

BMRB validation of the assignments highlighted several unusual chemical shifts, all of which were carefully checked and appear to be correct. The sidechain proton resonances of E50 are shifted to unusually high field (H β 3 1.38, H γ 2 1.57, H γ 3 0.57 ppm). A three-dimensional structure predicted from the chemical shift assignments by the CS23D server (Wishart et al. 2008) has the E50 sidechain placed above the plane of the F51 aromatic ring, where ring current effects may explain the upfield shifts. The Q67 sidechain amide nitrogen chemical shift is also unusually far downfield (N ϵ 2 117.9 ppm), but in this case the CS23D predicted structure did not provide an easy rationalization.

Figure 2 shows an alignment of the *P. aeruginosa* AcpP amino acid sequence with the *E. coli* AcpP sequence. The secondary structure predicted from the *P. aeruginosa* AcpP chemical shifts by TALOS+ (Shen et al. 2009) is shown above the alignment. The secondary structure reported by the PDB of the holo *E. coli* AcpP NMR structure (PDB ID 2K92, Wu et al 2009) is shown below the alignment. The positions of the major helices in both proteins are essentially the same, indicating that the three dimensional structure of *P. aeruginosa* AcpP is very similar to the *E. coli* AcpP structure. TALOS+ predicts a single residue (E54) in β -strand conformation; an obvious artifact, since existence of a β -strand requires more than one residue, which may have been caused by the downfield shifts of the backbone N and C β resonances. The loop between the first and second helix in the *E. coli* AcpP contains two turns, one of which matches the TALOS+ predicted helix in *P. aeruginosa* AcpP. Similarly the final *E. coli* AcpP helix is extended by residues classified as turn and 3_{10} helix which in *P. aeruginosa* AcpP are predicted to be helical by TALOS+. TALOS+ is trained to discriminate between turns and helices but the differences between these two secondary structure elements are likely to be subtle, and they may even interconvert in solution. Identifying the true secondary structure of these residues in *P. aeruginosa* AcpP awaits a full structure determination.

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Figure 1.

^1H - ^{15}N HSQC spectrum of *P. aeruginosa* AcpP labeled with assignments. The crosspeak pairs for the sidechain amides of asparagine and glutamine are linked by horizontal lines. The resonances for R7e (7.34, 119.9 ppm), S26 (7.67, 126.0 ppm), G34 (7.24, 124.5 ppm), and I55 (9.67, 110.0 ppm) are aliased from their true positions.

Figure 2. Alignment and secondary structure of the *P. aeruginosa* AcpP and *E. coli* AcpP sequences. The *P. aeruginosa* AcpP secondary structure was predicted by TALOS+ from the chemical shifts and appears above the sequences. TALOS+ does not discriminate between α and 3_{10} helices and does not predict turns. The *E. coli* AcpP secondary structure was obtained from the holo *E. coli* AcpP NMR structure (Wu et al 2009) using DSSP and appears below the sequences.