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^1H , ^{13}C and ^{15}N resonance assignments and secondary structure analysis of translation initiation factor 1 from *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen and a primary cause of infection in humans. *P. aeruginosa* can acquire resistance against multiple groups of antimicrobial agents, including β -lactams, aminoglycosides and fluoroquinolones, and multidrug resistance is increasing in this organism which makes treatment of the infections difficult and expensive. This has led to the unmet need for discovery of new compounds distinctly different from present antimicrobials. Protein synthesis is an essential metabolic process and a validated target for the development of new antibiotics. Translation initiation factor 1 from *P. aeruginosa* (Pa-IF1) is the smallest of the three initiation factors that acts to establish the 30S initiation complex to initiate translation during protein biosynthesis, and its structure is unknown. Here we report the ^1H , ^{13}C and ^{15}N chemical shift assignments of Pa-IF1 as the basis for NMR structure determination and interaction studies. Secondary structure analyses deduced from the NMR chemical shift data have identified five β -strands with an unusually extended β -strand at the C-terminal end of the protein and one short α -helix arranged in the sequential order $\beta 1$ – $\beta 2$ – $\beta 3$ – $\alpha 1$ – $\beta 4$ – $\beta 5$. This is further supported by ^{15}N – $\{^1\text{H}\}$ hetero NOEs. These secondary structure elements suggest the Pa-IF1 adopts the typical β -barrel structure and is composed of an oligomer-binding motif.

Keywords

Pseudomonas aeruginosa; Translation initiation factor 1 (IF1); NMR assignments; Protein secondary structure

Biological context

Pseudomonas aeruginosa is a ubiquitous Gram-negative bacterium that is an opportunistic pathogen and a top cause of infection in humans (Stover et al. 2000). *P. aeruginosa* is a primary cause of infections in immunocompromised individuals such as cancer and AIDS patients or patients with severe burns or wounds. However, the most serious medical problem caused by *P. aeruginosa* is chronic lung colonization associated with cystic fibrosis patients. *P. aeruginosa* has an extraordinary ability to develop resistance to multiple classes of antibiotics rather rapidly over several generations and can even occur during the course of treating an infection (Driscoll et al. 2007; Lister et al. 2009). At a time when the need for

new antibiotics is dramatically increasing there is an unmet need for the discovery of new compounds that have a substantially different mode of action and different targets than current antibiotics.

Protein synthesis is an essential metabolic process and a validated target for the development of antibiotics (McCoy et al. 2011). Protein synthesis involves four consecutive phases—initiation, elongation, termination and ribosome recycling. Of these phases, translation initiation is the rate limiting and most highly regulated phase (Laursen et al. 2005). This critical step involves the formation of the 30S initiation complex and requires the three protein initiation factors, IF1, IF2 and IF3. These three initiation factors are currently being explored as targets for the development of new antibiotics in our laboratories, but their structures are unknown. IF1 is the smallest of the initiation factors and IF1 from *Escherichia coli* has been extensively studied. IF1 plays an important role in the initiation of protein synthesis by binding at the A-site of the 30S ribosomal subunit thereby preventing the initiator tRNA from binding at that site. Several amino acids, especially Arg70 at the C-terminal end of the protein were identified as critical for IF1 functionality (Sette et al. 1997). However, these results are inconsistent with the crystal structure of a complex of IF1 with the 30S ribosomal subunit from *Thermus thermophilus* (PDB ID 1HR0) in which the equivalent residue makes no direct contact with the 30S subunit, suggesting that IF1 from different species may exhibit distinct interaction with the 30S ribosomal subunit (Carter et al. 2001). In this work, we report NMR assignments of IF1 from *P. aeruginosa* (Pa-IF1) at pH 5.1 as a first step toward determining its atomic resolution structure and interaction with the 30S ribosomal subunit for elucidating the structural basis of protein translation initiation in *P. aeruginosa*.

Experimental procedures

Expression and purification of *P. aeruginosa* IF1

The DNA encoding *P. aeruginosa* translation initiation factor 1 (Pa-IF1) was inserted between the *NheI* and *XhoI* restriction site in a pET-24b(+) plasmid (Novagen). This placed the gene encoding IF1 upstream of a sequence encoding six histidine residues (LEHHHHHH). The plasmid was transformed into Rosetta 2(DE3) *E. coli* competent cells (Novagen) for overexpression. Uniformly ¹⁵N-labeled and ¹³C, ¹⁵N-labeled IF1 were expressed in *E. coli* by using a high cell density method (Sivashanmugam et al., 2009). Bacterial cultures overexpressing Pa-IF1 were grown in 2L of LB (Luria–Bertani) media to an optical density (A_{600}) of 0.8–1.0. The cells were harvested by centrifugation (6000 rpm, 15 min, 4 °C) and re-suspended in 1 liter of M9 Minimal Media containing 1 g of ¹⁵NH₄Cl for ¹⁵N-labeled Pa-IF1 or 1 g of ¹⁵NH₄Cl and 4 g of ¹³C-glucose for ¹⁵N/¹³C-labeled Pa-IF1. Growth was continued for 40 min at 37 °C. Expression of target protein was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to 0.5 mM. Growth of bacterial cultures was continued for 4 h post-induction, and the bacteria were harvested by centrifugation as above. Overexpressed Pa-IF1 protein was purified using nickel–nitrilotriacetic acid (Ni–NTA) affinity chromatography. Pa-IF1 was purified to greater than 98 % homogeneity as determined by SDS-PAGE.

NMR spectroscopy

All forms of Pa-IF1 (unlabeled, ^{15}N - or $^{15}\text{N}/^{13}\text{C}$ -labeled) were exchanged to NMR buffer [20 mM phosphate (pH 5.1), 100 mM KCl, 8 % D_2O or 100 % D_2O] using a Millipore Amicon Ultra Centrifugal Filter *Ultracel-3 K* (Millipore #UFC900324, 3 kDa cut-off), concentrated to approximate 1.0 mM and transferred to an NMR Shigemi tube after removal of any precipitates by centrifugation. All NMR experiments were performed at 298 K on a Bruker Ultrashield Plus 600 MHz spectrometer equipped with a double resonance broad band room-temperature probe (BBO), a Bruker Avance 700 or 600 MHz spectrometer both equipped with a four channel interface and triple resonance cryogenic probes (TXI) with triple-axis (X, Y, Z) pulsed field gradients. The ^{15}N - ^1H HSQC spectrum (Fig. 1) was recorded using a sample of ^{15}N -labeled Pa-IF1 with 256 (F1) $\times$ 1024 (F2) complex points. Backbone and side-chain NMR chemical shift assignments were obtained by analyzing the following spectra: HNCACB, CBCA(CO)NH, HNCO, HBHA(CO)NH, and ^{15}N -HSQC-TOCSY. The ^{13}C -CT-HSQC, ^{13}C -HCCH-TOCSY and CCH-TOCSY spectra were used to assign the additional side-chain aliphatic ^1H and ^{13}C resonances. For aromatic side-chain chemical shift assignments, ^{13}C -CT-HSQC-TOCSY, ^{13}C -HSQC-NOESY spectra along with 2D ^1H - ^1H NOESY and TOCSY were used. The ^{15}N - $\{^1\text{H}\}$ NOE experiments were performed using ^{15}N -labeled Pa-IF1 at 298 K using standard pulse sequences as described (Farrow et al. 1994). ^{15}N - $\{^1\text{H}\}$ NOE values were obtained by recording two sets of spectra in the presence and absence of a 3 s proton saturation period. The NOE experiments were repeated 3 times to calculate the average and standard deviation of the NOE values. The NMR data were processed using NMRPipe (<http://spin.niddk.nih.gov/NMRPipe/>) and analyzed using Sparky (<https://www.cgl.ucsf.edu/home/sparky/>).

Assignments and data deposition

The ^1H , ^{15}N HSQC spectrum of Pa-IF1 is shown in Fig. 1. All non-proline residues exhibited strong backbone amide resonances with uniform intensities and a large chemical shift dispersion, indicative of a well-ordered three dimensional protein structure. Following a sequential assignment procedure, all backbone resonances (^1HN , $^1\text{H}\alpha$, $^1\text{H}\beta$, ^{15}N , $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and ^{13}CO) of Pa-IF1 (except for the first residue and two Prolines) were assigned. In addition 85 % of side chain aliphatic and aromatic ^1H and ^{13}C assignments were completed. The chemical shift values for the ^1H , ^{13}C and ^{15}N resonances of Pa-IF1 have been deposited into the BioMagResBank (<http://www.bmrb.wisc.edu/>) under accession number 26649.

Secondary structure elements of Pa-IF1 were identified by analysis of TALOS+ factors (Shen et al. 2009) derived using C, $\text{C}\alpha$, $\text{C}\beta$, $\text{H}\alpha$ and N chemical shifts (Fig. 2a). Positive and negative values of the TALOS+ factors represent β -strand and α -helix secondary structures, respectively. Secondary structure analysis deduced from the NMR chemical shift data reveals five β -strands, $\beta 1$: residues 7–16, $\beta 2$: 21–26, $\beta 3$: 31–36, $\beta 4$: 52–57, $\beta 5$: 64–70 and one short helix $\alpha 1$: 39–42 arranged in the sequential order $\beta 1$ – $\beta 2$ – $\beta 3$ – $\alpha 1$ – $\beta 4$ – $\beta 5$. These secondary structure elements suggest that Pa-IF1 adopts the typical β -barrel structure and topology seen in other prokaryotic IF1 proteins (Sette et al. 1997) and is an oligomer-binding motif. The amino acid sequence of *P. aeruginosa* IF1 is most similar to that of *E. coli* (86 % identity) and the secondary structures of *P. aeruginosa* IF1 are also similar to that

of IF1 from *E. coli*. An interesting difference in secondary structures was observed for non-conserved residues at the C-terminus of the protein. The difference is represented by an extended β -strand ($\beta 5$: 64–70) which is shortened in IF1 from *E. coli* (Sette et al. 1997). As shown in Fig. 2b, TALOS+ Index analysis predicts this β -strand with average TALOS+ Index of 0.82. Previous mutagenesis experiments have indicated that the C-terminal end is crucial for the functionality of prokaryotic initiation factor 1 in *E. coli* (Spurio et al. 1991). The structural difference at the C-terminal end of *P. aeruginosa* initiation factor 1 might indicate that Pa-IF1 functions differently in the translation initiation stage.

The secondary structures of Pa-IF1 are supported by the backbone $^{15}\text{N}\{-^1\text{H}\}$ heteronuclear NOE results shown in Fig. 2b. The backbone $^{15}\text{N}\{-^1\text{H}\}$ NOE provides information about the motion of individual N–H bond vectors. Those that undergo motion faster than the overall tumbling of molecules show a decreased NOE intensity relative to the average observed for the majority of the residues. The N-terminal end of Pa-IF1 was observed to have $^{15}\text{N}\{-^1\text{H}\}$ NOEs of less than 0.5 (some with negative values) which is indicative of large-scale flexibility. The residue 28 in the linker between strands $\beta 2$ and $\beta 3$ showed a significantly reduced $^{15}\text{N}\{-^1\text{H}\}$ NOE value, indicating the linker is considerably flexible. A similar reduction in $^{15}\text{N}\{-^1\text{H}\}$ NOEs was also observed for the residues in the flexible region connecting strands $\beta 3$ and $\beta 4$, however, this is not the case for the residues in the linking segments between strands $\beta 1$ and $\beta 2$, or $\beta 4$ and $\beta 5$ which are less flexible. The average NOE for residues in secondary structural regions is 0.75. Intriguingly, the average $^{15}\text{N}\{-^1\text{H}\}$ NOE for residues 64–70 in $\beta 5$ strand is 0.73, indicating this extended strand is fairly rigid and less flexible than that of IF1 from *E. coli*.

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Fig. 2.

Secondary structure analysis based on the Pa-IF1 NMR assignment data and $^{15}\text{N}\{-^1\text{H}\}$ heteroNOE measurements. **a** TALOS+-predicted secondary structure distribution along the protein sequence (positive and negative values of TALOS+ factors correspond to β -strand and α -helix secondary structures, respectively). The expected secondary structure elements of Pa-IF1 are represented above. **b** Plot of backbone heteronuclear $^{15}\text{N}\{-^1\text{H}\}$ NOE values versus residue number for Pa-IF1