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Structure of a putative acetyltransferase (PA1377) from *Pseudomonas aeruginosa*

Gene PA1377 from *Pseudomonas aeruginosa* encodes a 177-amino-acid conserved hypothetical protein of unknown function. The structure of this protein (termed pitax) has been solved in space group *I*222 to 2.25 Å resolution. Pitax belongs to the GCN5-related *N*-acetyltransferase family and contains all four sequence motifs conserved among family members. The β -strand structure in one of these motifs (motif A) is disrupted, which is believed to affect binding of the substrate that accepts the acetyl group from acetyl-CoA.

1. Introduction

When the genome of *Pseudomonas aeruginosa* PAO1 was first published in 2000, approximately 45% of the ORFs were classed as representing genes for which no function could be determined (Stover *et al.*, 2000; <http://www.pseudomonas.com>). Recently, we and others reported the crystal structure of a conserved hypothetical protein from *P. aeruginosa* (locus ID PA4866, termed pita; Davies *et al.*, 2005, 2007; PDB code 1yvo; B. Nocek, X. Xu, A. Savchenko, A. Edwards & A. Joachimiak, unpublished work). Pita belongs to the GNAT (GCN5-related *N*-acetyltransferase) family and efforts have been made to identify its function (Davies *et al.*, 2007). When the pita sequence was used to conduct a *BLAST* search of the *Pseudomonas* genome, a 177-amino-acid protein displaying 28% sequence identity to pita was returned (Fig. 1). This protein (locus ID PA1377, termed pitax) is also a conserved hypothetical protein. The function of pitax is unknown; however, preliminary studies (F. Chauviac, R. Tata & P. Brown, unpublished data) have revealed that it is an acetyltransferase, catalysing the transfer of an acetyl group from acetyl-CoA to an acceptor substrate. In this communication, we report the crystal structure of pitax to 2.25 Å resolution and compare it with that of pita.

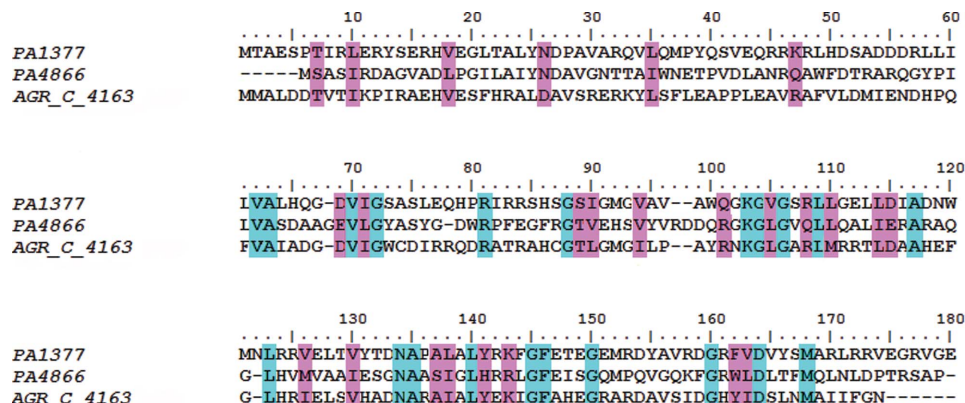
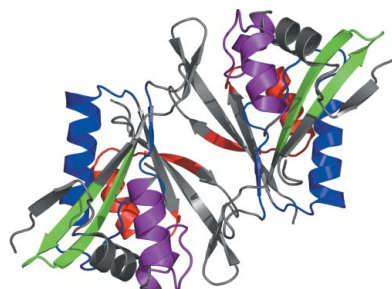


Figure 1
Multiple sequence alignment. PA1377, *P. aeruginosa* conserved hypothetical protein (pitax); accession No. Q9I3W7. PA4866, *P. aeruginosa* conserved hypothetical protein (pita); accession No. Q9HUU7. AGR_C_4163, *A. tumefaciens* probable acetyltransferase; accession No. Q8UD38. Residues coloured light blue are identical, while those coloured light pink display 70% conservation. The multiple sequence alignment was performed using *ClustalW* (Thompson *et al.*, 1994) and the figure was generated with *BioEdit* (Hall, 1999).

2. Materials and methods

2.1. Cloning, protein expression, purification and crystallization

PA1377 (pitax) was amplified by PCR using primers 1377F (5'-GGATGACCCATATGACCGCCGAATCGCCGAC-3') and 1377R (5'-AAAAGGATCCGTCGCGCCCGCCCCTCACT-3') with chromosomal DNA from *P. aeruginosa* PAC1 as template. Amplifications were performed in 50 μ l diluted RedTaq Readimix (Sigma Ltd) with 0.2 mM primers and 100 ng chromosomal DNA. The program was as follows: 60 s at 367 K, followed by 30 cycles of 30 s at 367 K, 30 s at 331 K, 60 s at 345 K and then 5 min at 345 K. The PCR product was cut with *Bam*HI and *Nde*I and ligated with *Bam*HI/*Nde*I-cut pET24a (Novagen Ltd). The ligation mixture was used to transform *Escherichia coli* JM109 and transformants containing recombinant plasmids were selected on LBkan medium and identified by colony PCR. After determination of the DNA sequence of the insert, the recombinant plasmid was used to transform *E. coli* BL21 (DE3).

E. coli BL21 (DE3) (pETpitax) was grown overnight in 1 l LBkan medium containing 1 mM IPTG. Cells were harvested by centrifugation and resuspended in 20 ml 50 mM Tris pH 8.5 and 1 mM EDTA (TE) at 277 K. All subsequent procedures were carried out at 273–277 K. Cells were broken by sonication using an MSE ultrasonic oscillator. 0.25 g streptomycin sulfate was added and the solution was centrifuged for 15 min at 10 000g to remove debris. The supernatant was loaded onto a Q-Sepharose column (2 \times 15 cm) equilibrated with TE. Protein was eluted with a 500 ml linear 0–0.5 M gradient of NaCl in TE. Fractions containing pitax were bulked and concentrated by ultrafiltration (Amicon Ultra-15 device, Millipore) to 1 ml. The solution was loaded onto an FPLC MonoBead MonoQ HR 10/10 column equilibrated with TE and eluted with an NaCl gradient (87.5–175 mM) at a flow rate of 2.0 ml min⁻¹. Fractions containing pitax were bulked and concentrated by ultrafiltration to 900 μ l. Purification was monitored by SDS-PAGE and pitax was identified by its molecular weight (20 067 Da).

Crystals were grown using the hanging-drop vapour-diffusion method. The reservoir solution contained 500 μ l 0.1 M Tris-HCl pH 6.5, 1.6 M ammonium sulfate and 0.1% (w/v) sodium azide. The drops contained 1 μ l protein solution at 13 mg ml⁻¹, to which an equal volume of reservoir solution was added. The drops were kept at 291 \pm 0.5 K and crystals up to 370 μ m in length appeared after 1–2 d. Crystals were flash-cooled in liquid nitrogen using a cryoprotectant comprising 0.1 M Tris-HCl pH 6.5, 1.6 M ammonium sulfate, 30% (v/v) glycerol and 0.1% (w/v) sodium azide.

2.2. Data collection, structure determination and refinement

Data were collected at Station 10.1, Synchrotron Radiation Source, Daresbury, UK (Cianci *et al.*, 2005), integrated with *MOSFLM* (Leslie, 1992) and scaled with *SCALA* (Collaborative Computational Project, Number 4, 1994). Data-collection and processing statistics are presented in Table 1.

The pitax sequence was used to perform a *BLAST* search against the Protein Data Bank (Berman *et al.*, 2000) in order to find a suitable search model for molecular replacement, as early attempts to solve the structure using the coordinates of pita were not successful. A probable acetyltransferase from *Agrobacterium tumefaciens* (gene AGR_C_4163; PDB code 2ge3; C. Chang, X. Xu, J. Gu, A. Savchenko, A. Edwards & A. Joachimiak, unpublished work) displaying 43% sequence identity to pitax was chosen (Fig. 1). Protein atoms from one subunit of the 2ge3 structure were used as a search model and the structure was solved with *MOLREP* (Vagin & Teplyakov, 1997). *CNS* v. 1.1 (Brünger *et al.*, 1998) was used for refinement and manual

Table 1

Data-processing statistics.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.488
Space group	<i>I</i> 222
Unit-cell parameters (Å, °)	$a = 79.71, b = 110.93, c = 134.48,$ $\alpha = \beta = \gamma = 90$
Resolution range (Å)	64.3–2.25 (2.31–2.25)
Unique reflections	28683 (2089)
Completeness (%)	99.9 (99.9)
Redundancy	6.7 (6.6)
$I/\sigma(I)$	21.0 (2.8)
R_{merge} (%)	9.2 (61.3)
Solvent content (%)	50.0
Molecules per ASU	3
Wilson B factor (Å ²)	39.5

Table 2

Refinement statistics.

Resolution range (Å)	64.3–2.25
No. of reflections	28683
No. of protein atoms per ASU	3843
No. of water molecules per ASU	265
Average B factor (Å ²)	
Protein atoms	34.8
Water molecules	38.1
Glycerol molecules	52.6
Azide ions	50.3
Sulfate ions	50.3
R_{cryst} (%) (all reflections)	19.1
R_{free} (%) (5% of reflections)	22.6
R.m.s.d. bond lengths (Å)	0.014
R.m.s.d. bond angles (°)	1.37
σ_A coordinate error (Å)	0.29
Ramachandran plot analysis (structure analysed with <i>MolProbity</i>)	
No. of residues in favoured regions (%)	97.9
No. of residues in allowed regions (%)	99.8
Outliers (%)	0.2

model building was performed with *QUANTA* (Molecular Simulations Inc., 2000) and *Coot* (Emsley & Cowtan, 2004) using σ_A -weighted $2F_{\text{obs}} - F_{\text{calc}}$ and $F_{\text{obs}} - F_{\text{calc}}$ electron-density maps. Protein atoms were modelled before water molecules, glycerol molecules, azide and sulfate ions were incorporated into the structure. Topology and parameter files for glycerol and azide were obtained from the HIC-Up database (Kleywegt & Jones, 1998). The three molecules of the asymmetric unit were refined independently. The refined model was analysed using *PROCHECK* (Laskowski *et al.*, 1993), *SFCHECK* (Vaguine *et al.*, 1999), *CNS* (Brünger *et al.*, 1998), *Coot* (Emsley & Cowtan, 2004) and *MolProbity* (Lovell *et al.*, 2003). The refined model comprised protein residues 7–52 and 54–170 for chain A, 6–52 and 54–169 for chain B, and 5–52 and 54–170 for chain C. The model also contained 265 water molecules, five glycerol molecules, six sulfate ions and four azide ions. Refinement statistics are presented in Table 2.

2.3. DALI server search

The pitax dimer was used to perform a structural alignment search with the *DALI* server (Holm & Sander, 1994) and hits from the search were returned in order of Z score. The Z score represents the statistical significance of the structural alignment and a score of less than 2 indicates structural dissimilarity (Holm & Sander, 1994). 128 alignments with Z scores ranging from 2 (the lowest) to 20.1 (the highest) were returned from the search; of these, structures with alignments with Z scores above 5 (55 structures in total) were used for more detailed comparison with pitax.

3. Results and discussion

3.1. Pitax is a GNAT-family member

Searching the Pfam database (Finn *et al.*, 2006) revealed that pitax is a member of the GCN5-related *N*-acetyltransferase (GNAT) superfamily (PF00583). Four conserved sequence motifs (motifs A–D) have been identified in GNAT-family members (Neuwald & Landsman, 1997) and examination of the sequence of pitax confirmed the presence of all four of these motifs.

3.2. Dimerization

Many GNAT-family members exist in a dimeric form [*e.g.* pita from *P. aeruginosa* (Davies *et al.*, 2007), glucosamine-6-phosphate *N*-acetyltransferase GNA1 from *Saccharomyces cerevisiae* (Peneff *et al.*, 2001), aminoglycoside acetyltransferase AAC9(6')II from *Enterococcus faecium* (Burk *et al.*, 2003) and RimL *N*^α-acetyltransferase from *Salmonella typhimurium* (Vetting *et al.*, 2005)]. The crystal structure of pitax suggests that it may also exist as a dimer. In pitax,

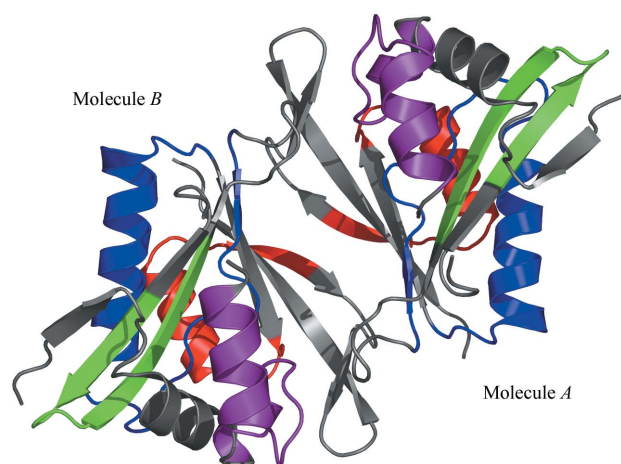


Figure 2
The pitax dimer. Molecules *A* and *B* of the asymmetric unit are shown. The GNAT motifs defined by Neuwald & Landsman (1997) are coloured, from the N-terminus to the C-terminus, as follows: purple (motif *C*), green (motif *D*), blue (motif *A*) and red (motif *B*). All other atoms are coloured grey. This figure was generated with PyMOL (DeLano, 2002).

two of the three molecules (*A* and *B*) of the asymmetric unit form a dimer and are related to one another by an approximate twofold rotation axis of 179.2° (Fig. 2), burying an area of approximately 2600 Å² at their interface, as calculated by CNS (Brünger *et al.*, 1998). The third molecule (*C*) of the asymmetric unit forms a structurally similar dimer with another molecule related by twofold crystallographic symmetry.

3.3. Overall fold

Pitax is an α/β -fold protein and its overall structure is best described as two regions of antiparallel β -sheet, each flanked by α -helices (Fig. 3*a*). The first antiparallel β -sheet (strands β 1–4) wraps around helix α 4 on one side and is flanked on its opposite face by helices α 1–3. The second β -sheet (strands β 5–8) is flanked by helix α 5. The four GNAT-superfamily motifs are highlighted in Figs. 2 and 3(*a*).

Comparison of the structures of pitax and pita showed that they are similar (r.m.s.d. of 2.1 Å over 163 C^α atoms; Fig. 3). However, structural differences were observed in regions contained within motifs *C* and *A*. In pitax motif *C* comprises two helices (α 2 and α 3) separated by a loop region (Fig. 3*a*), whereas in pita this motif comprises a loop region followed by a helix (α 2) (Fig. 3*b*). Structural variation in motif *C* is not unprecedented as this motif is not as well conserved as the other motifs among GNAT-family members, at least in terms of sequence similarity (Neuwald & Landsman, 1997). Motif *A*, on the other hand, is highly conserved (Neuwald & Landsman, 1997). This motif comprises a β -strand followed by an α -helix and some of its residues provide important contacts for binding of acetyl-CoA (Modis & Wierenga, 1998; Angus-Hill *et al.*, 1999; Wybenga-Groot *et al.*, 1999). The β -strand and α -helix comprising motif *A* (β 4 and α 3) are clearly visible in pita (Fig. 3*b*), whereas in pitax, while helix α 4 is present, strand β 4 is considerably shortened (Fig. 3*a*). Since the catalytic site of pita lies in this region, this difference may well be significant in determining the location or specificity of the acetyl acceptor-binding site.

3.4. The conformation of strand β 4 in pitax may affect substrate binding

Preliminary kinetic studies have demonstrated that pitax is an acetyltransferase and weakly catalyses the acetylation of L-methio-

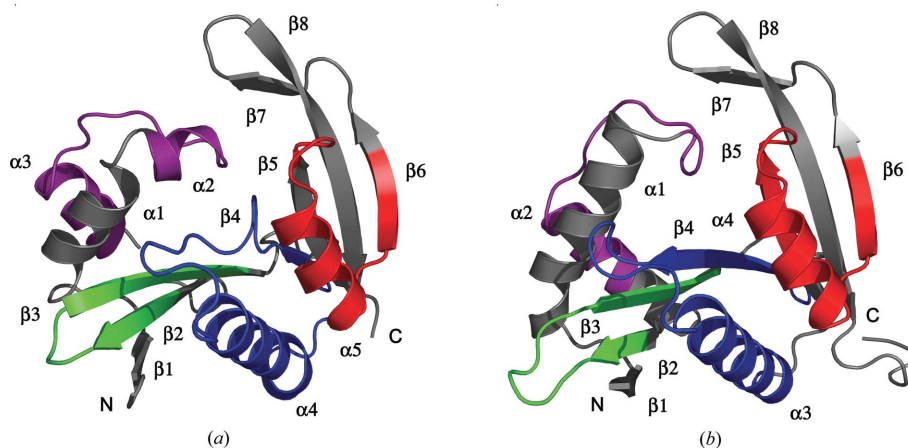


Figure 3
Structure of the pitax and pita monomers. (*a*) Structure of the pitax monomer. α -Helices and β -strands are labelled α 1–5 and β 1–8. Molecule *B* of the structure is shown. (*b*) Structure of the pita monomer. α -Helices and β -strands are labelled α 1–4 and β 1–8. Coordinates from molecule *B* of the pita native structure are shown (PDB code 2j8m; Davies *et al.*, 2007). In both (*a*) and (*b*) the GNAT motifs defined by Neuwald & Landsman (1997) are coloured, from the N-terminus to the C-terminus, as follows: purple (motif *C*), green (motif *D*), blue (motif *A*) and red (motif *B*). All other atoms are coloured grey. This figure was generated with PyMOL (DeLano, 2002).

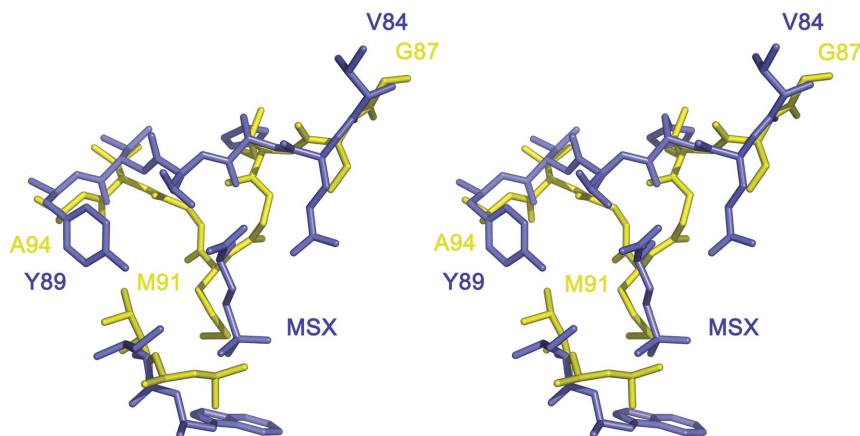


Figure 4

The location of the pitax active site may differ from that in pita. The structure of pitax (yellow) was superposed globally onto the structure of pita in complex with one of its substrates, L-methionine sulfoximine (blue; PDB code 2j8r; Davies *et al.*, 2007). Residues are numbered according to their respective protein sequences. Met91 in pitax clearly occludes the equivalent region occupied by L-methionine sulfoximine (labelled MSX) in pita. This figure was generated with *PyMOL* (DeLano, 2002).

nine and L-methionine sulfoxide (F. Chauviac, R. Tata & P. Brown, unpublished data). The substrates of pitax bear some structural similarity to the substrates of pita: L-methionine sulfoximine and L-methionine sulfone. Because of the overall structural similarity between these proteins and their substrates, the structure of pitax was superposed onto the structure of pita in complex with L-methionine sulfoximine (Davies *et al.*, 2007) in order to try to locate the substrate-binding site of pitax (Fig. 4).

In pitax, strand β_4 , which constitutes part of motif A, is disrupted. The protein main chain deviates from the plane of the β -strand (in pita) and causes a methionine residue (Met91) to occlude the region that in pita is the acceptor substrate-binding site (Fig. 4). Thus, either the location of the substrate-binding site in pitax must be different from that of pita or a conformational change is required to allow substrate to bind.

A surface representation of pitax generated in *PyMOL* (DeLano, 2002) using protein atoms only revealed the presence of a pocket. When the structure of a probable acetyltransferase from *A. tumefaciens* in complex with acetyl-CoA (PDB code 2ge3; Chang *et al.*, unpublished work) was superposed onto pitax, acetyl-CoA was found to occupy this pocket. However, this region in pitax putatively occupied by acetyl-CoA did not provide any clues as to where the acceptor substrate might bind, as potential access to substrate by the acetyl group was blocked by the presence of Met91. However, Met91 is flanked by two glycine residues, which may facilitate conformational change, allowing the acetyl group access to a bound substrate molecule.

The structures returned from the *DALI* server search were compared with pitax in order to investigate whether any other acetyltransferase possessed a similar conformation to that of the disrupted strand β_4 . However, it was found that while the majority contained small differences in strand β_4 , none of these caused the plane of the β -strand to deviate. The only protein with a similar conformation to pitax in this particular region was FemA from *Staphylococcus aureus* (Z score 10, r.m.s.d. 3.7 Å, 10% sequence identity over 138 residues; Benson *et al.*, 2002). FemA belongs to the N-acetyltransferase clan (CL0257 in the Pfam database; a clan being a group of families with a single evolutionary origin; Finn *et al.*, 2006) but does not bind acetyl-CoA.

4. Conclusions

We have solved the crystal structure of a conserved hypothetical protein (PA1377, termed pitax) from *P. aeruginosa* to 2.25 Å resolution. We are examining further the substrate specificity of pitax and will soak putative substrates into crystals in order to obtain enzyme–substrate complex structures. Residues important for substrate binding and catalysis will also be studied by site-directed mutagenesis. Parallel studies to determine the physiological role of pitax are being carried out by observing the properties of a mutant bacterium in which the pitax gene has been inactivated.

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