Biochimie 91 (2009) 484–489

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Structure and substrate specificity of acetyltransferase ACIAD1637 from *Acinetobacter baylyi* ADP1

Anna M. Davies, Renée Tata, Alison Snape, Brian J. Sutton, Paul R. Brown*

King's College London, Randall Division of Cell and Molecular Biophysics, London, SE1 1UL, UK

ARTICLE INFO

Article history:
Received 15 October 2008
Accepted 12 December 2008
Available online 24 December 2008

Keywords:
GNAT
Acetyltransferase
Methionine sulfoximine
Phosphinothricin

ABSTRACT

Gene ACIAD1637 from *Acinetobacter baylyi* ADP1 encodes a 182 amino acid putative antibiotic resistance protein. The structure of this protein (termed acepita) has been solved in space group P_2 to 2.35 Å resolution. Acepita belongs to the GCN5-related *N*-acetyltransferase (GNAT) family, and contains the four sequence motifs conserved among family members. The structure of acepita is compared with that of pita, its homologue from *Pseudomonas aeruginosa*. Acepita has a similar substrate profile to pita and performs a similar function.

1. Introduction

Recently, structures were independently determined for the 172 amino acid protein encoded by gene PA4866 (termed pita) from *Pseudomonas aeruginosa* ([8,9], and B. Nocek, X. Xu, A. Savchenko, A. Edwards and A. Joachimiak, unpublished work, PDB code 1YVO). Pita homologues are found in a wide variety of bacteria, including *Escherichia coli* (yncA/b1448), *Staphylococcus aureus* (SAR2609), *Salmonella typhimurium* (yncA/STM1590), *Agrobacterium tumefaciens* (AGR_C_1654), *Burkholderia cenocepacia* (BCAL1022) and *Acinetobacter baylyi* ADP1 [previously referred to as *Acinetobacter calcoaceticus* ADP1 or *Acinetobacter* sp. strain ADP1 but now reclassified [27]] (ACIAD1637). These hypothetical proteins belong to the GCN5-related *N*-acetyltransferase (GNAT) family, and a number have been assigned putative phosphinothricin acetyltransferase activity on the basis of sequence similarities to the phosphinothricin acetyltransferases synthesized by *Streptomyces hygroscopicus* [24] and *Streptomyces viridochromogenes* [30]. Phosphinothricin is a glutamate analogue formed in soil by the hydrolysis of demethylphosphinothricin, an intermediate in the biosynthetic pathway of the antibiotic bialaphos, found in *S. hygroscopicus* and *S. viridochromogenes*. It is a powerful inhibitor of glutamine synthetase which accounts for its use as a herbicide. Crops have been protected against its action through the introduction, by genetic engineering, of the *bar* gene, derived from *S. hygroscopicus* or *S. viridochromogenes*, that encodes phosphinothricin acetyltransferase [24].

Recent studies of pita from *P. aeruginosa* revealed that it does not act as a phosphinothricin acetyltransferase [9]. Instead, L-methionine sulfoximine (L-metsox) and L-methionine sulfone (L-metsone) were identified as substrates. It was also demonstrated that a mutant strain of *P. aeruginosa*, unable to synthesize pita due to a deletion in the PA4866 gene, did not grow on minimal media in the presence of L-metsox, because of L-metsox inhibition of glutamine synthetase, an essential enzyme for the assimilation of nitrogen [9].

The pita homologue from *A. baylyi* ADP1 is the protein product of gene ACIAD1637. This protein, termed acepita, is a 182 amino acid protein, and displays 47% sequence identity to pita (Fig. 1). In this communication we report the crystal structure of acepita to 2.35 Å resolution. We also report that L-metsox and L-metsone are substrates of acepita. A mutant strain of *A. baylyi* ADP1, in which the ACIAD1637 gene is disrupted by insertion of a kanamycin resistance gene, has been created. Like the pita-negative mutant *P. aeruginosa* strain, growth of this strain is also inhibited by L-metsox, indicating that acepita has a similar function to pita in protecting the organism against L-metsox.

2. Materials and methods

2.1. Chemicals

L-Metsox and L-metsone were obtained from Sigma–Aldrich. DL-phosphinothricin was purified from Basta™ (Bayer Crop Science) essentially by the method of Metzenberg et al. [21].

Abbreviations: GNAT, GCN5-related *N*-acetyltransferase; L-metsox, L-methionine sulfoximine; L-metsone, L-methionine sulfone.

* Corresponding author. King's College London, Randall Division of Cell and Molecular Biophysics, New Hunt's House, Guy's Campus, London Bridge, SE1 1UL London, UK. Tel.: +44 20 7848 6560; fax: +44 20 7848 6410.
E-mail address: paul.brown@kcl.ac.uk (P.R. Brown).

0300-9084/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2008.12.003

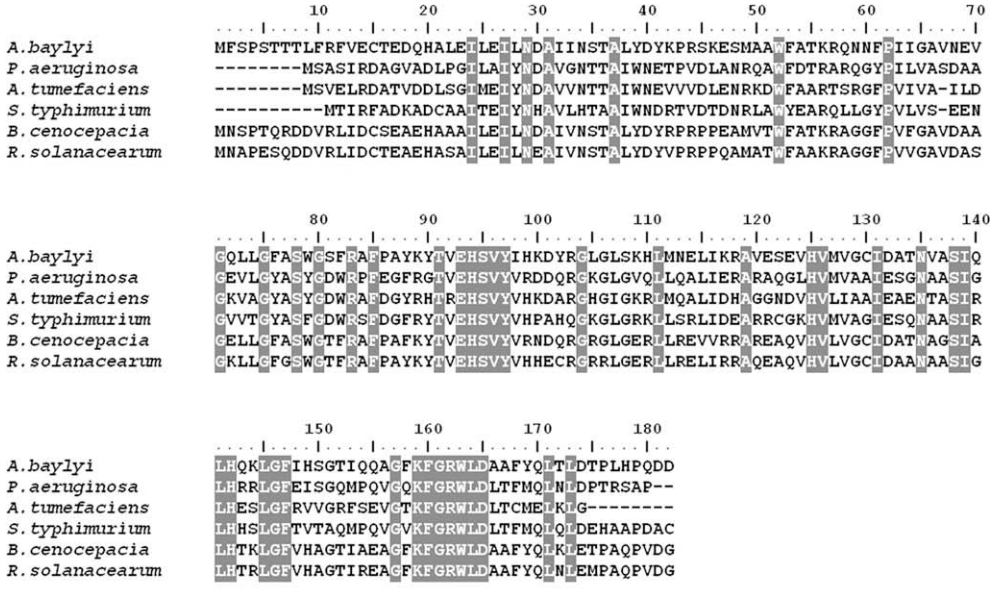

Fig. 1. Sequence alignment. *A. baylyi*, putative antibiotic resistance protein ACIAD1637 (acepita) from *A. baylyi* ADP1. Accession number Q6FBS8. *P. aeruginosa*, conserved hypothetical protein PA4866 (pita) from *P. aeruginosa*. Accession number Q9HUU7. *A. tumefaciens*, phosphinothricin acetyltransferase AGR_C_1654 from *A. tumefaciens*. Accession number A9CJR4. *S. typhimurium*, putative acetyltransferase yncA/STM1590 from *S. typhimurium*. Accession number Q8ZPD3. *B. cenocepacia*, acetyltransferase (GNAT) family protein BCAL1022 from *B. cenocepacia*. Accession number B4EA34. *R. solanacearum*, putative antibiotic resistance (acetyltransferase) protein RSc3133 from *R. solanacearum*. Accession number Q8XUQ3. Residues highlighted in grey are identical. The sequence alignment was performed using CLUSTAL W [23] and the figure was generated with BioEdit [15].

2.2. Cloning of the acepita gene, protein expression and purification

PCR protocols and media were as described in Davies et al. [9]. Sequences of primers used in amplifying regions of the *A. baylyi* ADP1 chromosome were based on its genome sequence [2]. The acepita gene ACIAD1637 from *A. baylyi* ADP1 (BD413, ATCC33305) was amplified by PCR using primers ACF (5′-CTTTTCTCATATGTTTTCTCCATCCACTAC-3′) and ACR (5′-CAAGGATCCTTATTAATCGTCTTGAGGGTG-3′). The PCR product was cut with BamHI and NdeI and cloned into BamHI/NdeI cut pET24a (Novagen). The ligation mixture was used to transform *E. coli* JM109 and transformants containing recombinant plasmid were selected on LBkan solid medium. Sequencing confirmed the identity of the inserted DNA. Recombinant plasmid was used to transform *E. coli* BL21 (DE3) to obtain *E. coli* BL21 (DE3) (pET(acepita)).

E. coli BL21 (DE3) (pET(acepita)) was grown overnight in 1 L LBkan medium containing 1 mM IPTG at 37 °C in two 2 L conical flasks in a rotary shaker. Subsequent steps were carried out at 4 °C. After centrifugation, cells were resuspended in 30 mL of 50 mM Tris pH 7.2, 1 mM dithiothreitol and 1 mM EDTA (TED). After breakage of cells by sonication in an MSE Soniprep 50 ultrasonicator, 0.25 g streptomycin sulphate was added and the mixture was centrifuged to remove debris and precipitated nucleic acid. The supernatant was 50% (w/v) saturated with ammonium sulphate, and the precipitate, obtained by centrifugation, was dissolved in TED, then dialysed against several changes of TED.

The supernatant was loaded onto a Q-Sepharose column (1.5 cm × 10 cm) previously equilibrated with TED. The protein was eluted with a linear gradient of 0–0.4 M NaCl in 400 mL TED. Fractions containing the OD_{280nm} peak, eluting at ~0.1 M NaCl, were bulked and concentrated using an Amicon Ultracentrifugal Filter Device (Millipore) with a 5000 MW cut off. The concentrated protein solution (2–3 mL) was applied to a Sephacryl S-200 Hiprep 26/60 gel filtration column (Amersham Biosciences) and eluted with TED. Fractions containing the major OD_{280nm} absorbing peak were bulked and concentrated by filter centrifugation. Homogeneity of the protein was established by SDS-PAGE.

2.3. Kinetic studies

Assays for acetyltransferase activity were based on the method of D'Halluin et al. [12]. The rate of formation of the reaction product, CoASH, was determined by its interaction with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) to form the yellow 5-thio-2-nitrobenzoic acid, whose formation was followed at 412 nm. Reactions were carried out at 37 °C in 50 mM Tris buffer pH 7.2 with DTNB (4 mg/mL) in a volume of 800 μL. Absorbance changes were measured with a Pye Unicam SP8-400 spectrophotometer.

Reaction rates were measured in duplicate over concentration ranges 0.02–11.0 mM for L-metsox and 0.03–3 mM for L-metsone, at 0.38 mM acetyl CoA. Reaction rates at concentrations of acetyl CoA between 0.004 mM and 0.4 mM were made at a fixed concentration of 2.8 mM L-metsox. For $K_{m(app)}$ and $k_{cat(app)}$ determinations data were fitted to the equation $v = V_m S/(K_m + S)$ using the Leonora program [7]. For L-metsox, v values were restricted to those obtained over the concentration range 0.03–3.0 mM because of substrate inhibition at higher concentrations. Appropriate blanks were included to show that no significant activity occurred in the absence of substrate. The molar extinction coefficients for 5′-thio-2-dinitrobenzene and acepita (monomer) were 13,600 M^{-1} cm^{-1} at 412 nm and 27,055 M^{-1} cm^{-1} at 280 nm respectively [14].

Table 1
Data processing statistics. Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.488
Space group	P_2
Unit-cell parameters (Å, °)	$a = b = 78.41$, $c = 197.76$ $\alpha = \beta = 90$, $\gamma = 120$
Resolution range (Å)	68–2.35 (2.41–2.35)
Unique reflections	56,442 (3962)
Completeness (%)	99.6 (94.7)
Redundancy	5.4 (3.6)
$I/\sigma(I)$	17.5 (4.0)
R_{merge} (%)	8.7 (25.3)
Solvent content (%)	57.0
Molecules per ASU	6
Wilson B factor (Å^2)	41.4