Protein J (2012) 31:359–365
DOI 10.1007/s10930-012-9412-y

Characterization of the indole-3-glycerol phosphate synthase from *Pseudomonas aeruginosa* PAO1

Monica L. Gerth · Laura V. Nigon · Wayne M. Patrick

Published online: 4 May 2012

Abstract *Pseudomonas aeruginosa* is an opportunistic pathogen that causes chronic infections in the lungs of individuals with cystic fibrosis. It is intrinsically resistant to many antibiotics, and resistance is emerging rapidly to those drugs that currently remain efficacious. Therefore, there is a pressing need to identify new anti-pseudomonal drug targets. To this end, we have characterized the *P. aeruginosa* indole-3-glycerol phosphate synthase (PaIGPS). PaIGPS catalyzes the fifth reaction in the synthesis of tryptophan from chorismate—a reaction that is absent in mammals. PaIGPS was expressed heterologously in *Escherichia coli*, and purified with high yields. The purified enzyme is active over a broad pH range and has the highest turnover number of any characterized IGPS ($k_{cat} = 11.1 \pm 0.1\ s^{-1}$). These properties are likely to make PaIGPS useful in coupled assays for other enzymes in tryptophan biosynthesis. We have also shown that deleting the gene for PaIGPS reduces the fitness of *P. aeruginosa* strain PAO1 in synthetic cystic fibrosis sputum (relative fitness, $W = 0.89 \pm 0.02$, $P = 0.001$). This suggests that de novo tryptophan biosynthesis may play a role in the establishment and maintenance of *P. aeruginosa* infections, and therefore that PaIGPS is a potential target for the development of new anti-pseudomonal drugs.

M. L. Gerth
New Zealand Institute for Advanced Study, Massey University, Private Bag 102 904, North Shore Mail Centre, Auckland 0745, New Zealand

L. V. Nigon · W. M. Patrick (✉)
Institute of Natural Sciences, Massey University, Private Bag 102 904, North Shore Mail Centre, Auckland 0745, New Zealand
e-mail: w.patrick@massey.ac.nz

Keywords *Pseudomonas aeruginosa* PAO1 · Tryptophan biosynthesis · Indole-3-glycerol phosphate synthase

Abbreviations

CdRP	1-(*o*-carboxyphenylamino) 1-deoxyribulose 5-phosphate
CF	Cystic fibrosis
IGP	Indole-3-glycerol phosphate
IGPS	Indole-3-glycerol phosphate synthase
PRPP	5-Phosphoribosyl-1-pyrophosphate
SCFM	Synthetic cystic fibrosis sputum medium

1 Introduction

The Gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic pathogen that causes a range of infections in immunocompromised patients. *P. aeruginosa* lung infections are a particularly significant cause of morbidity and mortality in cystic fibrosis (CF) patients [13]. Moreover, *P. aeruginosa* is a multidrug resistant superbug: it possesses intrinsically high levels of resistance to many antibiotics, and resistance to all available anti-pseudomonal drugs has been observed in clinical isolates [3]. As with other superbugs (such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*), there is an urgent need to identify new strategies and targets for antibiotic development [6].

In 1956, Edward Garber proposed a "nutrition-inhibition hypothesis" of pathogenicity, in which he reasoned that infection not only required a pathogen to avoid the host's defence mechanisms, but also required that it could utilize the host environment as a growth medium [10]. The implication is that virulence can be diminished by interfering with the biosynthetic requirements of a bacterium at

its site of infection [5, 11]. Indeed, the first commercially available antibacterial drugs—the sulfonamides—target the biosynthetic pathway for folate [4].

Inspired by this early work, we have characterized an enzyme from the tryptophan biosynthesis pathway of *P. aeruginosa* and assessed its potential as a novel drug target. The tryptophan biosynthesis pathway is absent in mammals, and has been validated as a drug target in *Mycobacterium tuberculosis* [33]. Tryptophan is by far the least abundant amino acid in the sputum of CF patients [28], and genes for its synthesis are among those induced to sustain high-cell-density replication in the lung [34]. Sub-lethal concentrations of the macrolide antibiotic, azithromycin, also induce expression of tryptophan biosynthesis genes in *P. aeruginosa* strain PAO1 [25]. These observations suggest that: (1) *P. aeruginosa* may need to synthesize its own tryptophan in order to use CF sputum as an effective growth medium; and (2) inhibitors of tryptophan biosynthesis may prove to be useful anti-pseudomonal drugs (alone, or in combination with azithromycin).

The biosynthesis of tryptophan from chorismate requires the expression of seven *trp* genes (*trpEGDFCBA*). The roles of these *trp* genes are conserved throughout all the bacterial phyla in which they have been characterized; however, there is considerable variation in their operon organization and regulation [23]. In *P. aeruginosa* PAO1, *trpE* and *trpF* are produced as single-gene transcripts, whereas *trpGDC* and *trpBA* are in multi-gene operons [7].

In this study, we have focused on characterizing the product of the *trpC* gene, indole-3-glycerol phosphate synthase (IGPS). This enzyme (EC 4.1.1.48) catalyzes the fifth reaction in tryptophan biosynthesis (Fig. 1), in which indole-3-glycerol phosphate (IGP) is formed from

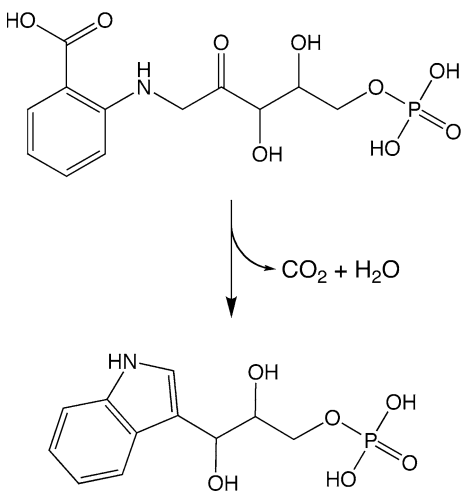

Fig. 1 The IGPS-catalyzed reaction. IGPS catalyzes the conversion of CdRP (*top*) into IGP (*bottom*). The formation of the pyrrole ring of the indole, together with the release of CO_2, effectively render the reaction irreversible

1-(*o*-carboxyphenylamino) 1-deoxyribulose 5-phosphate (CdRP). We have cloned, expressed, purified and characterized the *P. aeruginosa* IGPS enzyme (PaIGPS). It is highly active in vitro, with a broad pH-activity profile and a turnover number which exceeds that reported for any other IGPS. We have deleted the *trpC* gene from the *P. aeruginosa* PAO1 chromosome, and measured the effect of its deletion on the bacterium's fitness in synthetic CF sputum medium (SCFM; [28]). Overall, our study provides the first kinetics data for any IGPS from the genus *Pseudomonas*, and suggests that inhibiting tryptophan biosynthesis may have deleterious effects on the growth of *P. aeruginosa* in the lungs of CF patients.

2 Materials and Methods

2.1 Materials

All molecular biology enzymes were purchased from New England Biolabs (Ipswich, MA, USA). Oligonucleotides were from Integrated DNA Technologies (Coralville, IA, USA). 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) was from Melford Laboratories (Ipswich, Suffolk, UK). Isopropyl-β-D-thiogalactopyranoside (IPTG) was from Gold Biotechnology (St. Louis, MO, USA). Benzonase nuclease was from EMD Chemicals (San Diego, CA, USA). Talon metal affinity resin was from Clontech (Mountain View, CA, USA). Protease inhibitor cocktail, chicken egg white lysozyme and anthranilate were from Sigma Chemical Co. (St. Louis, MO, USA). 5-Phosphoribosyl-1-pyrophosphate (PRPP) was from Carbosynth (Compton, Berkshire, UK).

2.2 Cloning, Expression and Purification of PaIGPS

The *trpC* gene, encoding PaIGPS, was amplified from 1 µL of an overnight culture of *P. aeruginosa* PAO1, using Phusion polymerase and the primers Pse_trpC.for (5′-AACGACATATGAGTGTGCCGACGGTTCTG-3′; NdeI site underlined) and Pae_trpC.rev (5′-AGCGCACTAGTGTCAGGATCGGCGCCCAGC-3′; SpeI site underlined). The product was cloned into expression vector pLAB101, using NdeI and SpeI. This vector had been constructed previously, by modifying the cloning cassette of pMS401 [29] to incorporate NdeI and SpeI sites. The resulting plasmid, pLAB101-*trpC*, was used to transform the expression host, *E. coli* strain KK-8(pDM). This strain lacks the entire *trp* operon [17], ensuring that *E. coli* IGPS could not contaminate our enzyme preparations.

PaIGPS expression was induced in mid-log phase cultures ($OD_{600} \approx 0.5$), by adding IPTG (1 mM). The cultures were incubated at 28 °C for an additional 5 h. Cells

were harvested by centrifugation and the pellets stored at −80 °C. Each pellet (~3.0 g wet cell weight from 250 mL culture) was resuspended in 10 mL of lysis buffer (50 mM potassium phosphate, 300 mM NaCl and 1 mM β-mercaptoethanol, pH 7.0). Protease inhibitor cocktail (150 µL), Benzonase nuclease (37.5 U) and lysozyme (0.2 mg mL^{-1}, final concentration) were added. After 20 min incubation at 4 °C, cells were lysed by sonication on ice, and the lysates were clarified by centrifugation (21,000*g*, 4 °C, 30 min). The clarified lysate was mixed with 900 µL Talon metal affinity resin (50 % w/v slurry) and the mixture was gently agitated at 4 °C for 60 min to allow the His_6-tagged protein to bind the resin. The resin was washed twice with 10 bed volumes of lysis buffer, before being transferred to a gravity flow column. After two further washes with 5 bed volumes of lysis buffer containing 5 mM imidazole and 10 mM imidazole, respectively, the purified protein was eluted with 5 bed volumes elution buffer (50 mM potassium phosphate, 300 mM NaCl, 1 mM β-mercaptoethanol and 150 mM imidazole, pH 7.0). Amicon Ultra centrifugal filter units (10 kDa molecular weight cut-off; Millipore, Billerica, MA) were used to exchange the purified protein into storage buffer (40 mM Tris–HCl, 200 mM NaCl, 0.5 mM $MgCl_2$, 2 mM β-mercaptoethanol, 2 mM ethylenediaminetetraacetic acid, pH 8.0). Aggregates were removed by filtration through a sterile 0.22 µm filter (Millex-GV; Millipore). Sterile glycerol was added to a final concentration of 10 % (v/v). PaIGPS concentrations were quantified by measuring A_{280} (ε = 5,960 M^{-1} cm^{-1}, calculated according to [27]). Aliquots were stored at −80 °C. Activity assays verified that these storage conditions, combined with a freeze/thaw cycle, did not lead to any loss of activity.

2.3 Activity Assays

PaIGPS activity was quantified using a spectrophotometric assay [19]. To determine the pH-activity profile at 25 °C, assays (total volume, 500 µL) were done with PaIGPS (50 nM) in 200 mM NaCl, 0.5 mM $MgCl_2$ and 0.5 mM β-mercaptoethanol that was buffered with Tris–HCl and Tris base (50 mM, total concentration). Tris–HCl and Tris base were mixed in appropriate ratios to generate buffers at pH = 7.0, 7.4, 7.8, 8.0, 8.2, 8.6 and 9.0. The pH of each buffer was verified at its final ionic strength and composition. The *Acinetobacter baylyi* TrpD and *E. coli* TrpF enzymes (both 1 µM) were used to synthesize the substrate, CdRP, in the cuvette at a concentration of 100 µM, from a corresponding amount of anthranilate and a five-fold molar excess of PRPP. The expression and purification of TrpD and TrpF have been described previously [30]. The rate of formation of the product, IGP, was followed by measuring the increase in A_{278} (ε_{IGP} = 5,590 M^{-1} cm^{-1}, based on the measurement for N–Ac-Trp-NH_2 [27]).

Steady-state kinetic parameters at 37 °C were determined using the assay described above, except that PaIGPS was present at 10 nM concentration, and the reaction was buffered by 50 mM Tris–HCl, pH 8.0 (adjusted so that pH = 8.0 at 37 °C, using ΔpH = –0.03 units/ °C). Controls showed activity to be proportional to enzyme concentration. Initial reaction rates were measured with seven substrate concentrations, in the range 0–100 µM. Measurements were made in triplicate and corrected for background. Assays were repeated with two batches of PaIGPS, purified from separate cultures. Kinetic parameters (k_{cat} and K_M) were determined by fitting data directly to the Michaelis–Menten equation, using non-linear regression analysis in GraphPad Prism (GraphPad, La Jolla, CA, USA). Values are reported as the mean ± standard error.

2.4 Generation of *P. aeruginosa* Δ*trpC*

The *trpC* gene was deleted from the *P. aeruginosa* PAO1 chromosome using gene splicing by overlap extension PCR (SOE-PCR) and two-step allelic exchange. First, a knockout construct was made by amplifying ~800 bp from the regions that flank the 5′ and 3′ ends of *trpC*, using the PCR primer pairs: MLG200.for (5′-AGATCTCGAACATCTTCAACGTGTCCTC–3′, BglII site underlined) and MLG201.rev (5′-GTTCGACAGCGCGGATCACTGTGCGTTCTCCTC-3′); and MLG202.for (5′-GGAGAACGCACAGTGATCCGCGCTGTCGAACCCCAT-3′) and MLG203.rev (5′-ACTAGTTCGCCTTGTACGTCAGGCATATTC-3′, SpeI site underlined). The two PCR products were assembled using SOE-PCR with the outside primers MLG200.for and MLG203.rev. The resulting product (~1.6 kb) was TA-cloned into pCR8/GW/TOPO (Invitrogen), confirmed by sequencing and then subcloned into the suicide vector pUIC3 [31], using BglII and SpeI. The method for introducing the resulting construct, pUIC3-Δ*trpC*, into *P. aeruginosa* PAO1, and then screening for colonies with the markerless Δ*trpC* mutation, was described previously [12]. The deletion was confirmed by PCR and DNA sequencing.

2.5 Competitive Fitness Assays

Freezer stocks of *P. aeruginosa* PAO1 Δ*trpC*, and a PAO1 strain that was neutrally marked with *lacZ* [12], were struck on LB agar to obtain single colonies. Eight colonies of each strain were used to inoculate 5 mL aliquots of SCFM [28]; another eight of each were picked into SCFM that had been supplemented with tryptophan to a final concentration of 100 µM; and a final eight colonies of each were picked into LB medium. Each clone was acclimated to its competition

environment at 37 °C for 24 h. Competitions were started by mixing a 2.5 µL aliquot of a PAO1 Δ*trpC* culture with a 2.5 µL aliquot of a *lacZ*-marked PAO1 culture, in 5 mL of fresh medium (*i.e.* either SCFM, SCFM + Trp, or LB). Initial and final frequencies of each competitor were determined by plating on LB agar supplemented with X-gal (40 µg mL^{-1}), at $t = 0$ h and $t = 24$ h. Across the 24 fitness assays (3 conditions × 8 replicates), the initial inoculum for each strain at $t = 0$ h gave an average cell density of $(2.1 \pm 0.2) \times 10^6$ cells mL^{-1} (mean ± SEM). For each replicate, the fitness of PAO1 Δ*trpC*, relative to *lacZ*-marked PAO1, was expressed as the ratio of the two strains' Malthusian parameters [22].

3 Results

3.1 Expression and Purification of PaIGPS

We amplified the *P. aeruginosa trpC* gene and cloned it into expression vector pLAB101. This vector encodes a C-terminal His_6 tag and a three-residue linker (Thr-Ser-Gly) between PaIGPS and the tag. We expressed PaIGPS in *E. coli* and purified the recombinant protein from the soluble cell lysate, using immobilized metal affinity chromatography (IMAC). The protein was expressed to very high levels, and was highly soluble. As a result, we routinely obtained ~30 mg of purified PaIGPS per liter of bacterial culture. This yield is 5–10 times greater than those reported from heterologously expressing other IGPS enzymes [8, 9, 15, 24], including from our own efforts to express and purify the *E. coli* enzyme [30]. SDS-PAGE showed a protein of the expected molecular mass (31.4 kDa) and >95 % purity (Fig. 2a).

3.2 Steady-State Kinetics

The activity of purified PaIGPS was measured by monitoring the absorbance increase at 278 nm, upon conversion of CdRP to the indole-containing product, IGP. Before determining the kinetic parameters of the enzyme, we investigated the pH dependence of its activity (Fig. 2b). The assays revealed a broad shoulder of maximum activity, between pH 7.0 and pH 8.2. Activity began to decrease above pH 8.2, and was 70 % of maximum at pH 9.0. This decrease at the basic limb of the plot is consistent with the known mechanism of IGPS, where the ε-amino group of an active site lysine must be protonated, in order to act as a general acid during catalysis [16].

Initial assays with substrate concentrations in the range 0-300 µM showed that PaIGPS is ~2-fold more active at the optimum growth temperature of *P. aeruginosa* (37 °C) than it is at 25 °C. Therefore, we determined the steady-state

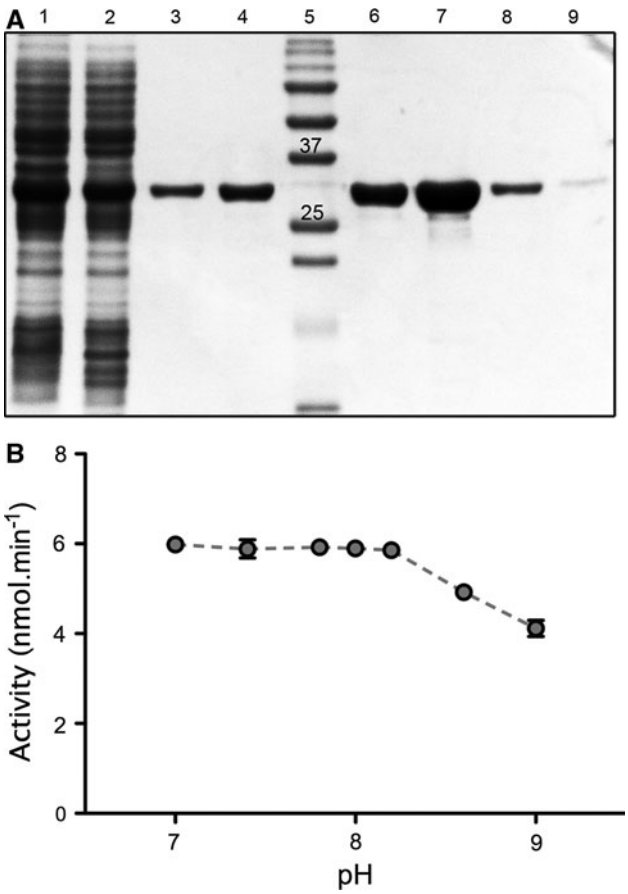

Fig. 2 Purification and characterization of PaIGPS-His_6. **a** The enzyme was purified using IMAC and visualized with Coomassie blue after separation on a 12 % SDS-PAGE gel. *Lane 1* whole cell extract. *Lane 2* soluble fraction. *Lanes 3–4* 5 and 10 mM imidazole washes. *Lane 5* molecular weight marker (Bio-Rad Precision Plus; 37 and 25 kDa markers are indicated). *Lanes 6–9* elution fractions. **b** pH dependence of PaIGPS. Measurements were made in triplicate and corrected for background. Where not visible, *error bars* are contained within the symbol

kinetic parameters of PaIGPS at 37 °C and pH 8.0. The enzyme was highly active, with a turnover number (k_{cat}) of 11.1 ± 0.1 s^{-1} and a K_M for CdRP of 11.3 ± 0.2 µM. While the K_M is higher than the sub-micromolar values reported for the *E. coli*, *Thermotoga maritima* and *Sulfolobus solfataricus* enzymes, the turnover number for PaIGPS is the highest reported to date (Table 1). Overall, the catalytic efficiency of PaIGPS ($k_{cat}/K_M = 9.8 \times 10^5$ M^{-1} s^{-1}) is comparable to those reported previously for other IGPS enzymes.

We have shown that PaIGPS can be expressed at very high levels in *E. coli*, that it is amenable to one-step, high-yield purification, that it is active over a range of temperatures, and that it has a broad pH-activity profile. In addition to its importance as a component of a key biosynthetic pathway in a pathogen, we therefore anticipate that PaIGPS will be useful in coupled activity assays for other enzymes in tryptophan biosynthesis.

Table 1 Steady-state kinetic parameters of microbial IGPS enzymes

Source organism	Sequence identity* (%)	Assay temperature (°C)	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M (M^{-1} s^{-1})	References
P. aeruginosa	100	37	11.1 ± 0.1	11.3 ± 0.2	9.8×10^5	This work
E. coli	38	25	3.6	0.42	8.6×10^6	[9]
M. tuberculosis	37	25	0.16 ± 0.01	55 ± 9	2.9×10^3	[8]
T. maritima	31	60	3.24	0.053	6.1×10^7	[24]
S. solfataricus	28	60	0.98	0.069	1.4×10^7	[15]

*Percent sequence identity with the *P. aeruginosa* PAO1 IGPS protein, calculated using pairwise ClustalW alignments

3.3 Contribution of PaIGPS to Bacterial Fitness

Informed by Garber's nutrition-inhibition model, we set out to test whether inhibiting tryptophan biosynthesis would be a useful strategy for reducing the virulence of *P. aeruginosa* in the CF lung environment. To begin, we constructed a strain of *P. aeruginosa* PAO1 with an unmarked deletion of the annotated *trpC* gene (locus PA0651 [36]). As expected, *P. aeruginosa* Δ*trpC* was unable to grow on Minimal Medium P (MMP; [12]) agar plates, even after extended incubation periods (up to 7 days at 37 °C). In contrast, supplementing MMP with tryptophan (10 μM) resulted in the growth of large colonies (~1 mm diameter) after 24 h incubation. This result confirmed that tryptophan biosynthesis is abolished in the Δ*trpC* strain, and that locus PA0651 is likely to encode the only active IGPS enzyme in the *P. aeruginosa* genome.

Next, the relative fitness (*W*) of the Δ*trpC* strain was measured in competition with the wild-type PAO1 ancestor, in three different growth media (Fig. 3). There was no cost associated with the *trpC* deletion in tryptophan-replete Luria–Bertani (LB) medium ($W = 1.03 \pm 0.02$, $P = 0.19$). In contrast, the Δ*trpC* strain had a significantly reduced fitness in SCFM ($W = 0.89 \pm 0.02$, $P = 0.001$). This fitness defect was abolished when SCFM was supplemented with tryptophan to a final concentration of 100 μM ($W = 0.99 \pm 0.01$, $P = 0.53$). SCFM medium accurately mimics the nutritionally rich growth environment of CF sputum [28]. It contains some tryptophan (10 μM), but the implication from our results is that this is insufficient to support maximal *P. aeruginosa* growth rates, without additional tryptophan that is synthesized by the bacterium de novo.

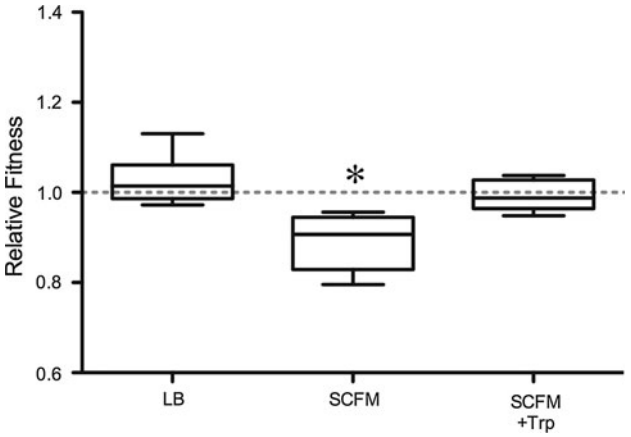

Fig. 3 Relative fitness, *W*, of PAO1 Δ*trpC* in competition with *lacZ*-marked PAO1. Values <1 (*dashed line*) indicate a lower relative fitness of the Δ*trpC* strain. Data are from eight replicates in each of three environments: Luria–Bertani broth (LB); synthetic CF sputum medium (SCFM); and SCFM with 100 μM tryptophan (SCFM + Trp). Key: median, *horizontal lines in boxes*; interquartile range, *boxes*; 90th and 10th percentiles, *vertical bars*. Median values with significant deviation from 1 ($P < 0.01$ for a two-tailed test with the null hypothesis that $W = 1$, calculated using the *t* distribution and 7 d.f.) are indicated (*asterisk*)

3.4 Homology Model of PaIGPS

If PaIGPS can be validated as a drug target, then knowledge of its structure will be essential for designing inhibitors. Structures have been reported for the IGPS enzymes from *E. coli* [35], *S. solfataricus* [15] and *T. maritima* [21]. Each possesses the common $(\beta\alpha)_8$ barrel architecture, and each is functional as a monomer (albeit fused to the *trpF* gene product, phosphoribosylanthranilate isomerase, in *E. coli*). PaIGPS (NCBI accession number NP_249342) comprises 278 amino acids and shows 38 % sequence identity with the *E. coli* enzyme. It is less similar in sequence to the other structurally-characterized IGPS enzymes (Table 1).

We submitted the PaIGPS sequence to the Phyre2 protein structure prediction server [18]. Predictably, Phyre2 could use the *E. coli* structure (PDB ID 1pii) to model 99 % of the residues in PaIGPS at >90 % confidence (Fig. 4). In the *E. coli* and *S. solfataricus* enzymes, 15 active site residues make contacts with the substrate (CdRP) and/or product (IGP) [16]. A structure-based alignment suggests that 13 of these amino acids are identical in the *E. coli* and *P. aeruginosa* enzymes, with two conservative substitutions (I146 in PaIGPS replaces M137 in *E. coli* IGPS; and E248 replaces S237). Three particularly important catalytic

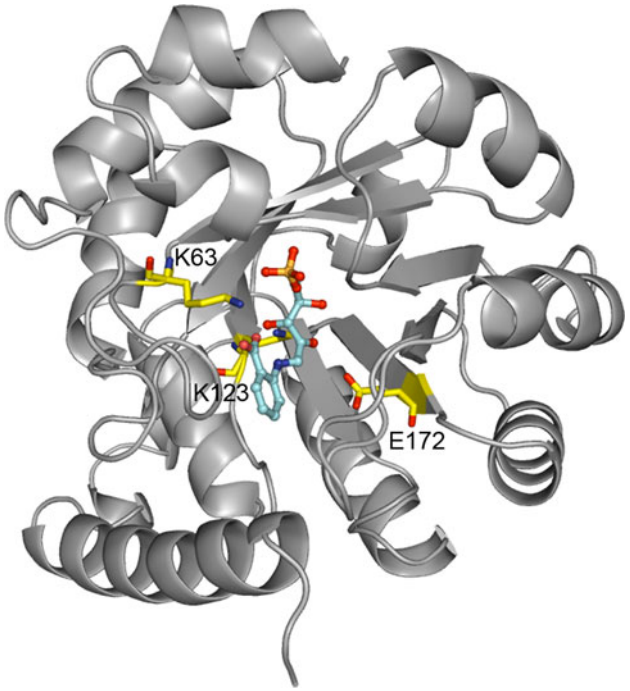

Fig. 4 Homology model of the PaIGPS structure. Residues predicted to be important for catalysis are highlighted as sticks (*yellow backbone*). The CdRP substrate (*blue backbone*) was modeled into the active site by overlaying the PaIGPS structure on the structure of the CdRP-bound *S. solfataricus* structure (PDB ID 1lbl)

residues are a lysine acting as a general acid, a glutamate acting as a general base, and a lysine that forms a salt bridge to the anthranilate moiety of the substrate [16]. Our model identifies these residues as K123, E172 and K63, respectively, in PaIGPS (Fig. 4).

4 Discussion

We have described the first experiments characterizing an enzyme from the tryptophan biosynthesis pathway of the opportunistic pathogen, *P. aeruginosa*. PaIGPS is highly active, with a k_{cat} exceeding those for any other characterized IGPS (Table 1). It has been hypothesized that enzymes evolve to maximize k_{cat} when they are substrate-saturated [20]. Moreover, it has been shown that the majority of *E. coli* biosynthetic enzymes are substrate-saturated; that is, the intracellular concentrations of their substrates are greater than their K_M values [1]. Therefore, it seems reasonable to infer that the kinetic parameters for PaIGPS indicate a particularly active pathway for tryptophan biosynthesis in *P. aeruginosa*.

If evolution has acted to maximize flux through the tryptophan biosynthesis pathway in *P. aeruginosa*, then it must reflect adaptation to at least one of the environments inhabited by the bacterium (including soil and water, in addition to animal tissues). We have reported the first evidence that abolishing tryptophan biosynthesis reduces the fitness of *P. aeruginosa* in conditions that are designed to mimic the CF lung environment (Fig. 3).

In keeping with Garber's nutrition-inhibition model [10], it is tempting to speculate that the heaviest biosynthetic demands on *P. aeruginosa* are associated with initiating a lung infection, and with the associated growth to very high cell densities in CF sputum. The decrease in fitness that we observed ($W = 0.89$) corresponds to a two-fold reduction in cell numbers (compared to the competitor, *lacZ*-marked PAO1) over the course of 24 h growth in SCFM. Put another way, wild-type *P. aeruginosa* PAO1 underwent an average of 9.0 divisions in 24 h, while on average, the competing Δ*trpC* strain completed 8.0 divisions. Previous models have shown that even smaller reductions in fitness can be critical for preventing the establishment of *E. coli* urinary tract infections [14, 26]. In *P. aeruginosa*, deleting the gene for a candidate drug target from the alanine utilization pathway (*dadA*) showed a similar fitness effect to the one that we observed for our Δ*trpC* strain. Over 7 days of growth in rat lungs, the *P. aeruginosa* Δ*dadA* strain completed ~1.6 fewer divisions than wild-type PAO1 [2]. In contrast, however, deleting genes involved in histidine utilization resulted in no loss of fitness in SCFM [12].

To our knowledge, the data in Fig. 3 are the first evidence—albeit preliminary—that the amino acid biosynthesis enzymes of *P. aeruginosa* may be useful drug targets. Ultimately, it will be necessary to assess the fitness of our Δ*trpC* strain in a rat infection model, as described previously for *P. aeruginosa* Δ*dadA* [2]. With this in mind, it is noteworthy that SCFM was formulated by averaging the compositions of 12 sputum samples from CF patients. While the *average* tryptophan concentration was 10 μM (and therefore, this was the concentration used in the recipe for SCFM), tryptophan was actually only detected in three of the 12 sputum samples [28]. The complete absence of tryptophan in the other nine sputum samples suggests that, in many clinical cases, inhibiting PaIGPS may be even more deleterious to the growth of *P. aeruginosa* than we observed in SCFM. Our result is also consistent with microarray data showing that *trp* genes are induced during growth at high cell densities in the lungs of CF patients [34].

Overall, the implication from this study is that inhibiting PaIGPS (or other enzymes for tryptophan biosynthesis) may be a useful therapeutic strategy for alleviating *P. aeruginosa* infections, particularly in the lungs of CF patients. A novel inhibitor of the *M. tuberculosis* IGPS has recently been identified [32], providing a starting point for future drug discovery efforts. We are optimistic that improving our knowledge of the amino acid biosynthesis

pathways in *P. aeruginosa* will offer new insights and targets for chemotherapy.

Acknowledgments The authors gratefully acknowledge financial support for this work from the Marsden Fund.

References

1. Bennett BD, Kimball EH, Gao M, Osterhout R, Van Dien SJ, Rabinowitz JD (2009) Nat Chem Biol 5:593–599
2. Boulette ML, Baynham PJ, Jorth PA, Kukavica-Ibrulj I, Longoria A, Barrera K, Levesque RC, Whiteley M (2009) J Bacteriol 191:6329–6334
3. Breidenstein EB, de la Fuente-Núñez C, Hancock RE (2011) Trends Microbiol 19:419–426
4. Brown GM (1962) J Biol Chem 237:536–540
5. Brown SA, Palmer KL, Whiteley M (2008) Nat Rev Microbiol 6:657–666
6. Cooper MA, Shlaes D (2011) Nature 472:32
7. Crawford IP (1989) Annu Rev Microbiol 43:567–600
8. Czekster CM, Neto BA, Lapis AA, Dupont J, Santos DS, Basso LA (2009) Arch Biochem Biophys 486:19–26
9. Eberhard M, Tsai-Pflugfelder M, Bolewska K, Hommel U, Kirschner K (1995) Biochemistry 34:5419–5428
10. Garber ED (1956) Am Nat 90:183–194
11. Garber ED (1960) Ann N Y Acad Sci 88:1187–1194
12. Gerth ML, Ferla MP, Rainey PB (2012) Environ Microbiol doi: 10.1111/j.1462-2920.2011.02691.x [Epub ahead of print]
13. Gómez MI, Prince A (2007) Curr Opin Pharmacol 7:244–251
14. Gordon DM, Riley MA (1992) Mol Microbiol 6:555–562
15. Hennig M, Darimont B, Sterner R, Kirschner K, Jansonius JN (1995) Structure 3:1295–1306
16. Hennig M, Darimont BD, Jansonius JN, Kirschner K (2002) J Mol Biol 319:757–766
17. Ivens A, Mayans O, Szadkowski H, Wilmanns M, Kirschner K (2001) Eur J Biochem 268:2246–2252
18. Kelley LA, Sternberg MJ (2009) Nat Protoc 4:363–371
19. Kirschner K, Szadkowski H, Jardetzky TS, Hager V (1987) Methods Enzymol 142:386–397
20. Klipp E, Heinrich R (1994) J Theor Biol 171:309–323
21. Knöchel T, Pappenberger A, Jansonius JN, Kirschner K (2002) J Biol Chem 277:8626–8634
22. Lenski RE, Rose MR, Simpson SC, Tadler SC (1991) Am Nat 138:1315–1341
23. Merino E, Jensen RA, Yanofsky C (2008) Curr Opin Microbiol 11:78–86
24. Merz A, Knöchel T, Jansonius JN, Kirschner K (1999) J Mol Biol 288:753–763
25. Nalca Y, Jänsch L, Bredenbruch F, Geffers R, Buer J, Häussler S (2006) Antimicrob Agents Chemother 50:1680–1688
26. Nilsson AI, Berg OG, Aspevall O, Kahlmeter G, Andersson DI (2003) Antimicrob Agents Chemother 47:2850–2858
27. Pace CN, Vajdos F, Fee L, Grimsley G, Gray T (1995) Protein Sci 4:2411–2423
28. Palmer KL, Aye LM, Whiteley M (2007) J Bacteriol 189: 8079–8087
29. Patrick WM, Blackburn JM (2005) FEBS J 272:3684–3697
30. Patrick WM, Matsumura I (2008) J Mol Biol 377:323–336
31. Rainey PB (1999) Environ Microbiol 1:243–257
32. Shen H, Wang F, Zhang Y, Huang Q, Xu S, Hu H, Yue J, Wang H (2009) FEBS J 276:144–154
33. Smith DA, Parish T, Stoker NG, Bancroft GJ (2001) Infect Immun 69:1142–1150
34. Son MS, Matthews WJ Jr, Kang Y, Nguyen DT, Hoang TT (2007) Infect Immun 75:5313–5324
35. Wilmanns M, Priestle JP, Niermann T, Jansonius JN (1992) J Mol Biol 223:477–507
36. Winsor GL, Lam DK, Fleming L, Lo R, Whiteside MD, Yu NY, Hancock RE, Brinkman FS (2011) Nucleic Acids Res 39:D596–D600