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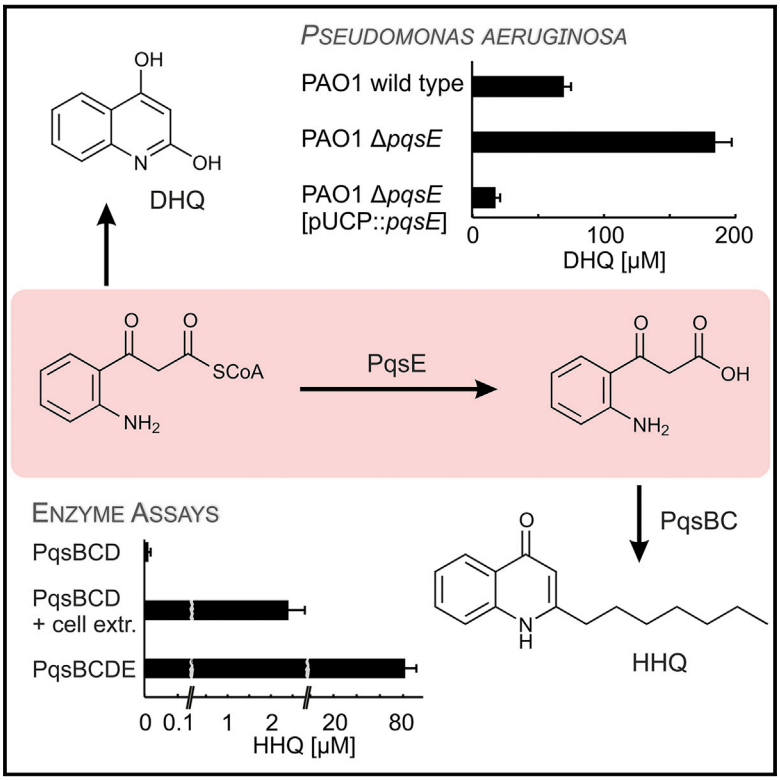
PqsE of *Pseudomonas aeruginosa* Acts as Pathway-Specific Thioesterase in the Biosynthesis of Alkylquinolone Signaling Molecules

Graphical Abstract



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In Brief

Drees and Fetzner demonstrate that PqsE of *Pseudomonas aeruginosa* acts as thioesterase in alkylquinolone biosynthesis. By hydrolyzing the intermediate 2-aminobenzoylacetyl-CoA, which tends to decompose to 2,4-dihydroxyquinoline, PqsE balances the levels of quorum-sensing signal molecules and secondary metabolites deriving from this pathway.

Highlights

- The biosynthesis of 2-alkyl-4(1*H*)-quinolones (AQs) was reconstituted in vitro
- Contrary to the current notion, PqsE contributes to AQ synthesis besides PqsABCD
- PqsE hydrolyzes the biosynthetic intermediate 2-aminobenzoylacetyl-coenzyme A
- PqsE balances the levels of metabolites deriving from the AQ biosynthetic pathway

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PqsE of *Pseudomonas aeruginosa* Acts as Pathway-Specific Thioesterase in the Biosynthesis of Alkylquinolone Signaling Molecules

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SUMMARY

Pseudomonas aeruginosa **uses the alkylquinolones PQS (2-heptyl-3-hydroxy-4(1*H*)-quinolone) and HHQ (2-heptyl-4(1*H*)-quinolone) as quorum-sensing signal molecules, controlling the expression of many virulence genes as a function of cell population density. The biosynthesis of HHQ is generally accepted to require the *pqsABCD* gene products. We now reconstitute the biosynthetic pathway in vitro, and demonstrate that in addition to PqsABCD, PqsE has a role in HHQ synthesis. PqsE acts as thioesterase, hydrolyzing the biosynthetic intermediate 2-aminobenzoylacetyl-coenzyme A to form 2-aminobenzoylacetate, the precursor of HHQ and 2-aminoacetophenone. The role of PqsE can be taken over to some extent by the broad-specificity thioesterase TesB, explaining why the *pqsE* deletion mutant of *P. aeruginosa* still synthesizes HHQ. Interestingly, the *pqsE* mutant produces increased levels of 2,4-dihydroxyquinoline, resulting from intramolecular cyclization of 2-aminobenzoylacetyl-coenzyme A. Overall, our data suggest that PqsE promotes the efficiency of alkylquinolone signal molecule biosynthesis in *P. aeruginosa* and balances the levels of secondary metabolites deriving from the alkylquinolone biosynthetic pathway.**

INTRODUCTION

The γ-proteobacterium *Pseudomonas aeruginosa* is a ubiquitous opportunistic pathogen frequently associated with nosocomial infections, especially among immunocompromised individuals. In patients with cystic fibrosis, it is the leading cause of morbidity and mortality (Emerson et al., 2002). Once established in the host, it produces a large array of virulence factors and forms biofilms that are difficult to combat (Gellatly and Hancock, 2013; Taylor et al., 2014). Synthesis of numerous virulence factors is controlled by a process called quorum sensing (QS), whereby the bacteria communicate via diffusible chemical signal molecules to coordinate their behavior within the population. The sophisticated QS network of *P. aeruginosa* involves the two *N*-acylhomoserine lactone-based Las and Rhl systems, and the Pqs system that is based on specific 2-*n*-alkyl-4(1*H*)-quinolones (AQs). 2-Heptyl-3-hydroxy-4(1*H*)-quinolone, termed the *Pseudomonas* quinolone signal (PQS), is the major AQ signal, but its biosynthetic precursor 2-heptyl-4(1*H*)-quinolone (HHQ) also acts as signal molecule (Déziel et al., 2004; Pesci et al., 1999; Xiao et al., 2006). AQ signaling is involved in the control of virulence factor production and influences biofilm maturation (reviewed in Heeb et al., 2011; Huse and Whiteley, 2011; Nadal Jimenez et al., 2012).

The AQ signaling molecules belong to a family of more than 50 compounds that share the 4-hydroxy-2-alkylquinoline structure (Lépine et al., 2004). Biosynthesis of AQs is generally accepted to require the anthranilate-coenzyme A (CoA) ligase PqsA (Coleman et al., 2008), the condensing enzyme PqsD, which has been proposed to form 2-aminobenzoylacetyl-CoA from anthraniloyl-CoA and malonyl-CoA (Zhang et al., 2008), and the PqsBC protein involved in coupling of 2-aminobenzoylacetate (2-ABA) to an octanoyl moiety to produce HHQ (Dulcey et al., 2013) (Figure 1). Two other bioactive secondary metabolites, 2-aminoacetophenone (2-AA) and 2,4-dihydroxyquinoline (DHQ), also derive from the AQ biosynthetic pathway. Formation of DHQ was shown to require PqsA and PqsD (Zhang et al., 2008), and 2-AA is thought to result from decarboxylation of 2-ABA (Dulcey et al., 2013) (Figure 1). While DHQ does not act as a signal in the AQ-based QS system and apparently has no antimicrobial effects (Lépine et al., 2007), it was observed to inhibit the viability of mouse lung epithelial cells, suggesting that it contributes to the pathogenicity of *P. aeruginosa* infection (Zhang et al., 2008). 2-AA promotes chronic infection phenotypes of *P. aeruginosa* by silencing acute virulence functions (Kesarwani et al., 2011) and by mediating persister cell accumulation (Que et al., 2013). Moreover, it modulates the innate immune response of mammalian hosts (Bandyopadhaya et al., 2012) and mediates host metabolic dysregulation that results in mitochondrial dysfunction (Tzika et al., 2013).

HHQ biosynthesis in a heterologous background requires expression of the *pqsABCD* genes (Niewerth et al., 2011; Xiao et al., 2006). However, in *P. aeruginosa* these genes form an operon together with *pqsE*. Mutants of *P. aeruginosa* deficient in *pqsE* produce wild-type levels of PQS and HHQ (Déziel et al., 2004; Gallagher et al., 2002), an observation that supported the notion that PqsE is not involved in the AQ biosynthetic pathway. PqsE was termed the "PQS response protein" because its disruption negatively affected the production of some PQS-mediated exoproducts such as pyocyanin and rhamnolipid (Déziel et al., 2005; Diggle et al., 2003; Farrow et al., 2008;