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Crystallization and preliminary X-ray analysis of the C-terminal fragment of PorM, a subunit of the *Porphyromonas gingivalis* type IX secretion system

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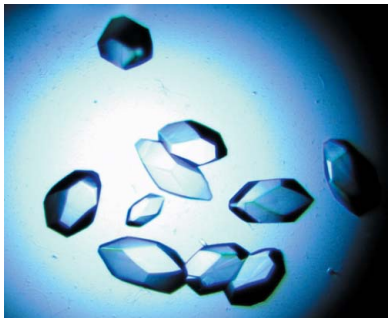
PorM is a membrane protein involved in the assembly of the type IX secretion system (T9SS) from *Porphyromonas gingivalis*, a major bacterial pathogen responsible for periodontal disease in humans. The periplasmic domain of PorM was overexpressed in *Escherichia coli* and purified. A fragment of the purified protein was obtained by limited proteolysis. Crystals of this fragment belonged to the tetragonal space group $P4_32_12$. Native and MAD data sets were recorded to 2.85 and 3.1 Å resolution, respectively, using synchrotron radiation.

1. Introduction

Periodontal disease, the major cause of tooth loss in industrial nations, is one of the most frequently occurring infectious diseases in humans (Armitage, 1996). The Gram-negative anaerobic bacterium *Porphyromonas gingivalis* is a major periodontal pathogen (Bostanci & Belibasakis, 2012). Tissue damage caused by *P. gingivalis* is mainly induced by a cocktail of secreted specialized toxin proteins, the gingipains (Fitzpatrick *et al.*, 2009). The active release of gingipains at the bacterial cell surface is catalyzed by a recently identified multi-protein complex called the type IX secretion system (T9SS; Sato *et al.*, 2010). This secretion system is composed of at least 14 subunits that are thought to assemble a trans-envelope channel that specifically recruits the gingipains and transports them to the cell surface (Sato *et al.*, 2010; McBride & Zhu, 2013). In *P. gingivalis*, 16 genes have been shown to be involved in gingipain cell-surface display. Among these genes, *porX* and *porY* encode a two-component system that is thought to positively regulate the expression of the 14 structural genes *porK*, *porL*, *porM*, *porN*, *porP*, *porQ*, *porT*, *porU*, *porV*, *porW*, PG26, PG27, PG0534 and *sov* (Ishiguro *et al.*, 2009; Sato *et al.*, 2010; Glew *et al.*, 2012; Saiki & Konishi, 2010*a*). Although 14 components of this secretion system have been identified to date, very little is known about the structure of the trans-envelope apparatus and its mechanism of recruitment, selection and transport of gingipains. The PorT and Sov proteins fractionate with the outer membrane (Nguyen *et al.*, 2009; Saiki & Konishi, 2010*b*), while the membrane-associated PorK, PorL, PorM and PorN proteins are part of a >1.2 MDa membrane complex (Sato *et al.*, 2010).

Here, we present the crystallization and preliminary X-ray analysis of the C-terminal fragment of the recombinant PorM periplasmic domain.



2. Materials and methods

2.1. Macromolecule production

The sequence corresponding to the periplasmic domain of the PorM protein (residues 36–516; hereafter denoted pPorM) was amplified from *P. gingivalis* ATCC33277 (NCBI Protein and Gene accession Nos. PGN_1674 and gi:188595218, respectively) using the primers 5′-CCGAGAACCTGTACTTCCAATCA*GATGGTTTCG-ACAAAGTGGATAAG* and 5′-CGGAGCTCGAATTCGGATCCT-*TATTAGTTCACAATTACTTCAATGGC* (sequences annealing on the *porM* gene are italicized) and cloned into the pLIC03 vector (kindly provided by BioXtal; unpublished work) (Table 1). The pLIC03 vector was designed for ligation-independent cloning (LIC; Aslanidis & de Jong, 1990) and is a derivative of the pET-28a+