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## Cloning, purification and characterization of the 6-phospho-3-hexulose isomerase YckF from *Bacillus subtilis*

The enzyme 6-phospho-3-hexulose isomerase (YckF) from *Bacillus subtilis* has been prepared and crystallized in a form suitable for X-ray crystallographic analysis. Crystals were grown by the hanging-drop method at 291 K using polyethylene glycol 2000 monomethyl-ether as precipitant. They diffract beyond 1.7 Å using an in-house Cu K $\alpha$  source and belong to either space group  $P6_522$  or  $P6_122$ , with unit-cell parameters  $a = b = 72.4$ ,  $c = 241.2$  Å, and have two molecules of YckF in the asymmetric unit.

Received 29 January 2001

Accepted 4 May 2001

### 1. Introduction

6-Phospho-3-hexulose isomerase (PHI) catalyses the isomerization of D-arabino-3-hexulose-6-phosphate to D-fructose-6-phosphate, as illustrated in Fig. 1. PHI is a key enzyme in the fixation of carbon, in the form of formaldehyde, by methylotrophic bacteria. This process is part of the ribulose monophosphate (RuMP) pathway, the key function of which is the condensation of formaldehyde with ribulose-5-phosphate by 3-hexulose-6-phosphate synthase (HPS) to form D-arabino-3-hexulose-6-phosphate, which in turn is isomerized by PHI to fructose-6-phosphate. The subsequent metabolism of fructose-6-phosphate leads to the regeneration of the pentose phosphate acceptor and the net production of triose phosphate (Strøm *et al.*, 1974). Until recently, it was believed that such enzyme systems were restricted solely to methylotrophic organisms. However, the recent explosion of information arising from genome-sequencing projects has led to the identification of similar genes in non-methylotrophs such as *B. subtilis* (Kunst *et al.*, 1997; Reizer *et al.*, 1997; Yasueda *et al.*, 1999). Investigation at the gene-expression level suggests that in this organism these gene products play a specific role in detoxification. For example, when *B. subtilis* was cultured in the presence of formaldehyde, the expression of PHI (*i.e.* YckF) and HPS (*i.e.* YckG) was induced. The observation that methanol, formate and methylamine did not elicit a similar response and the fact that induction of YckF and YckG expression was dependent on another gene, *yckH*, strongly suggest that these three genes function as a specific detoxification system for formaldehyde in this organism (Yasueda *et al.*, 1999).

The *yckF* gene from *B. subtilis* consists of a 558 bp open reading frame (ORF) which encodes a 19.96 kDa protein (Kunst *et al.*, 1997). YckF exhibits significant homology (34

and 37%, respectively) with functional PHIs from the obligate methylotrophic bacterium *Methylomonas aminofaciens* 77a (Sakai *et al.*, 1999) and the facultative methylotrophic bacterium *Mycobacterium gastri* MB19 (Mitsui *et al.*, 2000; Fig. 2). YckF also shares 22.8% identity with the putative CP0226 gene of *Chlamydomonas pneumoniae* AR39 and three putative genes from *Haemophilus influenzae*, HI0143 (18%), HI1678 (18.6%) and HI0754 (14.8%). Similarity (16.2%) also exists to a fructose-6-phosphate aminotransferase gene, *glmS*, from *B. subtilis*. Although PHI enzymes have been purified and characterized from a range of organisms (Beardmore *et al.*, 1982; Ferenci *et al.*, 1974; Kato *et al.*, 1977; Sakai *et al.*, 1999; Mitsui *et al.*, 2000), little is known about their catalytic mechanism. Metal-ion dependency is a common feature amongst previously characterized sugar isomerases, with cobalt, magnesium and manganese predominantly implicated (Watt, 1998). However, the PHI from *Methylococcus capsulatus* has been shown to be fully active in the absence of cations and to be inhibited by various divalent cations (Ferenci *et al.*, 1974). However, it is currently unknown whether YckF is active in the absence of cations. Despite the importance of PHI in terms of carbon fixation, detoxification and its potential in biotransformation applications, little is known about its structure or function. In this paper, we present the cloning and over-expression of the *yckF* gene using an *Escherichia coli* expression system, together with its purification, crystallization and preliminary X-ray diffraction analysis.

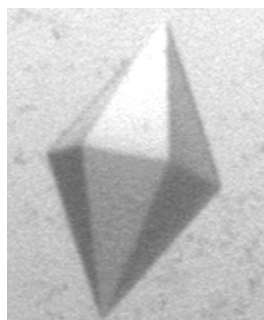
### 2. Experimental

#### 2.1. Cloning, expression and purification

PfuTurbo DNA polymerase (Stratagene) and the oligonucleotide primers 5'-CATAT-



340 nm. Recombinant YckF had a specific activity of 193  $\mu\text{mol}$  of  $\text{NADP}^+$  reduced per minute per milligram of pure enzyme. Crystals of YckF belong to space group  $P6_522$  or  $P6_122$ , with unit-cell parameters  $a = b = 72.4$ ,  $c = 241.2$  Å, and most likely have two protein molecules in the asymmetric unit. Crystals of YckF diffracted very strongly in the home laboratory, but the resolution of such data was constrained to 2.6 Å owing to the long  $c$  axis. 202 840 observations were merged to give 12 384 unique reflections, with an  $R_{\text{merge}}$  ( $\sum_{hkl} \sum_i |I_{hkl} - \langle I_{hkl} \rangle| / \sum_{hkl} \sum_i I_{hkl}$ ) of 0.019, a mean  $I/\sigma(I)$  of 48.9, a multiplicity of observation of 4.2 and a completeness of 94% in the 12–2.6 Å resolution range. The data were very strong, with an  $R_{\text{merge}}$  of 0.027 and a mean  $I/\sigma(I)$  of 32.4 and a multiplicity of observation of 4.4 in the outer resolution shell (2.69–2.6 Å). Assuming that there are two molecules in the asymmetric unit, the crystal packing density was determined to be  $2.07$  Å<sup>3</sup> Da<sup>-1</sup>, which corresponds to a solvent content of 40.2% (Matthews, 1968). Alternatively, though less likely, one molecule in the asymmetric unit



**Figure 3**  
A single crystal of YckF from *B. subtilis*. This hexagonal crystal with dimensions  $0.1 \times 0.1 \times 0.2$  mm was obtained using 20 mM Na HEPES buffer pH 7.4 containing 0.8 M sodium formate, 1 mM EDTA, 25% (w/v) polyethylene glycol 2000 mono-methylether and 10% (v/v) MPD.

corresponds to a density and solvent content of  $4.15$  Å<sup>3</sup> Da<sup>-1</sup> and 70.1%, respectively.

The structure of YckF will allow illumination of the catalytic mechanism and substrate specificity of this important family of proteins and will give an insight into the closely related homologues of unknown function from various pathogenic bacteria such as *H. influenzae* and *C. pneumoniae*. These putative enzymes, although distantly related, appear to possess the same catalytic machinery as YckF from *B. subtilis*. Structural data will also allow the rational design of catalysts for specific biotransformations using PHI with other RuMP pathway enzymes. Selenomethionine-substituted protein is currently being produced to aid rapid structure determination.

The authors would like to thank the BBSRC Wellcome Trust and EU for financial assistance. GJD is a Royal Society University Research Fellow. Collaboration between the Universities of York and Sunderland was assisted by the provision of a BBSRC Structural Biology Centre award to the York Structural Biology Laboratory.

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