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Production, crystallization and preliminary X-ray analysis of CTP:inositol-1-phosphate cytidylyltransferase from *Archaeoglobus fulgidus*

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Archaeoglobus fulgidus, a hyperthermophilic archaeon, accumulates di-*myo*-inositol phosphate (DIP) in response to heat stress. Recently, the pathway for biosynthesis of DIP has been elucidated in this organism and involves a bifunctional enzyme that contains two domains: CTP:inositol-1-phosphate cytidylyltransferase (IPCT) as a soluble domain and di-*myo*-inositol-1,3'-phosphate-1-phosphate synthase (DIPPS) as a membrane domain. Here, the expression, purification, crystallization and preliminary X-ray diffraction analysis of the IPCT domain from *A. fulgidus* in the apo form are reported. The crystals diffracted to 2.4 Å resolution using a synchrotron source and belonged to the orthorhombic space group $P2_12_12$, with unit-cell parameters a = 154.7, b = 83.9, c = 127.7 Å.

Keywords: CTP:inositol-1-phosphate cytidylyltransferase; *Archaeoglobus fulgidus*; compatible solutes; CDP-inositol; di-*myo*-inositol phosphate.

1. Introduction

Di-*myo*-inositol phosphate (DIP) is a compatible solute that is widespread amongst microorganisms adapted to hot environments and has never been found in mesophilic organisms (Santos *et al.*, 2007). In general, DIP increases notably in response to heat stress, suggesting that it plays a role in thermoadaptation (Borges *et al.*, 2010).

A novel pathway for DIP synthesis, which involves a phosphorylated intermediate, has been proposed by Borges *et al.* (2006) and has been fully corroborated by the subsequent identification and characterization of the key genes and enzymes (Rodrigues *et al.*, 2007; Rodionov *et al.*, 2007). The first step is catalysed by CTP:inositol-1-phosphate cytidylyltransferase (IPCT) and involves the condensation of L-*myo*-inositol-1-phosphate and CTP to yield CDP-inositol; this product is coupled to a second molecule of L-*myo*-inositol-1-phosphate to yield the phosphorylated intermediate di-*myo*-inositol-1,3'-phosphate-1-phosphate (DIPP) in a reaction catalyzed by DIPP synthase; finally, DIPP is dephosphorylated to DIP by the action of a phosphatase.

The IPCT and DIPPS (di-*myo*-inositol-1,3'-phosphate-1-phosphate synthase) activities are present in a single polypeptide chain in *Archaeoglobus fulgidus* and in most organisms that are known to accumulate DIP, although the two activities are separated in *Hyperthermus butylicus* and *Thermotoga* spp. The C-terminal DIPPS domain is believed to be membrane-associated, with three predicted transmembrane