FEBS Letters 586 (2012) 3452–3457

FEBS Letters

journal homepage: www.FEBSLetters.org

Structure of the GcpE (IspG)–MEcPP complex from *Thermus thermophilus*

Ingo Rekittke [a], Hassan Jomaa [a,*], Ulrich Ermler [b,*]

[a] Institut für Klinische Immunologie und Transfusionsmedizin, Justus-Liebig-Universität Giessen, Langhansstraße 7, 35392 Giessen, Germany
[b] Max-Planck-Institut für Biophysik, Max-von-Laue-Straße 3, D-60438 Frankfurt am Main, Germany

ARTICLE INFO

Article history:
Received 21 June 2012
Revised 24 July 2012
Accepted 27 July 2012
Available online 9 August 2012

Edited by Stuart Ferguson

Keywords:
Isoprenoid biosynthesis
GcpE
Iron–sulfur cluster
X-ray structure
Drug design

ABSTRACT

Isoprenoid precursor biosynthesis occurs through the mevalonate or the methylerythritol phosphate (MEP) pathway, used i.e., by humans and by many human pathogens, respectively. In the MEP pathway, 2-C-methyl-D-erythritol-2,4-cyclo-diphosphate (MEcPP) is converted to (*E*)-1-hydroxy-2-methyl-but-2-enyl-4-diphosphate (HMBPP) by the iron–sulfur cluster enzyme HMBPP synthase (GcpE). The presented X-ray structure of the GcpE–MEcPP complex from *Thermus thermophilus* at 1.55 Å resolution provides valuable information about the catalytic mechanism and for rational inhibitor design. MEcPP binding inside the TIM-barrel funnel induces a 60° rotation of the [4Fe–4S] cluster containing domain onto the TIM-barrel entrance. The apical iron of the [4Fe–4S] cluster ligates with the C3 oxygen atom of MEcPP.

1. Introduction

Isoprenoids – vital for all organisms – represent a large family (>55 000) of compounds including i.e., dolichol, quinones, carotenoids and sterols [1]. All isoprenoids are derived from two universal precursors – isopentenyl diphosphate and dimethylallyl diphosphate – that are biosynthesized in nature by two different pathways. The well-established mevalonate pathway is used by animals, fungi, archaea and some bacteria and the more recently discovered 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway (Fig. 1) by most bacteria and parasitic protozoa of the phylum apicomplexa [2–4]. Plants utilize both pathways, the mevalonate pathway in the cytosol and the MEP pathway in the plastids. Since humans exclusively use the mevalonate pathway, the enzymes of MEP pathway are attractive targets for the development of antimicrobial and herbicidal drugs [2,5].

The penultimate step of the MEP pathway is the conversion of 2-C-methyl-D-erythritol-2,4-cyclo-diphosphate (MEcPP) into (*E*)-1-hydroxy-2-methyl-but-2-enyl-4-diphosphate (HMBPP) by the HMBPP synthase, termed GcpE or IspG (Fig. 1). The [4Fe–4S] cluster containing enzyme GcpE occurs in two variants distinguished by a molecular mass of ca. 44 and 79 kDa, respectively [6–10]. The physiological electron donor for reducing the iron–sulfur cluster appears to be either a ferredoxin or a flavodoxin depending on the organism [11,12]. Dithionite and/or 5-deazaflavin are frequently used as electron donor for *in vitro* studies [7,8,10]. Various mechanistic proposals involving cationic, radical, anionic, epoxide and ferraoxetane intermediates for the reductive ring-opening reaction have been reported mainly based on EPR and NMR spectroscopic studies [13–16]. The catalytic mechanism appears to be similar in both GcpE variants [17]. X-ray structures of GcpE from *Aquifex aeolicus* and *Thermus thermophilus* published recently [18,19] revealed for the first variant a homodimeric enzyme with each monomer being composed of two spatially separated domains connected by a solvent-exposed linker of five amino acids (286–290) (Fig. 2A, Supplementary Fig. 1). The TIM-barrel domain (4–285) consists of a canonical $(\beta\alpha)_8$ barrel (β1–8, α1–8) enlarged by a N-terminal β-hairpin located at the barrel bottom and a helix-loop-helix protrusion after strand 5. It was assumed that the substrate binding site is situated inside a funnel-shaped pocket created at the C-terminal side of the eight parallel β-strands. The C-terminally fused α/β-domain consists of a five-stranded mixed β-sheet (β1′-5′) flanked by three helices (α1′-3′) that hosts the [4Fe–4S] cluster at the C-terminal strand end of the β2′α2′β3′ motif. Dimeric GcpE is built up by a head-to-tail arrangement of the two subunits such that the α/β-domain of one subunit is attached

Abbreviations: GcpE, (*E*)-1-hydroxy-2-methyl-but-2-enyl-4-diphosphate synthase; MEP, 2-C-methyl-D-erythritol-4-phosphate; MEcPP, 2-C-methyl-D-erythritol-24-cyclo-diphosphate; HMBPP, (*E*)-1-hydroxy-2-methyl-but-2-enyl-4-diphosphate

* Corresponding author. Fax: +49 641 985 41509 (H. Jomaa), fax: +49 69 6303 1002 (U. Ermler).
E-mail addresses: hassan.jomaa@uniklinikum-giessen.de (H. Jomaa), ulrich.ermler@biophys.mpg.de (U. Ermler).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2012.07.070

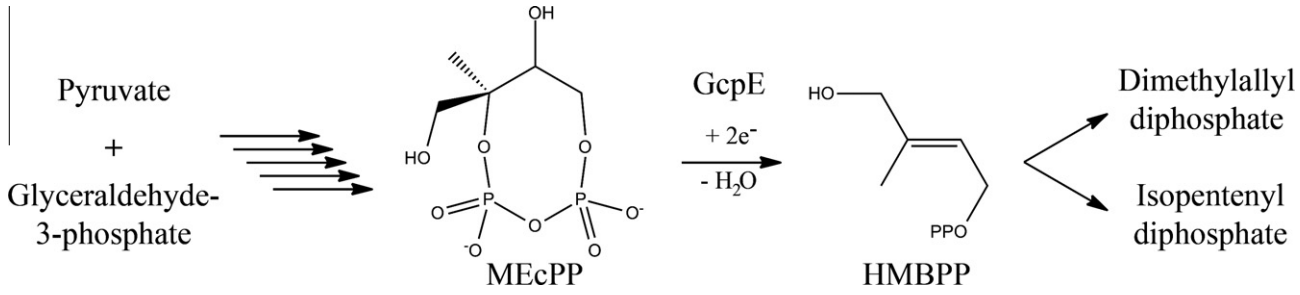

Fig. 1. Reaction of GcpE. GcpE catalyzes the penultimate step of the MEP pathway by converting MEcPP into HMBPP.

to the TIM-barrel domain of the counter one and vice versa [18,19]. In addition, the two TIM-barrel domains form an extended interface and are therefore considered as the rigid core of GcpE (Fig. 2A).

To establish the structural basis for understanding the GcpE reaction and for the design of knowledge-based inhibitors we solved the X-ray structure of the *T. thermophilus* GcpE–MEcPP complex at 1.55 Å resolution. We describe the conformation of the complex in a closed state, the binding mode of the substrate and discuss the mechanistic implications of the structural results.

2. Materials and methods

2.1. Enzyme production

Cloning, expression and purification of GcpE of *T. thermophilus* were described in detail [7,19]. Briefly, the PQETtGcpE vector, containing untagged GcpE, was transformed into TOP10 *E. coli* cells (Invitrogen). Cell cultivation was performed in LB broth medium (Roth) supplemented with 150 μg ml^{-1} ampicilin and 300 μM $FeCl_3$ at 37 °C. DEAE, Source 15Q and Superdex200 columns were applied for purification. GcpE was stored at a concentration of ca. 10 mg ml^{-1} in 30 mM Tris–HCl, pH 7.5 and 150 mM NaCl. All experimental steps after cell cultivation were carried out under oxygen exclusion.

2.2. Crystallization and X-ray structure analysis

Crystallization screens were performed at 18 °C using the sitting drop vapor diffusion method combined with the random microseeding [20], thereby utilizing GcpE-crystals grown in 30% (v/v) MPD and 20% (v/v) ethanol (sitting drop) for seed production [21]. Best diffracting crystals grew in 0.6 μl enzyme solution, containing 5 mM MEcPP, and 0.6 μl precipitant composed of 45% pentaerythritol propoxylate 426, 0.1 M MES pH 6.0, 0.4 M KCl, 0.1% NaN_3 (JBScreen Pentaerythritol 1, C6, Jena Bioscience) and 0.1 μl seed stock. Data were collected at the Swiss-Light source beamline PXII and processed with XDS [22]. Phases were determined by PHASER [23] using the TIM-barrel and α/β-domains as separated search models. The structure was refined using REFMAC [24] and PHENIX [25]. Crystal parameters, data collection and refinement statistics are listed in Supplementary Table 1. Figs. 2–5 were produced with PYMOL (Schrödinger, LLC). The atomic coordinates and structure factors of GcpE–MEcPP have been deposited in the Protein Data Bank, www.pdb.org with ID code 4G9P.

3. Results and discussion

3.1. Global structural differences between the GcpE–MEcPP and GcpE structures

Recombinant untagged *T. thermophilus* GcpE was crystallized in presence of 5 mM MEcPP under strictly anaerobic conditions; the resulting X-ray structure was refined to R/R_{free}-factors of 17.7/20.3% at 1.55 Å resolution (Supplementary Table 1). The asymmetric unit contains one monomer and the functional homodimer is built up by a crystallographic twofold axis present in the space group $P6_522$.

The GcpE–MEcPP complex and (substrate-free) GcpE structures are distinguished by a large-scale rearrangement of the two α/β-domains relative to the two TIM-barrel core (Fig. 2) which turned the homodimeric enzyme from an open and rather mobile into a closed and compact form. The rotation angle is ca. 60° (to open *A .aeolicus* GcpE ca. 50° [18]) considering the TIM-barrel core as fixed reference point. Ala289, a residue of the interdomain linker, serves as the hinge point. In this open-to-closed process already predicted [18,19] and very recently substantiated [17], the α/β-domain parked on the helix-loop-helix protrusion in the open GcpE structure swings from its edge towards the entrance of the counter

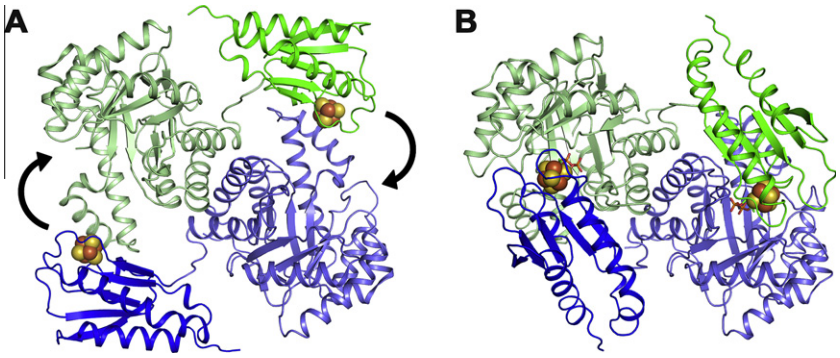

Fig. 2. Open-to-closed transition: (A) GcpE structure without substrate. The active site is formed between the TIM-barrel domain (lightgreen, blue) of one subunit and the α/β-domain of the counter subunit (lightblue, green). The [4Fe–4S] cluster of the α/β-domain is ca. 25 Å apart from the active site. An extended contact area is formed between helices 6, 7 and 8 of the TIM-barrel domains of both subunits. (B) The MEcPP–GcpE structure. The α/β-domain is rotated ca. 60° from the helix-loop-helix protrusion towards the funnel entrance of the TIM-barrel and locks the MEcPP binding site.