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Research review paper

Bacterial diguanylate cyclases: Structure, function and mechanism in exopolysaccharide biofilm development

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ABSTRACT

The ubiquitous bacterial cyclic di-guanosine monophosphate (c-di-GMP) emerges as an important messenger for the control of many bacterial cellular functions including virulence, motility, bioluminescence, cellulose biosynthesis, adhesion, secretion, community behaviour, biofilm formation and cell differentiation. The synthesis of this cyclic nucleotide arises from external stimuli on various signalling domains within the N-terminal region of a dimeric diguanylate cyclase. This initiates the condensation of two molecules of guanosine triphosphate juxtaposed to each other within the C-terminal region of the enzyme. The biofilm from pathogenic microbes is highly resistant to antimicrobial agents suggesting that diguanylate cyclase and its product – c-di-GMP – are key biomedical targets for the inhibition of biofilm development. Furthermore the formation and long-term stability of the aerobic granule, a superior biofilm for biological wastewater treatment, can be controlled by stimulation of c-di-GMP. Any modulation of the synthetic pathways for c-di-GMP is clearly advantageous in terms of medical, industrial and/or environmental bioremediation implications. This review discusses the structure and reaction of individual diguanylate cyclase enzymes with a focus on new directions in c-di-GMP research. Specific attention is made on the molecular mechanisms that control bacterial exopolysaccharide biofilm formation and aerobic granules.

Contents

Introduction

Bacterial biofilms are developed by aggregation of the bacterial cells, enclosed within a matrix of exopolysaccharides (EPSs), onto any inert

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solid material. These polysaccharides are bacterial exudates consisting of a range of structural homopolymers and branched heteropolymers that carry out many functions such as structural integrity, cell-surface adherence and protection against environmental stress (Stoodley et al., 2002). In tandem with this is a high concentration of 3′, 5′-cyclic diguanosine monophosphate [c-di-GMP] (Jenal and Malone, 2006), which is classified as an ubiquitous bacterial second messenger, synthesised from two molecules of guanosine triphosphate (GTP) by diguanylate cyclase (DGC) [EC.2.7.7.65] (Fig. 1). Degradation of c-di-GMP into either a linear 5′-phosphoguanylyl-(3′,5′)-guanosine (pGpG) or into two molecules of GMP is effected by a phosphodiesterase enzyme (PDE) [EC. 3.1.4.52] (Fig. 1). The steady-state concentration of c-di-GMP is, therefore, maintained by the activity of these two enzymes. Furthermore the conversion of free-swimming planktonic bacteria into a sessile biofilm-embedded community is controlled not only by levels of c-di-GMP but by activities of DGC and PDE (Tamayo et al., 2007; Mills et al., 2011; Römling et al., 2005, 2013).

Bacteria are capable of producing certain proteins and/or polysaccharides that are instrumental in biofilm development under various conditions. C-di-GMP, a chemical first identified in *Gluconacetobacter xylinus* as a regulator of cellulose synthesis (Ross et al., 1987), was recently identified to regulate biofilm dispersal even at a low concentration (McDougald et al., 2012). It is believed, therefore, that c-di-GMP levels are key intracellular regulators for exopolysaccharide production, and biofilm or aerobic granular stability (Hecht and Newton, 1995; Jenal, 2004; Römling et al, 2005; Simm et al., 2004; Kuchma et al., 2007; Merritt et al., 2010; Wan et al., 2013).

Bacterial cells that exist within biofilms are extremely tolerant to antimicrobial agents and indeed severe bacterial infections are manifested from the formation of biofilms associated with pathogenic bacteria (Mah and O'Toole, 2001; Mah et al., 2003). Progress in the use of effective antibiotics is still in its infancy and only a few that affect the infrastructure of persistent biofilms have been identified (Ueda and Wood, 2009; Antoniani et al., 2010). These facts point towards c-di-GMP metabolizing enzymes, especially diguanylate cyclase, as key biomedical targets for the inhibition of biofilm development (Wolfe and Visick, 2010; Römling et al., 2013).

This review discusses the structure and reaction of individual diguanylate cyclase enzymes with a focus on new directions in c-di-GMP research. Specific attention is made on the molecular mechanisms that control bacterial exopolysaccharide biofilm formation and aerobic granules.

Diguanylate cyclases

Structure

Despite the reputation that both c-di-GMP, and consequently the enzyme diguanylate cyclase, are ubiquitous in bacteria only limited research on structural aspects has reached the literature (Table 1). Furthermore as of the present date [October, 2014] 27 crystal structures of DGCs (or putative structural derivatives) from 11 different bacterial genera have been solved and deposited in the Protein Data Bank.

C-terminal

DGC activity is regulated by two highly conserved GGDEF (Gly-Gly-Asp-Glu-Phe) or GGEEF (Gly-Gly-Glu-Glu-Phe) motifs contained in a (approximately) 154 amino acid C-terminal domain of the enzyme. Two of these motifs are juxtaposed to one another, in an antiparallel arrangement, and can each accept a GTP substrate molecule (Paul et al., 2004; Chan et al., 2004; Wassmann et al., 2007). The secondary structure of these C-terminal domains is made from five α-helices and seven short β-strands with a sequence: α_1–β_1–α_2–α_3–β_2–β_3–α_4–

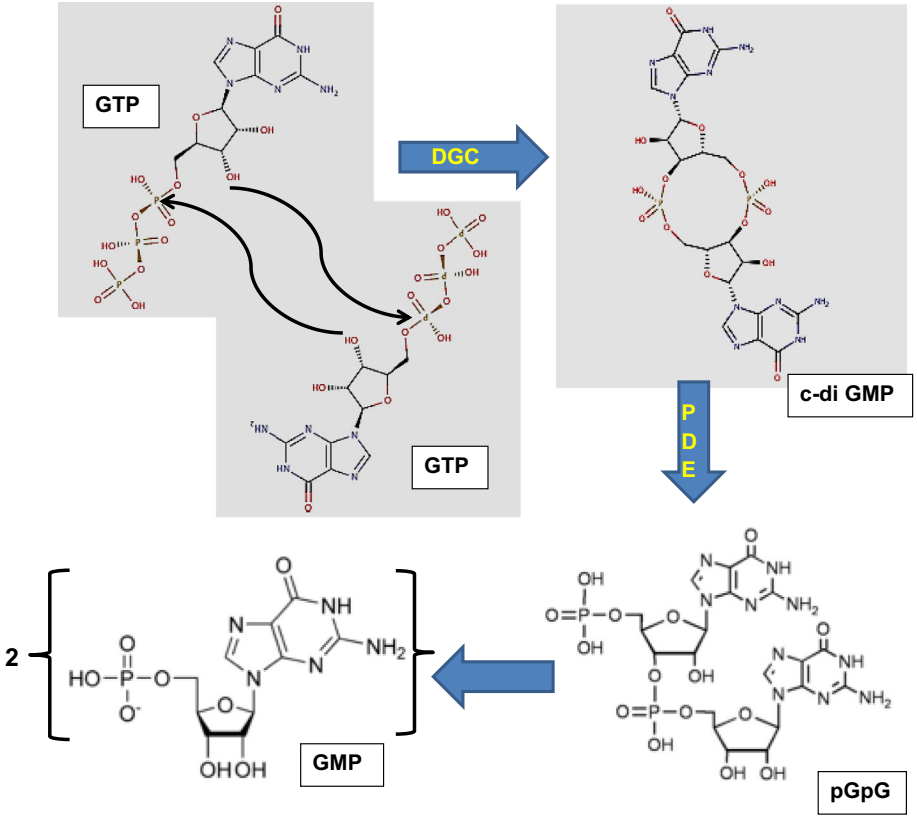

Fig. 1. Dimeric cyclisation of two guanosine triphosphate molecules (GTP) by diguanylate cyclase (DGC) to form cyclic-diguanylate monophosphate (c-di-GMP) and degradation into 5′-phosphoguanylyl-(3′,5′)-guanosine (pGpG) and eventually into two GMPs by phosphodiesterase (PDE).

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