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Minireview

Mycobacterial adenylyl cyclases: Biochemical diversity and structural plasticity

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Abstract **The conversion of adenine and guanine nucleoside triphosphates to cAMP and cGMP is carried out by nucleotide cyclases, which vary in their primary sequence and are therefore grouped into six classes. The class III enzymes encompass all eukaryotic adenylyl and guanylyl cyclase, and several bacterial and archaebacterial cyclases. Mycobacterial nucleotide cyclases show distinct biochemical properties and domain fusions, and we review here biochemical and structural studies on these enzymes from *Mycobacterium tuberculosis* and related bacteria. We also present an in silico analysis of nucleotide cyclases found in completely sequenced mycobacterial genomes. It is clear that this group of enzymes demonstrates the tinkering in the class III cyclase domain during evolution, involving subtle structural changes that retain the overall catalytic function and fine tune their activities.**

Keywords: cAMP; Comparative genomics; Biochemistry; Mutational analysis; Crystal structure; Phylogeny

1. Introduction

Nucleotide cyclases, the enzymes that convert NTP's into the respective 3′,5′-cyclic nucleoside monophosphates, are a large group of proteins in which the function of synthesizing these second messengers has evolved through convergent evolution [1]. Nucleotide cyclases synthesize cAMP in a metal-dependent manner, and can be grouped into six classes based on their primary amino acid sequences. Classes I, II, V and VI are exclusive to bacteria, while enzymes from class IV have thermostable properties and are found in archaebacteria as well [1,2]. Class III nucleotide cyclases, which includes all known guanylyl cyclases, have the widest phyletic distribution, being present in all eukaryotes as well as in several bacteria and archaebacteria [3].

Class III adenylyl and guanylyl cyclases are proteins with a central four stranded anti-parallel β-sheet (Fig. 1) structurally similar to the 'palm' domain of DNA polymerases, and α-helices on either side [4]. Residues important for catalytic activity were identified based on mutational and structural analyses of mammalian enzymes. Evidence from crystal structures of mammalian adenylyl cyclases indicate that two cyclase domains form a head-to-tail dimer and generate the active dimeric enzyme (Fig. 1). Catalytic activity requires the presence of conserved metal, substrate (either ATP or GTP) binding residues and transition-state stabilizing residues. These are often present in a single polypeptide chain giving rise to the family of mammalian receptor guanylyl cyclases and homodimeric bacterial nucleotide cyclases [5,6]. These cyclases are therefore composed of identical subunits and contain two perfectly symmetric catalytic sites. If the subunits are not identical, the dimeric enzyme has pseudosymmetric sites and usually only one of the two sites is catalytically competent (Fig. 1). For example, in the 12-transmembrane adenylyl cyclases, two tandem class III cyclase domains are found in a single polypeptide chain (called C1 and C2 by convention) [5]. The C1 domain provides residues that contribute to metal-binding while the C2 domain contains residues that confer nucleotide (substrate) specificity (lysine–aspartate pair in adenylyl cyclases or a glutamate–cysteine pair in guanylyl cyclases). The transition-state stabilizing asparagine–arginine pair of residues are also present in the C2 domain [5]. Thus, based on their functionalities in the mammalian membrane-bound adenylyl cyclases, cyclase domains have been classified as C1-like or C2-like [5]. The α and β subunits of eukaryotic soluble guanylyl cyclases are also functionally analogous to the C1 and C2 domains.

Bacterial class III adenylyl cyclases have diversified by means of various domains fusions, as well as variations in sequence in the catalytic cyclase domain [3,6,7]. In recent years, the adenylyl cyclases present in cyanobacteria and mycobacteria, have been well studied [6]. Among the adenylyl cyclases in *M. tuberculosis*, studies on Rv1625c highlighted a new role for substrate specifying residues in the maintenance of the oligomeric state of cyclases [8–11], Rv1264 revealed a mechanism for pH sensing [12,13], Rv1900c suggested that asymmetric heterodimeric cyclases could have evolved from homodimeric bacterial cyclases [14], Rv1647 showed that divergent adenylyl cyclases can have a predominantly hydrophobic dimeric interface [15] and Rv0386 could have a novel mechanism for substrate selectivity [16]. Given the recent explosion of information on mycobacterial cyclases, we review here the biochemical and structural aspects of these enzymes and comment on the insights provided by these enzymes on our understanding of class III nucleotide cyclases in general.

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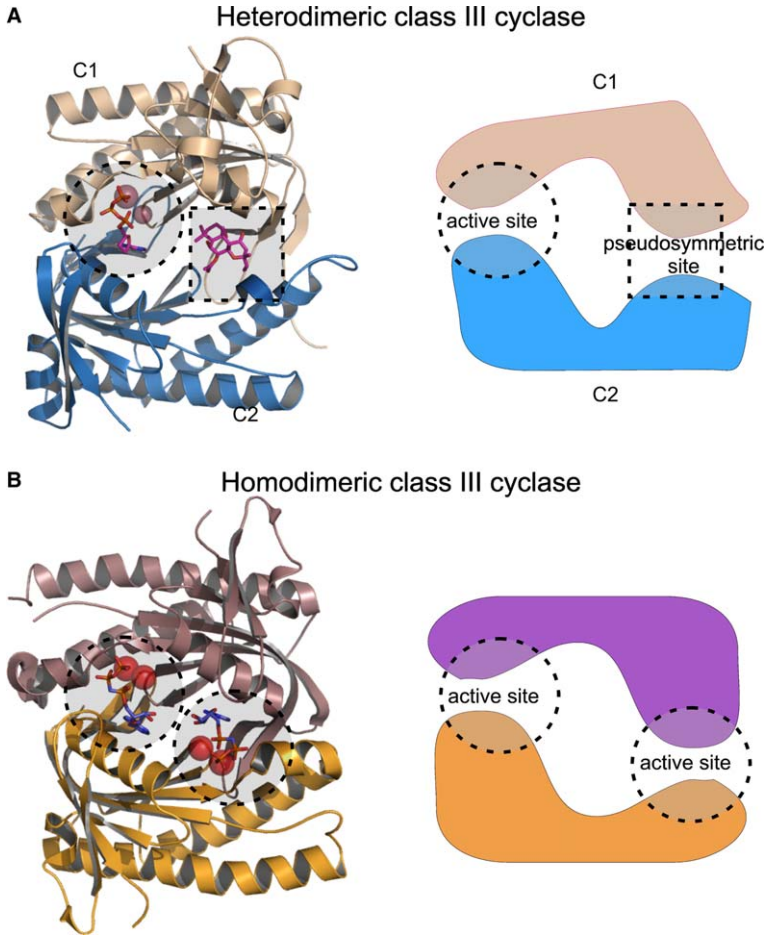

Fig. 1. Structure and schematic representation of class III cyclases. (A) Heterodimeric class III cyclases, e.g. 12- or 22-transmembrane adenylyl or guanylyl cyclases. The crystal structure of the mammalian adenylyl cyclase C1 and C2 domains showing forskolin and a P-site inhibitor is shown (PDB ID 1cjv). The right panel shows a schematic representation of the heterodimer with one active site (dotted circle; occupied by the P-site inhibitor in 1cjv) and a pseudosymmetric inactive site (dotted square; occupied by forskolin in 1cjv). Notice the overall structural similarity of the C1 and C2 chains and their head-to-tail dimerization. (B) Homodimeric class III cyclases, e.g. bacterial adenylyl cyclases and receptor guanylyl cyclases. Crystal structure of a *Spirulina* adenylyl cyclase is shown with two active sites occupied by ATP analogues (PDB ID 1wc5). The right panel shows the schematic homodimer. Metals are shown as spheres. Notice the overall similarities in the subunits of the homodimeric cyclase in B and the C1 and C2 chains in A.

2. Class III adenylyl cyclases in mycobacteria

The genome of *M. tuberculosis* H37Rv encodes 16 class III cyclases while the CDC1551 strain has 17 cyclases [17–19]. Several of these gene products were earlier annotated as "putative cyclases" or "conserved hypotheticals", but have now been characterized biochemically and structurally. All the identified mycobacterial class III cyclases are listed in Table 1 and a multiple sequence alignment of the cyclase domains found in the cyclase-like genes in *M. tuberculosis* is shown in Fig. 2.

Biochemically characterized cyclases from mycobacteria retain similar catalytic properties to the mammalian enzymes, in terms of the requirement for divalent cations, and dimerization as a prerequisite to generate the catalytic site. However, very precise allosteric regulation of the catalytic activities of some of the mycobacterial enzymes is evident, and Hill coefficients vary from 1 to as high as 4 [8,9]. A summary of the biochemical and structural features of the characterized enzymes is provided here. As described below, distinct features of individual enzymes highlight the lack of generalities and common properties in these proteins.

2.1. The first characterized adenylyl cyclase from mycobacteria: Rv1625c

Rv1625c was the first adenylyl cyclase to be characterized from *M. tuberculosis* H37Rv, perhaps because the catalytic domain of this protein has the highest sequence similarity with the mammalian enzymes [8,10]. Rv1625c is a six-transmembrane protein with a single class III cyclase domain (Table 1) and therefore topologically equivalent to half of the 12-transmembrane mammalian adenylyl cyclases. The purified catalytic domain of Rv1625c has high adenylyl cyclase activity, which is increased if the two catalytic domains are artificially linked by a flexible linker, suggesting that forceful dimerization stabilizes the enzyme [8].

Mutation of the metal-binding (D256 and D300; Fig. 2), substrate specifying (K296 and D365) and transition state stabilizing (R376) residues to alanine, led to significant decreases in adenylyl cyclase activity of the mutant Rv1625c proteins, indicating the conservation of the biochemical mechanism for cyclization in nucleotide cyclases [8]. Like the mammalian heterodimeric adenylyl cyclases, mixtures of artificial C1-like (K296A, D365A and R376A) and C2-like (D256A and