TITLE: NEW LINEAR AND CYCLIC DINUCLEOTIDES FORMATION BY WILD TYPE GGDEF DOMAINS

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ABSTRACT

cdiGMP is a second messenger that regulate several functions in cell such as transition from motile to sessile state, biofilm formation, virulence, flagella motility, etc. GGDEF protein domains are ubiquitous in bacteria, mainly in gram-negative bacteria. Canonical GGDEF domains function as a dimer where each monomer recognizes one GTP to form cdiGMP. There are approximately 85 thousand of GGDEF domain containing proteins in the pFAM database with some of them showing substitutions in residues important for substrate recognition or enzymatic catalysis. A part of this group of GGDEF proteins has been showed as a cdiGMP receptors but the observation that some of them have substitutions just in the recognition site lead us to hypothesize that these GGDEF domains could recognize others nucleotide triphosphates as a substrate. We cloned, expressed and purified some GGDEF domains from different bacteria including Xanthomonas axonopodis pv. citri. Using the purified enzymes, we perform enzymatic assays with different NTP substrates and the product formation was evaluated by HPLC-ESI-MS. We showed that other naturally occurring NTPs can be recognized as a substrates by GGDEF domains. XAC0610 and GSU1658 can use 2`dGTP as a substrate to form cdi2'dGMP and GSU1658 also can use ITP to form cdiIMP. Mixing different substrates, we were able to form different hybrid cyclic dinucleotides including c-GMP-2`dGMP, cGIMP and cAIMP. In our knowledge this is the first report of the formation of these compounds by wild type enzymes. In the case of Xac0610 we also observed that when similar amounts of GTP and 2`dGTP are mixed in the reaction, the majority product was the linear product pppGp2`dG. These linear products could perform not yet described functions in the organism. Enzymatic kinetic analysis will be performed to measure rate and affinity constants of these substrates for the enzymes in study.

Keywords: XAC0610; GSU1658; 2`dGTP; ITP; cdiGMP

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