Scholarly Article Template

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Abstract

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Introduction

DNA methylation is one of the most well-studied DNA modification involved in gene regulation. This epigenetic mechanism is essential for development, genome defense, genome imprinting, chromatin stability and elements transposable silencing. The enzyme responsible for cytosine methylation is the DNA-(cytosine-C5)-methyltransferase (DCMtase), classified into three subfamilies: DNMT1, DNMT2 and DNMT3a and 3b. DNMT enzyme family are divided into a N-terminal (regulatory region) and C-terminal, which it shares ten conserved residues motifs, where the catalytic domain and the coenzyme (S-adenosyl-l-methionine - AdoMet) binding site are found.

DNMT2 does not have a N-terminal regulatory region, which is present in the other DNMTs (Hermann et al., 2004; Yoder, 1998). Although it is the best conserved of DCMtase, the biological function of DNMT2 has not yet been clarified. Furthermore, some organisms like *Drosophila*, *Entamoeba* and *Dyctiostelium* seem to lose the DNMT1 and DNMT3a/b throughout its evolution, and the only DCMtase found is the DNMT2. DNMT2 is the smallest methyltransferase (approximately 400 amino acids) and the most conserved among animals, fungi, protists and plants (Ponger and Li, 2005; Jeltsch, 2002). With respect to their biological function, studies associated DNMT2 with RNA interference in *Dictyostelium* and covalent histones modification, longevity in *Drosophila* and DNA activity (Kuhlmann, 2005; Kunert, 2003; Katoh et al., 2006; Lin et al., 2005).

Basically, the reaction mechanism of the DNMTs is the same among the different families. The catalytic process is mediated by a nucleophilic attack on the target cytosine carbon-6. This attack is promoted by the catalytic cysteine present in the conserved motif PCQ in the DCMtase active site (Wu and Santi, 1985, 1987). From the protonation of the target cytosine in the N3 site mediated by the glutamic acid of the ENV motif, covalent bonding occurs that exposes the C5 position of cytosine to the nucleophilic attack, where the methyl group of the co-enzyme AdoMet will be transferred to the carbon-5. The intermediate state of covalent binding between DCMtase in complex with DNA is widely described (Klimasauskas et al., 1994a; Reinisch et al., 1995), highlighting the fundamental role of the PCQ motif, more specifically the cysteine catalysis (Chen et al., 1991; Everett et al., 1990; Hanck et al., 1993). In prokaryotic DCMtase this same motif has been demonstrated crucial to DNMT2 activity (Hurd et al., 1999; Wyszynski et al., 1992, 1993).

However, this event is only possible after the so-called base flipping process, in which the target cytosine is withdrawn from the double-helix context and positioned close to the AdoMet coenzyme binding site. For this, there are several crucial residues for the stabilization of the transient structure, such as PCQ and ENV motifs (Klimasauskas et al., 1994a; Reinisch et al., 1995) .

While on the one hand the mechanisms and catalytic processes of DNMT2 are widely described and known, on the other hand, the reasons why eukaryotic DNMT2 have low DNA activity are still unknown, despite the structural conservation of prokaryotic and eukaryotic catalytic domains. Some "hints" as to the reason for this affinity difference can be obtained by analyzing the tertiary structures of the target-recognizing domain (TRD) of different DNMT2. For example, HhaI and HaeIII (prokaryotic DNMT2 with DNA-MTase activity) are known to exhibit structural conservation in their catalytic domains, but differ in TRD structure and amino-acid composition, but still acting on DNA as substrate. Nevertheless, they eventually have different methylation target sequences from each other, where HhaI recognizes 5'-GCGC-3' and HaeIII 5'-GGCC-3' sites (Klimasauskas et al., 1994b; O'Gara et al., 1996). When comparing DNMT2 tertiary structures and the distribution of molecular electrostatic surface in different DCMtases, marked differences emerge within latter aspect, mainly in TRD region (Vieira et al., 2017), even in evolutionarily close species, as *D. melanogaster* that has preference for CT, CA and CC context (Kunert, 2003) and *D. willistoni* seems to methylate CG sites (Garcia et al., 2007) - the same context preference found in human DNMT2 (Hermann et al., 2003).

It is interesting to note that according to previous studies, Ehmeth (*Entamoeba histolytica* DCMTase) and human MT2 presented weak activity on DNA substrate and shown preference to RNA substrate, suggesting that in some species rose an alternative role to DNMT2: the tRNA methylation (Hermann et al., 2003; Fisher, 2004). More recently, it was found that DNMT2 can methylates more efficiently DNA fragments in a covalent DNA-RNA hybrids context (Kaiser et al., 2016).

The function performed by an enzyme underlies its structure, electrostatic potential surface and dynamics. As described previously, the structure of the catalytic domain of DNMT2 is very well known and described, but with respect to TRD, its aspects are still neglected. Since diverse crystallographic structures of different DNMT2 have been resolved, like HhaI (PDB: 1MHT), HaeIII (PDB: 1DCT), *Spodoptera frugiperda* (PDB: 4H0N) and human MT2 (PDB: 1G55), the elaboration of predicted structural models of other species has become possible with great accuracy through homology modeling. However, studies focusing on molecular structures fail to take into account the subtle aspects of the dynamics between the different enzymes, especially in the enzyme-substrate context.

Molecular dynamics (MD) simulations can provide a series of detailed information about a protein's behavior, its motions, conformation modifications and interactions with substrates. This information from the MD is supported by the laws of thermodynamics, which offers reliability in the results obtained when adequate force field and simulation time are provided and can be compared to experimental data. Thus, the present study aims to look more closely at the interactions that emerge within the DNMT2-DNA complex interactions of several DNMT2, through analyzes of protein-ligand all-atoms from MD simulations, seeking to shed light on the mechanisms involved in target substrate recognition and the inherent reasons for the differences found between the various DNMT2.

Results

The conformational changes between DNMT2

In none of the analyzed proteins were abrupt changes in conformation detected, indicating a structural stability at the macro level. In general, the DNMT2 present two main movements: (i) between the catalytic

and TRD pendulum domains; (ii) open-close. Using the main component Analysis (PCA), we verified that, somehow, all the structures present differences in the dynamic behavior of the structures apo vs. DNA-bound, and when compared with each other (**Figure 1**).

HhaI and HaeIII presented a lower motion amplitude along the dynamics when in the presence of DNA, being slightly more dynamically stable when the ligand was present. In contrast, eukaryotic DNMT2 are characterized by heterogeneous behaviors in dynamic when in the presence of the ligand and also differences when compared with each other. Among the eukaryotes, Ehmeth, rhoDNMT2 and SfDNMT2 shown the smallest differences in dynamics between PCAs (apo vs DNA-bound), whereas GsDNMT2 is notable for the remarkable change in PCA2 between apo and DNA-bound structures. The melDNMT2, wilDNMT2 and human DNMT2 shown lower amplitude in PCA1 in the presence of the substrate.

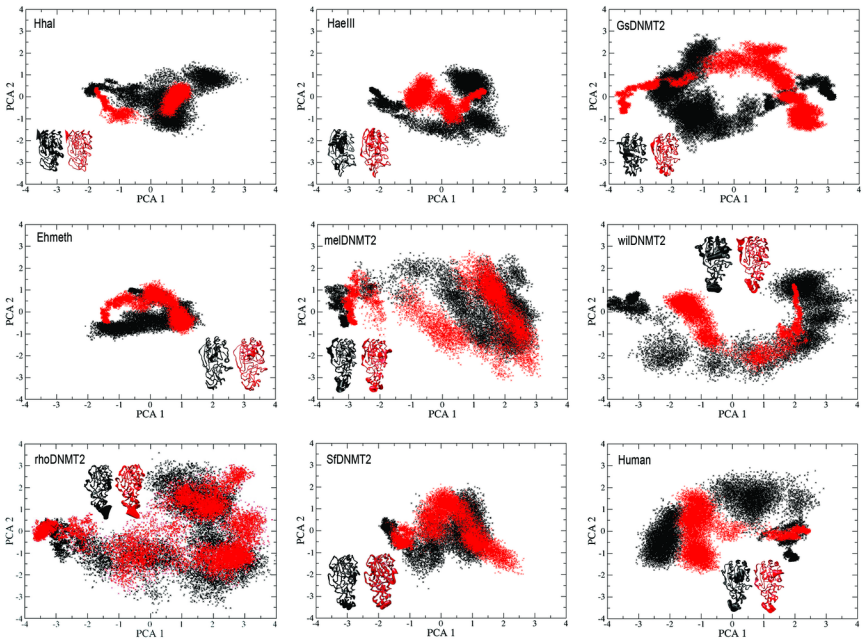

Figure 1: Projections of trajectory snapshots on first (PC1) and second (PC2) eigenvectors for investigated DNMTs (Apo-protein black and DNA-bound red). DNMT2 ribbons representation motion along the first eigenvector next to the chart (Apo-protein black and DNA-bound red).

DNMT2 Residues fluctuation from Molecular Dynamic Simulation

In general, the differences in atomic fluctuations of the DNA-bound and apo-DNMT2 found to indicate a loss of dynamics when the enzyme is linked to DNA, least in Ehmeth and rhoDNMT2, which it presents subtle dynamics gain. However, the observed differences were not uniform along the same enzyme, either between the different DNMT2 (**Figure 2**).

Interesting Ehmeth and rhoDNMT2 shown a gain of dynamics when linked to DNA. The biggest differences [Holo-Apo] were found in HhaI (-9.6376), HaeIII (-7.9525) and wilDNMT2 (-9.822), indicating the loss of

dynamics in the structure of these DNMT2 when DNA is present (**Table S1**). Even if the structures and phylogenetic relationships are close, there are differences in atomic fluctuation between related MT2, like HhaI and HaeIII, which seems to be according to with the differential target recognition mechanisms between them [10-12] and between melDNMT2, wilDNMT2 and rhoDNMT2.

There are two main regions in DNMT2 which show significant variations when compared apo- and holo-protein structure: the first is between residues 71-99, which correspond to PC loop (with the catalytic cysteine); and the second above residues 260-290, that belong to TRD C-terminal region. The handle connection region of melDNMT2 (between residues 165-205, approximately) seems to present a lower fluctuation when in the presence of DNA, while wilDNMT2 presented gain of dynamics in the presence of substrate (**Figure 2**). Regarding the PC loop region, between the eukaryotic DNMT2, wilDNMT2 presented the lowest dynamics in DNA-bound.

Acknowledgements

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