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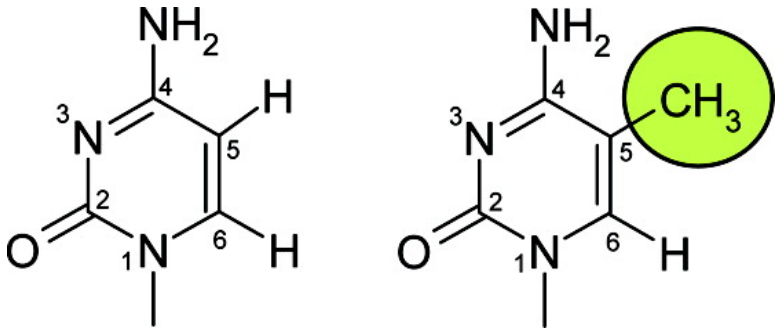

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C5-Methylation of Cytosine in B-DNA Thermodynamically and Kinetically Stabilizes BI

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In addition to the four-letter code (ACGT) of DNA, methylated bases are considered to be an extension of the genetic code. C5-methylcytosine (5mC) occurs in various prokaryotic and eukaryotic organisms. This epigenetic feature plays an important role for numerous biological functions of DNA.[1,2] It is involved in gene silencing,[3] genomic imprinting,[4] X chromosome inactivation,[5] and carcinogenesis.[2,6] Methylation occurs exclusively at CpG steps in a tissue-specific manner and is accomplished by a class of enzymes called DNA methyltransferases. The specific recognition of methylated bases is still a matter of discussion.[7] Experimental studies indicate that a single CpG methylation only leads to small structural changes.[8] Results of solid-state NMR experiments show altered dynamical properties of methylated DNA, with the strongest effect on the sugar−phosphate backbone.[9,10] Other biochemical experiments do not reveal an influence on the intrinsic flexibility, suggesting that the biological consequences are likely to be derived from local structural distortions.[11]

The phosphate units of DNA occupy either the BI or BII conformational substate.[12] Our group has investigated the dynamics of the DNA substates BI/BII by means of molecular dynamics and has shown that a base modification in the minor groove leads to a significant change in the BI/BII pattern.[13] In the case of 5-methylcytosine, the additive methyl group protrudes into the major groove. The aim of this study is to investigate the influence of 5-methylcytosine on the dynamic and kinetic properties of the DNA backbone and thus answer whether methylation has consequences on the indirect readout mechanism of DNA and the specific DNA−protein recognition.

For our MD simulations, we used the AMBER7 suite of programs with the parm99 force field.[14] All DNA molecules were created with NUCGEN on the basis of standard B-DNA parameters. The nucleotides were simulated in a rectangular unit cell containing about 4000−5000 TIP3P water models and equivalent sodium ions for charge neutralization. After 40 ps of equilibration, the systems were simulated under NPT conditions for 10−15 nanoseconds using (a) the particle mesh Ewald summation (PME) to treat long-range electrostatic interactions, (b) a 2 fs time step, (c) a 9 Å nonbonded cutoff, and (d) SHAKE constraints on all bonds involving hydrogen atoms. Coordinates were accumulated every picosecond. The parametrization of the 5-methylcytosine was carried out in analogy to the parameter development for cytosine in the AMBER force field. For details, see the Supporting Information.

The dodecanucleotide $d(GC)_6$ was calculated once with standard bases and once with the internal cytosines methylated. Both simulations were carried out for 15 ns and are stable after at most 1 ns. This was validated with the constant total energy of the system and the constant RMSD value for the DNA (see Supporting Information). For analysis, only data after this time was used.

We applied a simple method to gain thermodynamic and kinetic information from the coordinates of an MD trajectory. The idea was to count how often the difference between the ϵ- and the ζ-angle ($\epsilon-\zeta$ is a determinant for BI and BII[12]) of every nucleotide is in a certain angle space (16°), resulting in a histogram with two maxima defining the two phosphate conformations BI and BII. It is also known that the backbone angles α and γ can affect the BI/BII substate conformations.[15] Analyzing these angles, we observed a few noncanonical α/γ conformations, but in our case these findings did not influence the ($\epsilon-\zeta$)-values and, thus, our results. We assumed that our trajectories were long enough to sufficiently sample the phase space and thus yield the partition function (Z). Transforming the curves into free-energy graphs [$\Delta G = -RT^* \ln(Z)$] resulted in the ΔG and $\Delta G^{\ddagger}$ values for every base step. For better statistics, we averaged the free energies over all identical base steps, e.g., we summed all data points of GpC steps. The end standing bases were excluded. Thus, we had up to 150 000 data points (≅150 ns) for the simulated dodecamer for one specific step like GpC or CpG. To estimate the convergence of the free energy values, we carried out the analysis for two consecutive time blocks.

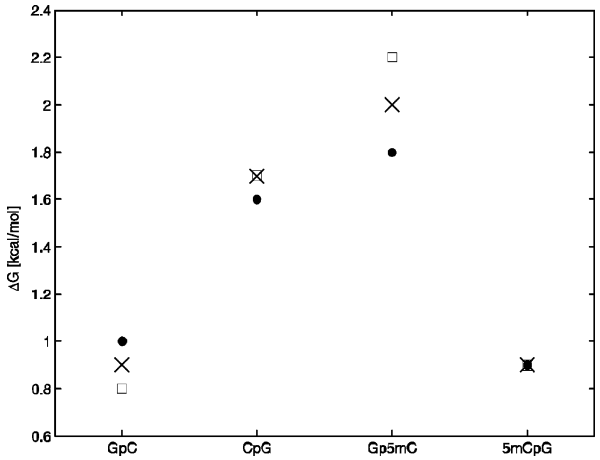

Figure 1. ΔG values [kcal/mol] for the BI/BII transition (defined by $\epsilon-\zeta$) of the base steps GpC/Gp5mC and CpG/5mCpG are plotted. Crosses correspond to averaged values over the whole trajectory (1−15 ns), squares contain data from 1 to 8 ns, and circles from 8 to 15 ns.

Because of methylation, the thermodynamic properties (free enthalpies) of the DNA backbone are significantly changed (Figure 1). In both simulations the two occurring base steps can be distinguished significantly due to their ΔG value. This confirms and quantifies the general sequence dependence of the substate interconversion of BI/BII in DNA. The $\Delta\Delta G$ value between the two possible base steps is −0.8 kcal/mol in $d(GC)_6$ and 1.1 kcal/mol in $d(G5mC)_6$. Through methylation, the GpC step is stabilized in BI by 1.1 kcal/mol, whereas the BI substate of CpG is destabilized by 0.8 kcal/mol (but BI is still the energetically favored substate). Thus, the overall thermodynamic picture, comparing the unmethylated and the methylated DNA, has completely changed.

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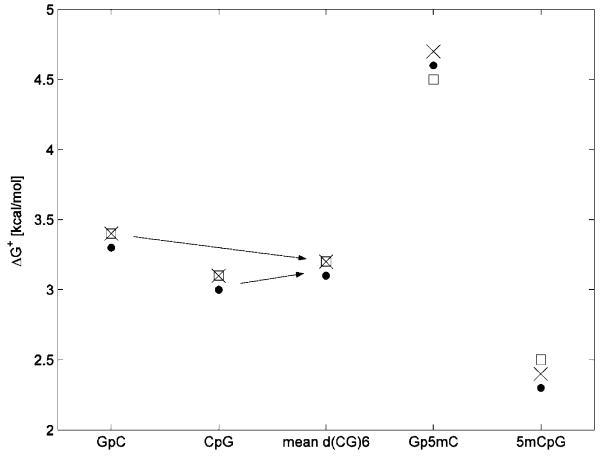

Figure 2. Averaged $\Delta G^{\ddagger}$ values [kcal/mol] for the BI/BII transition (defined by $\epsilon-\epsilon$) of the base steps GpC/Gp5mC and CpG/5mCpG are plotted. For the unmethylated sequence the two base steps are combined to one value. Crosses correspond to averaged values over the whole trajectory (1−15 ns), squares contain data from 1 to 8 ns, and circles from 8 to 15ns.

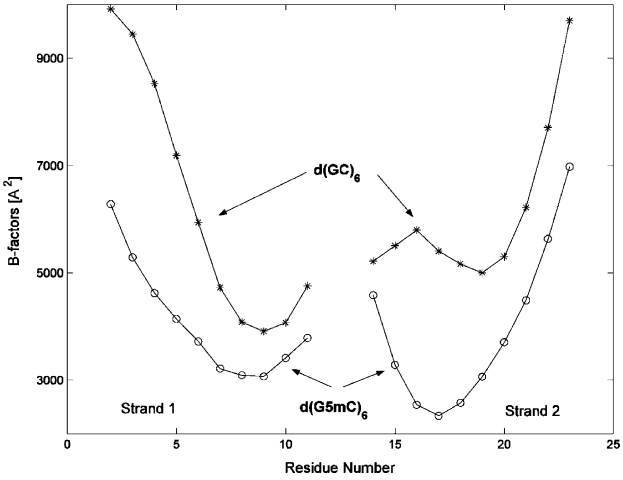

Figure 3. B-factors [Å^2] calculated with ptraj on a per-residue basis (rotational and translational contributions were not removed). The end standing residues were excluded. Open circles are equivalent to residues of methylated DNA; stars are equivalent to residues in nonmethylated DNA.

The background for this shift in equilibrium due to methylation is still not clear. Structural consequences are a decrease of 0.3 Å and an increase of 0.4 Å of the base-stacking distances of GpC and CpG, respectively, due to methylation. This is consistent with the different stacking distance values for the two substates BI/BII figured out by MD and confirms experimental data[16] where the major effect of the methyl substitution is in fact an increased base-stacking energy. The distance between the carbon atom of the cytosine methyl group and one free oxygen of the appropriate phosphate group is about 6.4 Å. This is the ideal distance for a water-bridged contact and, thus, a barrier for the phosphate flexibility to adopt the BII substate, as BII is defined by a rotation of the phosphate group toward the minor groove. For thymine, which has a methyl group at the same ring position as 5-methylcytosine, it is proposed that a water molecule is located between one free phosphate oxygen and the methyl group.[17]

The kinetic parameter $\Delta G^{\ddagger}$ (Figure 2) indicates that in the nonmethylated sequence the barrier for the substate interconversion is equal in GpC and CpG and thus not sequence-dependent. The averaged $\Delta G^{\ddagger}$ for $d(GC)_6$ is 3.2 kcal/mol. In the methylated sequence, the two base steps are separated by 2.3 kcal/mol. The barrier for the conversion from BI to BII in Gp5mC is hindered by 1.5 kcal/mol compared to the nonmethylated step GpC, and the barrier for the 5mCpG is lowered by 0.8 kcal/mol compared to CpG. Thus, one base step (GpC) is rendered significantly more rigid due to methylation. This is in good agreement with experimental data.[9,10] Analysis of the B-factors of the two sequences confirms our results, as the methylated structure seems to be more rigid overall (Figure 3).

Subsequently, we wanted to exclude the effect of the 5′ guanosine on the properties of the methylated cytosine, and thus, we calculated a second set of DNA molecules with a different sequence. We chose the tridecamer $d(ACGACGACGACGA)_2$ which has a new base step (ApC) upstream of the important cytosines and fulfills the criterion of biological relevance (contains CpG steps, which are the only ones to be methylated in vivo). Two simulations (10 ns each) were performed, one with standard cytosines, one with 5-methylcytosines. The same analyses as described for the GC sequence were applied on these trajectories. In this case, only five out of eight possible cytosine phosphate groups in the nonmethylated sequence showed good BI/BII statistics. For the methylated sequence, no Ap5mC step had the BII substate populated. These results indicate that for this special sequence the shift of equilibrium for the phosphate groups of the methylated bases as a consequence of methylation was even stronger. We extended our investigations on the substates of the Z-DNA backbone. Methylation is known to stabilize the left-handed Z conformation.[18] We suggest an impact of the methylation on the interconversion of the two occurring substates ZI/ZII. These results will be discussed elsewhere.

Thus, we can show by means of MD that methyl groups influence the thermodynamics and the kinetic properties of DNA. We surmise a link of methylation to the indirect readout mechanism which is known to be important for the selective recognition of 5-methylcytosine.

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Supporting Information Available: Parametrization and implementation details of 5-methylcytosine (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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