Analysis of Specificity of DNA Methyltransferase EcoVIII and Its Homologs

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The aim of the study was to determine the substrate specificity of bacterial DNA methyltransferases. As a model four isospecific DNA methyltransferases were used – M.EcoVIII, M.HindIII, M.LlaCI and M.BstZ1II. They all act in a sequence specific manner and although they originated from distinct unrelated bacterial species (Gram-negative and Gram-positive), they all catalyze transfer of a methyl group to the first adenine in the canonical sequence 5'-AAGCTT-3'/3'-TTCGAA-5'. Additionally, in the case of M.BstZ1II deletion variant devoid of the 60-aa fragment near the target recognition domain (TRD) was examined (M.BstZ1IIΔ).

Using factors prompting relaxation of the specificity (DMSO or glycerol) methylation of all possible secondary sites was assessed. In the case of all studied enzymes, modification of at least some secondary sites was detectable. It appeared that nucleotides in the centre of the canonical sequence are more important than those located at the poles – sites with substitutions in the 3rd position were the hardest substrates for all the proteins, however the determined substitutions hierarchy slightly differs according to the analyzed enzyme. In order to precisely characterize M.EcoVIII preferences towards nucleotide sequence kinetic parameters were set. The enzyme follows Michaelis-Menten kinetics and preferentially modifies hemimethylated substrate (K_m=24.4 nM). In contrast to others studied proteins, M.EcoVIII is able to modify the canonical site located at the single-stranded DNA. Methylation efficiency of secondary sites as well as ssDNA ranged 0,09-0,22 of level obtained for the unmethylated canonical sequence.

In the next step, I examined interactions with the DNA backbone close to or within the canonical sequence. The most important are contacts with bonds within the canonical sequence, especially G-C, C-T, T-T and between the target adenine from the 5' side. Removal of any of these contacts decreases the methylation level by at least 50%. M.EcoVIII and M.BstZ1II are capable of methylation of the canonical site located directly at the end of the DNA molecule, and only for M.EcoVIII and M.BstZ1IIΔ 3-nt distance from the oligo's end is sufficient for full modification. Polypurine tracts are not preferred flanks for any analyzed enzyme.

M.LlaCI, isomethylomer of M.EcoVIII, is able to specifically modify the canonical sequence in a DNA/RNA hybrid when the reaction is supplemented with glycerol. Enzyme only methylated the DNA strand, and the modification level was lower by an order of magnitude when compared to the DNA/DNA duplex. This unique feature may be applicable for DNA/RNA duplex labeling.

To determine which of the amino acid residues conserved among the TRDs of the isospecific methyltransferases are important for interactions with DNA, systematic analysis was performed. In case of M.EcoVIII substitution of any of the 16 residues did not totally abolish the enzyme's activity, however, mutants in residues W176, L181, P187, W190, Y195, N214 and N227 retained only 10-20% activity, while K172, K193, P194 and T200 exhibited over 80% activity.