Biochemical and structural characterization of a DNA N⁶-adenine methyltransferase from *Helicobacter pylori*

Supplementary Material

	Number of residues	Residues not visible in the structure
M1.HpyAVI Chain A	197	34-57, 160-170
M1.HpyAVI Chain B	185	32-58, 116, 154, 156-172, 232
M1.HpyAVI Chain C	185	32-61, 114, 157-171, 232
M1.HpyAVI Chain D	177	32-60, 111-117, 147, 149, 156-171, 232
M1.HpyAVI-AdoMet Chain A	201	33-58, 169-172, 232
M1.HpyAVI-AdoMet Chain B	194	32-58, 157-164, 230-232
M1.HpyAVI-AdoMet Chain C	204	32-56, 159-160, 232
M1.HpyAVI-AdoMet Chain D	188	32-59, 158-172, 232
M1.HpyAVI-AdoMet Chain E	193	32-58, 158-167, 231-232
M1.HpyAVI-AdoMet Chain F	194	32-58, 159-168, 232
M1.HpyAVI-AdoMet Chain G	200	33-56, 159-163, 230-232
M1.HpyAVI-AdoMet Chain H	193	32-58, 159-168, 231-232

Table S1. Residues of the asymmetric unit

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Duplex	Sequence (5'- to 3')
GAGG-1 ^a	CATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAAAAA
	GGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACAT
	TAACCTATAAAAATAGGCGTATCAC <u>GAGG</u> CCCTTTCGTC
GAGG-2 ^a	CTATTACGCCAGCTGGCGAAAGGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTT
	CCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTCGAGCTCGGTACCCGGGGAT <u>CCTC</u> TAGAGT
	CGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCG
GAAG-1 ^b	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGT
	TATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAAT
	GAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGC
GAAG-2 ^b	CGTTGTCA <u>GAAG</u> TAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTA
	CTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAG
	TGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAA
GGAG-1 ^c	GTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCG <u>CTCC</u> AAGCTGGG
	CTGTGTGCACGAACCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCA
	ACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAG
GGAG-2 ^c	AGAAATTCTTTCATCTCTTTCGGCGGCTGGTAACCCGGAACAAGTGTGCCATTGCTCAGCACAACTGC
	C <u>GGAG</u> TACCGCTAACGCCAAGCTGGACGCCAAGTGCGTAATGGTCGGCAATATCCACGTCGCAACT
	GGCTGGTGCGACGCTTTTACCTGCCATCACATCATCAAACGCTTTGTTTTTATCTTTCGCACACCAGA
N-Control-1 ^d	TAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCAT
	CCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCG
	ACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTG
N-Control-2 ^d	ATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATT
	TGATGCTCGATGAGTTTTTCTAAGAATTAATTCATGAGCGGATACATATTTGAATGTATTTAGAAAAA
	TAAACAAATAGGGGTTCCGCGCACATTTCCCCCGAAAAGTGCCACCTGAAATTGTAAACGTTAATATT

Table S2. Duplex DNA sequences used in the methyl transfer reaction

^a DNA substrates that contain 5'-GAGG-3' sequence; ^b DNA substrates that contain 5'-GAAG-3' sequence; ^c DNA substrates that contain 5'-GGAG-3' sequence; ^d DNA substrates that used as negative control.

BAD AVG GOOD

M1.HpyAVI M.MboIIA M.RsrI TTHA0409 cons	MIQI YHADAFEIIKDFYQQNLKVDAIITDPP 3 MLEI NKIHQMNCFDFLDQVENKSVQLAVIDPP 33 MANRSHHNAGHRAMNALRKSGQKHSSESQLGSSEIGTTRHVYDVCDCLDTLAKLPDDSVQLIICDPP 6 MRSFPKAKEAFSEGEKVSFGVHRLHVGDAREVLASFPEASVHLVVTSPP 4 * . :. : : : .* . : .**	1 2 7 9
M1.HpyAVI M.MboIIA M.RsrI TTHA0409	YNISVKNNFPTLKSAKRQGIDFGEWDKNFKLLEWIARYAPLVNPNGCMVIFCSYR8 8 YNLSKADW-DSFDSHNEFLAFTYRWIDKVLDKLDKDGSLYIFNTPF7 7 YNIMLADWDDHMDYIGWAKRWLAEAERVLSPTGSIAIFGGLQYQGEAG-SG 1 YWTLKRYE-DTPGQLGHIEDYEAFLDELDRVWREVFRLLVPGGRLVIVVGDVAVARRRFGRH 1	6 7 17 10
cons	* * . * .	
M1.HpyAVI M.MboIIA M.RsrI	-FISYIADFLEENGFVVKDFIQWVKNNPMPRNIHRRYVQDTEFALWAVKKKAKWVFNKP-KNEK -NCAFICQYLVSKGMIFQNWITWDKRDGMG-SAKRRFSTGQETILFFSKSKN-HTFNYDEVRVP DLISIISHMRQNSKMLLANLIIWNYPNGMSAQRFFANRHEEIAWFAKTKK-YFFDLDAVREP	48 38 78
TTHA0409	LVFPLHADIQVRCRKLGFDNLNPIIWHKHTNASLEVEGRGVFLGKPYEPGAIIKTEI-EYILMQRKPGG [78
cons	· * · · · * * · · · · *.: :	
M1.HpyAVI M.MboIIA M.RsrI TTHA0409 cons	YLRPLILKSPVVSGLEKTKHPTQKSLALMEKII 12 YESTDRIKHASEKGILKNGKRWFPNPNGRLCGEVWHFSSQRHKEKVNGKTV-KLTHITPKPRDLIERII 20 YDEETKAAYMKDKRLNPESVEKGRNPTNVWRMSRLNGNSLERVGHPTQKPAAVIERLV 21 YRKPTQEQREKSRLPKEDFHRFFRQIWDDIPGESTKDHPAPFPLELAERLV 22 * : * * : . : *:::	81 06 36 29
M1.HpyAVI M.MboIIA M.RsrI TTHA0409 cons	SIHTNPNDIVLDPFMGSGTTGLACKNLERNFIGIESEKEYFQTAKKRLNLF 2. RASSNPNDLVLDCFMGSGTTAIVAKKLGRNFIGCDMNAEYVNQAMFVLNQL 2. RALSHPGSTVLDFFAGSGVTARVAIQEGRNSICTDAAPVFKEYYQKQLTFLQDDGLIDKARSYEIVEGA 3. RMFSFVGDVVLDPFAGTGTTLIAAARWGRRALGVELVPRYAQLAKERFARE-V PGFSLEVLDGA : *** * *:*.* : : : : : : 2.	32 60 05 92
M1.HpyAVI M.MboIIA M.RsrI TTHA0409 cons	ANFGAALQRGDVAS 319 THPRR 297	

Figure S1. Multiple sequence alignment among M1.HpyAVI and other β -class N⁶ adenine or N⁴ cytosine MTases.

The highly flexible region of M1.HpyAVI (residues 33-58) is longer while the TRD of M1.HpyAVI (residues 136-166) is shorter in length, compared with the other three sequences. Residues coloring from blue through yellow to red indicate the similarity among sequences. The multiple sequence alignment was performed using T-coffee web server.



Figure S2. Structural and biochemical analyses of the conserved domain "FMGSG" for AdoMet binding

A. Cofactor binding affinity of wt-/mutants M1.HpyAVI proteins analyzed by microscale thermophoresis (MST). The binding affinity was determined between

fluorescently labelled M1.HpyAVI protein and unlabeled AdoMet. The bound fraction is shown on the *y*-axis against the protein concentration. AdoMet (15 nM to 1 mM) was titrated into a fixed concentration of M1.HpyAVI wt/mutant protein (800 nM). The dissociation constant (K_D) is yielded according to the law of mass action from the isotherm derived of the raw data: M1.HpyAVI-wt: 31 ± 4 µM; M1.HpyAVI-M196A : 46 ± 11 µM; M1.HpyAVI-G197A : 0 µM; M1.HpyAVI-S198A : 193 ± 23 µM; M1.HpyAVI-G199A : 38 ± 6 µM. Standard deviation for three replicates is indicated. Measurements were made with 40% LED and 40% laser power at 25°C. **B.** Methyl transfer activity of wide type protein and the mutants were quantified using radioactive assay. [³H]-methyl transferred to duplex DNA containing 5'-GAGG-3' was quantified by Beckman LS6500 for 10 min. Experiments were repeated for three times and data were corrected by subtraction of the background. **C.** Interaction of F195 and AdoMet in the cofactor-bound structure of M1.HpyAVI.





Figure S3. Dimer organization of M1.HpyAVI, M.MboIIA, M.RsrI and TTHA0409

The two monomers are marked in green and blue. The pictures were generated using *PyMol*.



Figure S4.The *Fo-Fc* omit map of the AdoMet in the M1.HpyAVI binary complex structure.

The pictures were generated using *PyMol*.