Base Pair Opening Dynamics in Methylated GATC Sites Catalyzed by Ammonia

Jongchul Bang, Sung-Hun Bae, Chin-Ju Park, Joon-Hwa Lee,†,* and Byong-Seok Choi*

Department of Chemistry and National Creative Research Initiative Center, KAIST, Daejeon 305-701, Korea
**E-mail: byongseok.choi@kaist.ac.kr*
*†Department of Chemistry, RINS, and Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju, Gyeongnam 660-701, Korea *E-mail: joonhwa@gnu.ac.kr*
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DNA methylation regulates various cellular process in most living organism.[1] *Escherichia coli* (*E. coli*) DNA adenine (*dam*) methyltransferase methylates the N6 positions of adenine within the 5'-GATC-3' sequence (Fig. 1).[2] The *dam*-methylation status of GATC sites plays a key role for control of cellular DNA process.[2] The *E. coli* SeqA protein specifically binds to newly synthesized hemimethylated GATC site at *oriC* region during replication and then prevents second replication process.[3] During DNA mismatch repair, the MutH protein selectively cleaves the unmethylated strand which is newly synthesized during replication, within two strands of hemimethylated GATC site.[4] The control of DNA process at GATC sites might be caused by unique structural or dynamic properties of *dam*-methylation on DNA. The structural and/or dynamic studies of the GATC site with various methylation modifications are required in order to elucidate the mechanism of specific recognition SeqA to hemimethylated GATC in a sea of fully methylated one. Recently, the kinetics and thermodynamics of base-pair openings of the unmethylated (UMe), hemimethylated (HMe), and fully methylated (FMe) GATC-containing DNA duplexes using tris(hydroxymethyl) aminomethane (TRIS) were reported.[5] This study suggested that the faster base-pair opening of the hemimethylated GATC duplex, relative to the fully methylated one, allows the hemimethylated GATC duplex to be recognized easily by the SeqA protein in the background of abundant fully methylated sites.[5] This study also implicated that the lower energy level of the partially opened state of the hemimethylated GATC site, relative to that of the fully methylated one, might be a key factor that contributes to specific complex formation between SeqA and hemimethylated GATC sites.

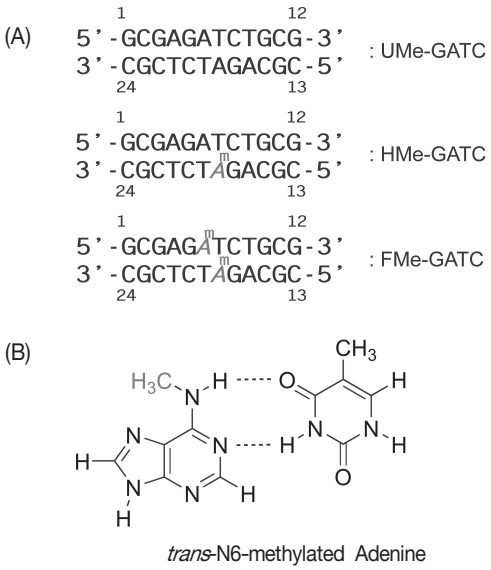

Figure 1. (A) Sequence and numbering of the dodecamer DNA duplexes that contain (from top to bottom) unmethylated, hemimethylated, and fully methylated GATC sites (referred to as the UMe-GATC, HMe-GATC, and FMe-GATC duplexes, respectively). A^m indicates an N6-methylated adenine. (B) The Watson-Crick base pairing between a *trans*-N6-methylated adenine residue and its complementary thymine residue.

NMR hydrogen exchange experiment has been used to probe the thermodynamics and kinetics for base pair opening in a variety of nucleic acids.[6] Ammonia is the most commonly used base catalyst in this study because of its high catalytic efficiency.[6,7] At high temperature, however, TRIS was used in order to minimize the severe line-broadening caused by strong base catalyst such as ammonia.[5,7] The catalytic efficiency of base catalyst is affected by its size and charge property.[8] Here, we performed ammonia-catalyzing imino proton exchange studies of the three GATC-containing DNA duplexes (Fig. 1) and compared with the result of TRIS-catalyzing exchange study previously reported,[5] to elucidate the size effect of base catalyst.

All DNA oligonucleotides were purchased from Genotech Inc. (Daejeon, Korea). The oligonucleotides were desalted using a Sephadex G-25 gel filtration column. DNA duplex samples were prepared by combining molar equivalent of the two strands in 90% H_2O/10% D_2O NMR buffer containing 10mM sodium phosphate (pH 6.8) and 100mM NaCl. For ammonia-catalyzed exchange experiments, the pH of buffer was change to 8.0. The ammonia concentration was increased from 0 to 100mM by addition of a 2M ammonia stock solution. NMR experiments were performed on a Varian Inova 600MHz spectrophotometer. All NMR data were processed and analyzed with program VNMRJ and FELIX2004 (Accelrys) as described.[9] Semi-selective inversion recovery 1-D NMR experiments were used to determine the determine the apparent longitudinal relaxation rate constants, R_{1a} (= $1/T_{1a}$), of the imino protons.

The formalism of imino proton exchange has been extensively described.[5-7,10] The apparent relaxation rate constant for imino protons (R_{1a}) can be represented by:

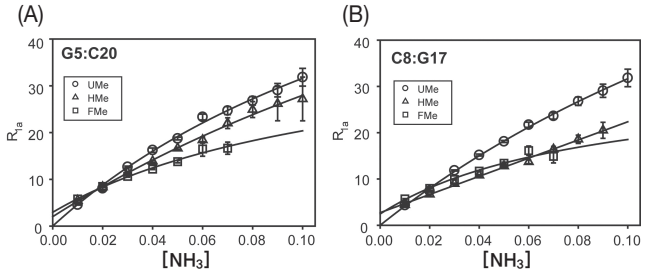

Figure 2. Ammonia-catalyzed exchange experiments carried out with the GATC duplexes. Apparent spin-lattice R_1 relaxation rate constants (R_{1a}) for the (A) G5 and (B) G17 imino protons as a function of the ammonia concentrations are shown. The solid lines are the best fits to (1), and the error bars represent the fitting errors during determination of R_{1a} using data obtained from inversion recovery experiments.

$$R_{1a} = R_1 + k_{AAC} + \frac{k_{op}k_i[\mathrm{B}]}{k_{cl}/\alpha + k_i[\mathrm{B}]} \quad (1)$$

where R_1 is the relaxation rate constant, k_{ACC} is the contribution of the intrinsic base, k_{op} and k_{cl} are the base pair opening and closing rate constants, respectively, α is the accessibility factor which depends on the base catalyst, k_i is the proton transfer rate constant by base catalyst, and [B] is the concentration of base catalyst.[5,7] The apparent equilibrium constant (αK_{op}) for the base pair opening can be calculated by $\alpha k_{op}/k_{cl}$.

The effects of [NH_3] on R_{1a} of the imino protons were measured by inversion recovery experiments and results for G5 and G17 imino protons are shown in Fig. 2. From these data, the base- pair life times ($\tau_0 = 1/k_{op}$) and αK_{op} for central four base pairs of three GATC-containing DNA duplexes were determined by curve fitting using Eq. 1 and listed in Table 1. These values were compared with the corresponding values which had been determined by TRIS-catalyzing imino proton exchange ex- periments.[5] The apparent equilibrium constants for the base pair opening of the G5·C20 and C8·G17 determined using ammonia ($\alpha_N K_{op}$) are very similar with the corresponding values ($\alpha_T K_{op}$) determined using TRIS (Table 1). The relative ratio (α_N/α_T) of these two corresponding values are in the range from 0.8 to 1.2, indicating that two base catalysts, ammonia and TRIS, have the same accessibility factors. The ammonia led to rapid exchange for most of the imino protons at 35°C, because the ammonia (pKa~9.3) is much stronger base catalyst than TRIS (pKa ~7.9 at 35°C).[7] This phenomenon is responsible for severe line-broadening under high concentration of ammonia. Thus, in this study, the base-pair life times were determined with very large standard deviations which meant that the values were relatively inaccurate compared to those determined from TRIS-catalyzing experiment.

Surprisingly, the αK_{op} of two A·T base pairs determined by ammonia are 2~3-fold smaller than those derived from TRIS-catalyzing experiment. This result might be caused by the fast exchange of the A·T base pairs which are relatively less stable than neighboring G·C base pairs. However, this experiment clearly showed that these two A·T base pairs in the FMe-GATC duplex are much more stable than the corresponding base pairs in the UMe- and HMe-GATC duplexes.

Table 1. Parameters for ammonia-catalyzing base pair opening of the GATC-containing DNA duplexes at 35°C

Base Pair	Duplex	NH_3-catalyzing		TRIS-catalyzing[a]		α_N/α_T[b]
		$\alpha_N K_{op}$ ($\times 10^{-6}$)	τ_0 (ms)	$\alpha_T K_{op}$ ($\times 10^{-6}$)	τ_0 (ms)	
G5·C20	UMe	0.91 ± 0.10	11 ± 3	0.73 ± 0.01	10 ± 2	1.2
	HMe	0.64 ± 0.08	9 ± 4	0.76 ± 0.02	24 ± 4	0.8
	FMe	0.59 ± 0.16	26 ± 16	0.59 ± 0.02	64 ± 7	1.0
A6·T19	UMe[c]	12.3 ± 3.5	0 ± 1	41.9 ± 0.7	1 ± 1	0.3
	HMe	7.7 ± 6.5	8 ± 9	17.5 ± 0.3	2 ± 1	0.4
	FMe[c]	4.9 ± 2.3	10 ± 6	8.3 ± 0.1	3 ± 1	0.6
T7·A18	UMe[c]	12.3 ± 3.5	0 ± 1	41.9 ± 0.7	1 ± 1	0.3
	HMe	8.0 ± 27.8	8 ± 9	18.7 ± 0.3	2 ± 1	0.4
	FMe[c]	4.9 ± 2.3	10 ± 6	8.3 ± 0.1	3 ± 1	0.6
C8·G17	UMe	0.80 ± 0.05	8 ± 2	0.79 ± 0.01	8 ± 1	1.0
	HMe	0.37 ± 0.04	0 ± 6	0.49 ± 0.01	9 ± 6	0.8
	FMe	0.65 ± 0.35	33 ± 32	0.64 ± 0.01	65 ± 5	1.0

[a]data of reference (5); [b]relative ratio of $\alpha_N K_{op}$ and $\alpha_T K_{op}$; [c]T19 and T7 resonances are overlapped each other

This implies that the ammonia-catalyzing exchange experiment performed at high temperature cannot give us the exact parameter for base pair opening but provides the relatively exact thermal stabilities of the interesting system.

In conclusion, we determined the parameters for base pair opening of three GATC-containing DNA duplexes using ammonia as base catalyst and the results were compared with the corresponding values derived from TRIS-catalyzing exchange experiment previously reported. Ammonia has the same accessibility factor with TRIS and both base catalysts give the similar αK_{op} for very stable base pairs. In contrast, it is difficult to determine the exact parameters for base pair opening of less stable base pairs using ammonia but the relative base pair stability can be estimated exactly.

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