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Slow rotational dynamics of cytosine NH_2 groups in double-stranded DNA

Xi Wang,

Binhan Yu,

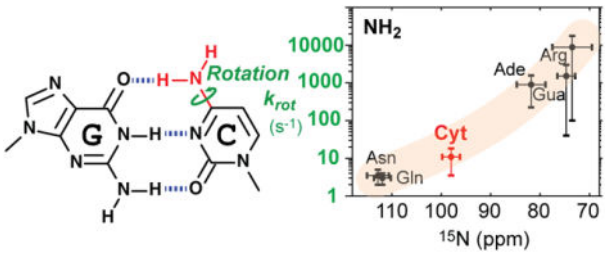
Junji Iwahara*

Department of Biochemistry & Molecular Biology, Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch, Galveston, TX 77555-1068

Abstract

Aromatic NH_2 groups are essential as hydrogen-bonding donors in secondary structures of DNA and RNA. Although rapid rotations of NH_2 groups of adenine and guanine bases were previously characterized, there has been a lack of quantitative information about slow rotations of cytosine NH_2 groups in Watson-Crick base pairs. In this study, using an NMR method we had recently developed, we determined the kinetic rate constants and energy barriers for cytosine NH_2 rotations in a 15-base-pair DNA duplex. Our data show that the rotational dynamics of cytosine NH_2 groups depend on local environments. Qualitative correlation between the ranges of ^{15}N chemical shifts and rotational timescales for various NH_2 groups of nucleic acids and proteins illuminates a relationship between the partial double-bond character of the C-N bond and the timescale for NH_2 rotations.

Graphical Abstract



Base pairing of nucleic acids is crucial for gene replication, recombination, transcription, and translation in life. The dynamic nature of base pairs has been revealed by nuclear magnetic resonance (NMR) spectroscopy.[1,2] Nucleic acids undergo conformational transitions between Watson-Crick base pair and Hoogsteen base pair.[3–6] Transient breakage of a base pair ('base-opening') can lead to an extrahelical conformation where a base is flipped out of double helix and recognized by enzymes for base excision repair.[7,8]

*Corresponding Author: j.iwahara@utmb.edu.

Supporting Information. Materials and methods; the Eyring plot of temperature dependence of k_{rot} rate constants; and Solvent-accessible surface areas of NH_2 groups in the 15-bp DNA.

Therefore, the dynamic properties of base pairs are important for our understanding of the functions of DNA and RNA as well as their interactions with proteins.

NH_2 groups of adenine (A), cytosine (C), and guanine (G) bases serve as hydrogen-bonding donors in base pairing. NH_2 rotations of A/G bases in DNA were studied through NMR line-shape analysis.[9,10] Despite their hydrogen-bonding with base-pair partners, adenine/guanine NH_2 groups rapidly rotate on a timescale of 10^{-2}–10^{-5} s, depending on temperature and hydrogen bonds. Due to the rapid exchange caused by the rotations, the two ^{1}H resonances of adenine/guanine NH_2 groups are severely broadened or averaged into a single resonance at physiological temperature.[9–11] By contrast, cytosine NH_2 groups typically exhibit two distinct ^{1}H resonances, clearly indicating that their rotations are slower.[11] It was proposed that cytosine NH_2 groups do not even rotate within Watson-Crick GC base pairs.[12]

In this communication, we report the kinetics and the energy barriers for rotations of cytosine NH_2 groups in double-stranded DNA. Nuclear magnetization transfer via the exchange between the two ^{1}H resonances of each NH_2 group is difficult to distinguish from transfer via cross-relaxation that occurs simultaneously. Recently, to investigate NH_2 rotations, we developed a new NMR method named “ODDS-*zz*-EXSY” (off-diagonal–diagonal swapping *zz*-exchange spectroscopy). This method cancels cross-relaxation, controls the positions of auto and exchange cross peaks, and allows us to accurately measure the rate constants for NH_2 rotations in the slow exchange regime.[13] In our prior work, we used ODDS-*zz*-EXSY to investigate slow rotations of protein Asn/Gln side-chain NH_2 groups. In our current study, we apply it to the rotational dynamics of cytosine NH_2 group in double-stranded DNA.

A ^{13}C,^{15}N-labeled 15 base-pair (bp) DNA duplex (Figure 1A) was used in this study. Details of the sample preparation are described in the Supporting Information (SI). This 15-bp DNA contains 8 cytosine bases that form hydrogen bonds with guanine bases. ^{1}H, ^{13}C, ^{15}N resonances were assigned as described in the SI. As shown in Figure 1B, the cytosine NH_2 groups exhibit well-resolved signals in the ^{1}H-^{15}N heteronuclear single-quantum coherence (HSQC) spectra. NMR signals from the NH_2 groups of the terminal cytosine (C0 and C15) were broadened, presumably due to the rapid hydrogen exchange, and therefore were excluded from further analysis. We investigated the behavior of the other 6 cytosine NH_2 groups in the 15-bp DNA.

Using the ODDS-*zz*-EXSY method,[13] we measured the kinetics of NH_2 rotations for these cytosine bases. Each ODDS-*zz*-EXSY experiment provides two 2D ^{1}H-^{1}H sub-spectra for NH_2 groups selectively. Examples of these sub-spectra are shown in Figure 1C. In one of the sub-spectra, the exchange cross peaks arising from the NH_2 rotations (which cause interconversion of the product operator terms $2H_z^aN_z$ and $2H_z^bN_z$ during the mixing time) are observed at off-diagonal positions whereas the auto cross peaks are observed at diagonal positions. Because the ODDS-*zz*-EXSY method cancels cross-relaxation, the off-diagonal cross peaks arise from exchange. These data clearly show that cytosine NH_2 groups undergo rotations in a slow exchange regime. In the other sub-spectrum, for which the $2H_z^aN_z$ and $2H_z^bN_z$ terms are interconverted through coherence transfer via two scalar $^1J_{NH}$ couplings,[13] the exchange cross peaks are located at the diagonal positions and the auto cross peaks are

at the off-diagonal positions. This feature of the ODDS-zz-EXSY method greatly facilitate quantitative analysis of auto cross peaks.[13]

We measured ODDS-*zz*-EXSY sub-spectra using 6 different mixing times. From the signal intensity ratios, we determined the rate constant k_{rot} for rotations of the cytosine NH_2 groups through nonlinear least-squares fitting with:[13]

$$\sqrt{\frac{I_{E1}I_{E2}}{I_{A1}I_{A2}}} = q + (1-q)\tanh(k_{rot}T_m) \quad [1],$$

where I_{E1} and I_{E2} are the intensities of two off-diagonal exchange cross peaks; I_{A1} and I_{A2} are those of two off-diagonal auto cross peaks; q is a parameter representing the incompleteness of the ODDS filter;[13] and T_m is the mixing time. Figure 1D shows examples of time courses and best-fit curves. At 25°C, the rate constants k_{rot} for cytosine NH_2 rotations ranged from 4.8 s^{-1} to 24 s^{-1} in the 15-bp DNA. Interestingly, even among cytosine bases in the middle region of the 15-bp DNA, significant variation in k_{rot} rate constants was observed. For example, the k_{rot} constant for C6 (15 ± 1 s^{-1}) was three times as large as that for C24 (4.8 ± 0.3 s^{-1}).

We measured the rate constants k_{rot} for all non-terminal cytosine NH_2 groups at 15, 20, 25, and 30°C. These rate constants are shown in Figure 2A. The rotation kinetics were faster at higher temperatures. Through fitting to the temperature dependence data using the Eyring equation, we determined the energy barriers for NH_2 rotations. The activation free energy ($\Delta G^{\ddagger}$) and its enthalpic ($\Delta H^{\ddagger}$) and entropic ($-T\Delta S^{\ddagger}$) components obtained for each cytosine NH_2 group are shown in Figure 2B. For the majority of cytosine NH_2 groups, the energy barrier for rotations about the C-N bond is largely enthalpic with only little or no entropic contribution.

Interestingly, the C7 NH_2 group exhibited a significant entropic contribution and a relatively small activation enthalpy ($\Delta H^{\ddagger}$). The smaller $\Delta H^{\ddagger}$ for this NH_2 group was obvious in a weaker dependence of its k_{rot} rate constant on temperature (see also Eyring plots in Figure S1 in the SI). The negative $\Delta S^{\ddagger}$ for this NH_2 group suggests that the transition state is entropically unfavorable, which might be caused by a larger degree of exposure of hydrophobic bases to solvent via a kink of DNA. Because a kink of DNA occurs more easily at C-A steps[15] and C7 is located at a C-A step in the middle of DNA, the unique $\Delta H^{\ddagger}$ and $-T\Delta S^{\ddagger}$ of this residue might be related to the deformability.

Although both C1 and C16 are located at a position second to a 5'-terminus, the k_{rot} rate constants of the C16 NH_2 group were larger than those of the C1 NH_2 group by a factor of 3. The activation free energy ($\Delta G^{\ddagger}$) for C1 NH_2 rotations was larger by 2.7 kJ/mol than that for C16 NH_2 rotations. The 3'-neighboring nucleotides (A2 and C17) are different for C1 and C16. A recent study shows that the base-stacking energies for CpA (TpG) steps are significantly more favorable than those for CpC (GpG) steps.[16] More stable base-stacking of CpA might impede rotations of the C1 NH_2 group.

Now, with our data on cytosine NH_2 rotations in double-stranded DNA, more comprehensive information is available about hindered rotations about sp^2 C-N bonds in

biomolecules. NMR spectroscopy has been used to study rotations about sp^2 C-N bonds of arginine (Arg), asparagine (Asn), and glutamine (Gln) in proteins.[13,17–24] Arg side-chain sp^2 C-N bond rotations are far faster than Asn/Gln side-chain NH_2 rotations. This difference can be ascribed to the different chemical structures between the guanidinium (Arg) and carbamoyl (Asn/Gln) moieties. However, it is less clear why NH_2 rotation timescales are different between A/G and C bases despite the similarity in local chemical structures. Since solvent-accessible surface areas of cytosine NH_2 groups are not significantly smaller than those of adenine/guanine NH_2 groups (Figure S2 in the SI), the slower NH_2 rotations for C bases are not due to a smaller degree of exposure to solvent.

The difference in the rotational dynamics may stem from different degrees of the partial double-bond character in the C-N bonds. Due to strong shielding effects of π electrons, ^{15}N chemical shifts qualitatively reflect the double-bond characters. For example, Lys side-chain amino groups single-bonded to sp^3 carbon exhibit ^{15}N chemical shifts around ~24 ppm in the NH_2 state (~33 ppm in the NH_3^+ state),[22] whereas ^{15}N chemical shifts of nitrogen atoms double-bonded to an sp^2 carbon atom are far larger (e.g., adenine N1, ~225 ppm; guanine N7, ~237 ppm). ^{15}N chemical shifts of cytosine NH_2 (~98 ppm) are larger than ^{15}N chemical shifts of adenine NH_2 (~80 ppm) and guanine NH_2 (~76 ppm). These ^{15}N chemical shifts suggest that the partial double-bond character in the C-N bond is stronger for cytosine NH_2 groups than for adenine and guanine NH_2 groups, which is consistent with the slower rotations of cytosine NH_2 groups. The same rule can also explain the difference between protein side-chain NH_2 groups: Arg NH_2 (^{15}N ~72 ppm) rotates far faster than Asn NH_2 (^{15}N ~113 ppm) and Gln NH_2 (^{15}N ~112 ppm). The qualitative correlation between the typical ranges of NH_2 rotation rates and ^{15}N chemical shifts is shown in Figure 3. These data suggest that the timescale of NH_2 rotations about a C-N bond is related to the degree of partial double-bond character in the C-N bond.

However, the observed variation in rates and barriers for rotations among cytosine NH_2 groups (Figure 2) should reflect local environments around individual NH_2 groups in the same DNA. Local conformational dynamics such as the base-opening process and transitions between Watson-Crick and Hoogsteen base pairs depend on sequence context.[3,25] DNA bendability also depends on sequence context.[15] Since the hydrogen bond of each cytosine NH_2 group remains in both Watson-Crick and Hoogsteen base pairs, base-opening dynamics that break the hydrogen bond may be more relevant to the cytosine NH_2 rotations in double-stranded DNA. The behavior of cytosine NH_2 groups may be even more diverse in RNA due to its structural diversity. The ODDS-*zz*-EXSY method is readily applicable to ^{15}N- or $^{13}C/^{15}N$-labeled RNA as well.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENT

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ABBREVIATIONS

HSQC	heteronuclear single-quantum coherence
ODDS-zz-EXSY	off-diagonal–diagonal swapping zz-exchange spectroscopy

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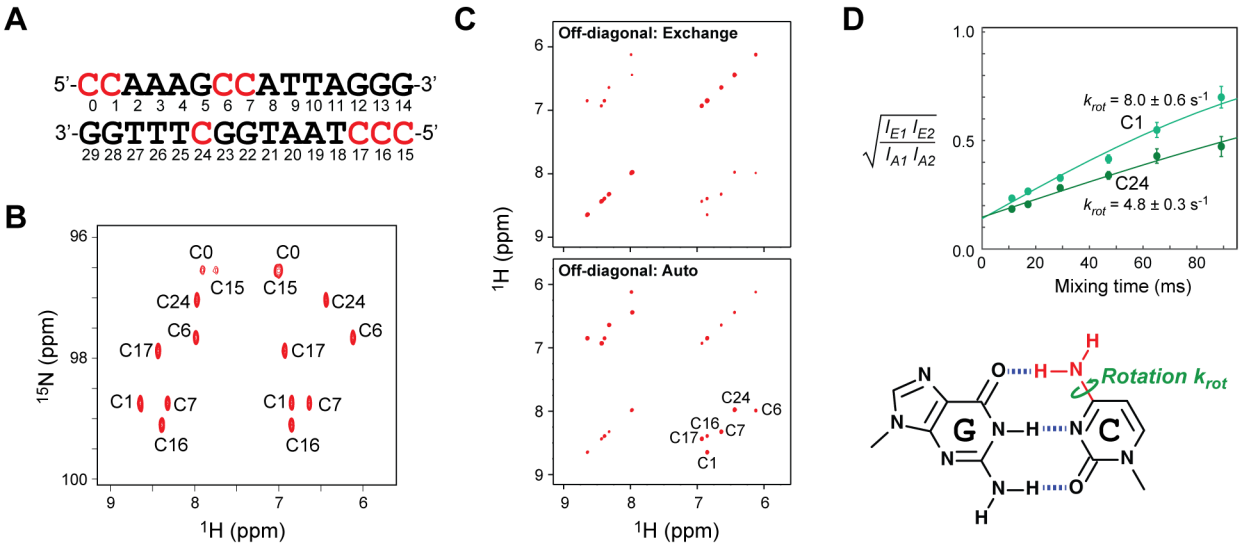

Figure 1.

NMR investigations of cytosine NH_2 rotations in double-stranded DNA. (**A**) 15-bp DNA used in this study. The residue numbering is based on that of Fernandez et al.[14] Cytosine and guanine nucleotides in this DNA were labeled by ^{13}C and ^{15}N isotopes. The sample solution contained 0.3 mM DNA, 20 mM potassium succinate (pH 5.8), 100 mM KCl, and 0.4 mM NaF. (**B**) ^{13}C-decoupled ^{1}H-^{15}N HSQC spectrum for cytosine NH_2 groups in the 15-bp DNA duplex at 25°C. The resonances were assigned as described in the Supporting Information. (**C**)Examples of ODDS-*zz*-EXSY sub-spectra for cytosine NH_2 groups of the $^{13}C/^{15}N$-labeled 15-bp DNA at 25°C (with a mixing time of 47 ms). In the second sub-spectrum, off-diagonal and diagonal cross peaks are swapped through coherence transfer between $2H_z^aN_z$ and $2H_z^bN_z$ terms via two $^1J_{NH}$ couplings.[13] (**D**) Ratios of intensities of off-diagonal exchange cross peaks and off-diagonal auto cross peaks for the NH_2 groups of C1 and C24 at 25°C. Solid curves represent the best-fit curves obtained through the fitting with Eq. 1. The k_{rot} constant represents a rate constant for a 180° rotation and is a half of the exchange rate constant k_{ex}.

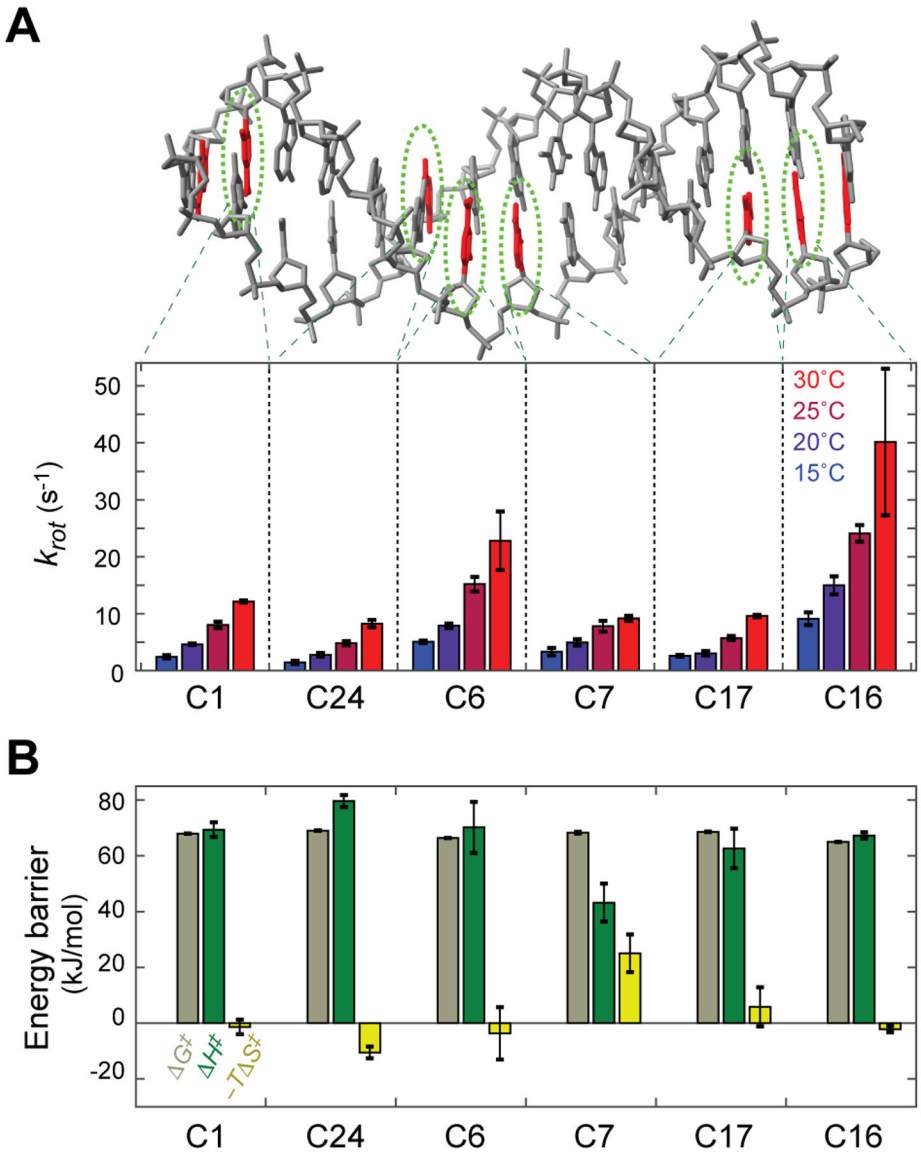

Figure 2.

Temperature dependence of cytosine NH_2 rotations in the 15-bp DNA duplex. (**A**) Rate constants (k_{rot}) for cytosine NH_2 rotations measured at 15, 20, 25, and 30°C. (**B**) Energy barriers for cytosine NH_2 rotations. The activation free energy and its enthalpic and entropic components were determined from the temperature dependence of k_{rot} constants.

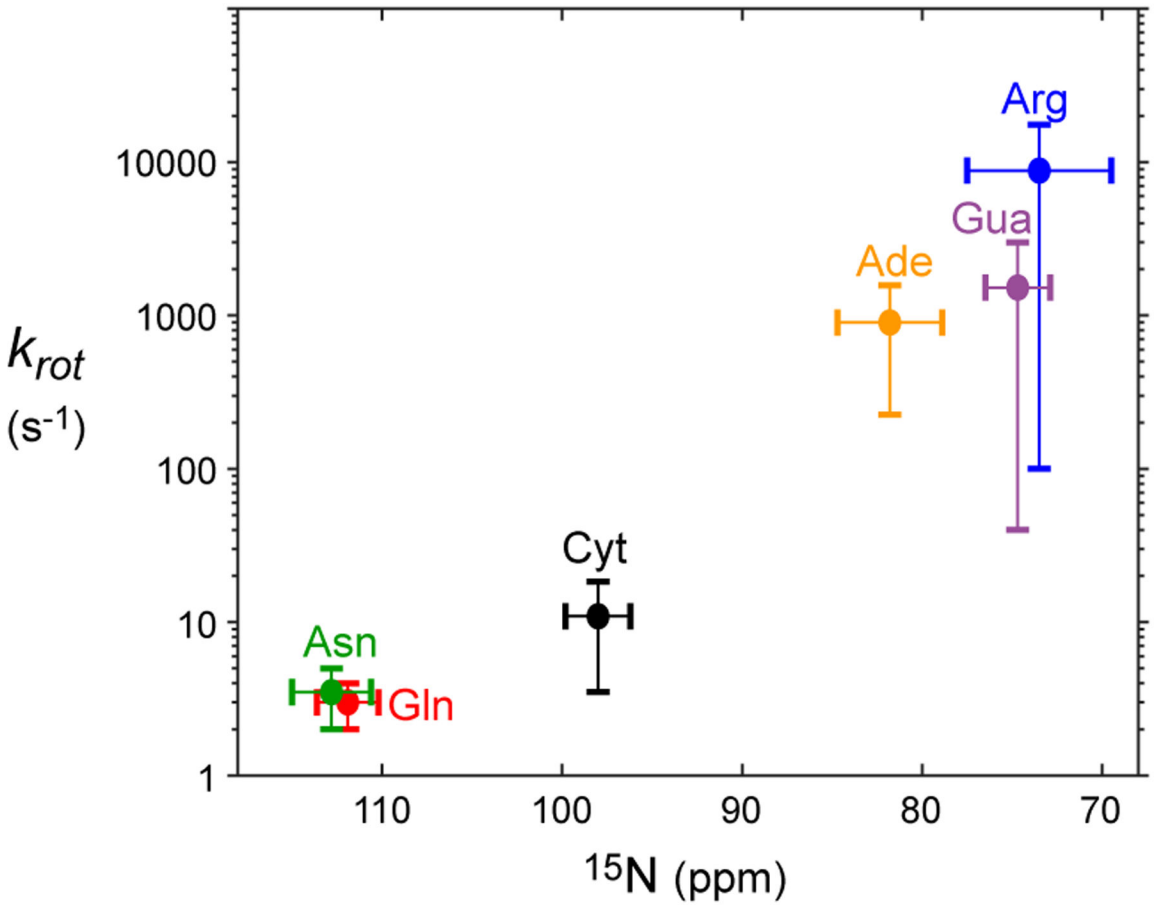

Figure 3.

Qualitative correlation between the ranges of ^{15}N chemical shifts and rotation rate constants k_{rot} for biomolecular NH_2 groups. The horizontal position and width of a cross represent the average and the standard deviation of ^{15}N chemical shifts of each NH_2 type in the Biological Magnetic Resonance Bank (BMRB) database. The vertical position and width represent a range of k_{rot} rate constants for Asp/Gln,[13] Arg,[23] cytosine (Cyt; current work), adenine (Ade),[10] or guanine (Gua)[9] NH_2 groups at 25°C. Because of the limited information and factors that influence NH_2 rotations (e.g., hydrogen bonds), the shown ranges of k_{rot} rate constants provide only qualitative information.