Mini Review

Base-pair Opening Dynamics of Nucleic Acids in Relation to Their Biological Function☆

Seo-Ree Choi a, Na-Hyun Kim a, Ho-Seong Jin a, Yeo-Jin Seo a, Juhyun Lee b, Joon-Hwa Lee a,⁎

a Department of Chemistry and RINS, Gyeongsang National University, Gyeongnam 52828, South Korea
b Department of Chemistry, KAIST, Daejeon 34141, South Korea

ABSTRACT

Base-pair opening is a conformational transition that is required for proper biological function of nucleic acids. Hydrogen exchange, observed by NMR spectroscopic experiments, is a widely used method to study the thermodynamics and kinetics of base-pair opening in nucleic acids. The hydrogen exchange data of imino protons are analyzed based on a two-state (open/closed) model for the base-pair, where hydrogen exchange only occurs from the open state. In this review, we discuss examples of how hydrogen exchange data provide insight into several interesting biological processes involving functional interactions of nucleic acids: i) selective recognition of DNA by proteins; ii) regulation of RNA cleavage by site-specific mutations; iii) intermolecular interaction of proteins with their target DNA or RNA; iv) formation of PNA:DNA hybrid duplexes.

© 2019 The Authors. Published by Elsevier B.V. on behalf of Research Network of Computational and Structural Biotechnology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords:
NMR
Nucleic acids
Base-pair opening
Hydrogen exchange
DNA
RNA

Contents
1. Introduction .............................................................. 797
2. Hydrogen Exchange Theory ....................................................... 798
  2.1. Hydrogen Exchange of the Base-Paired Imino Protons .... 798
  2.2. NMR Measurement of Hydrogen Exchange Rates ............ 800
3. Implications for Specific DNA Recognition. ...................... 800
  3.1. Recognition of Hemimethylated GATC Site ................. 800
  3.2. Recognition of Cyclobutane Pyrimidine Dimer .......... 801
4. Implications for Sequence-Specific RNA Cleavage ............ 801
  4.1. Biogenesis of miRNA156a . ........................................ 801
  4.2. Self-Cleavage of the P1 Duplex of the Tetrahymena Group I Ribozyme ... 802
5. Implications for DNA/RNA-Protein Interactions ............. 802
  5.1. B-Z Transition of DNA by Z-DNA Binding Proteins ........ 802
  5.2. B-Z Junction Formation in DNA by Z-DNA Binding Proteins ... 802
  5.3. Target Recognition of RNA Aptamer ......................... 802
6. Implications for DNA-PNA Hybrid Duplex Formation .... 802
7. Conclusion ................................................................ 802
8. Declarations of Competing Interest .............................. 802
Acknowledgements .................................................. 802
References .................................................................. 803

1. Introduction

Base-pair opening in DNA is a structural fluctuation that is required for its biological function in transcription, repair, and
recombination. RNA also undergoes conformational transitions that exhibit distinct structural and dynamic features required for proper function. The hydrogen-bonded imino protons of nucleic acids are a probe of the base-pair opening kinetics. Hydrogen exchange NMR experiments provide information on the thermodynamics and kinetics of base-pair opening and therefore represent a probe of the dynamic motions of the base-pairs. Analysis of the hydrogen exchange of imino protons employs a two-state (open/closed) model for the base-pair, where hydrogen exchange only occurs from the open state (Fig. 1) [1–3]. The opening \( k_{\text{op}} \) and closing \( k_{\text{cl}} \) rate constants and/or equilibrium constant for base-pair opening \( k_{\text{op}} = k_{\text{op}}/k_{\text{cl}} \) can be determined by measuring the exchange using an external catalyst. These experiments have been used to probe base-pair opening in various DNA duplexes [3–18], DNAs containing modified base such as 5-flourourasil, N6-methyl adenine, or modified guanine [19–21], UV-induced photoadduct-containing DNA [22], IHF-complexed DNA [23], interstrand cross-linked DNA [24], i-motif structure formed by the complementary C-rich DNA [25,26], various RNAs [15,27–36], peptide nucleic acids (PNAs) [37,38], and threose nucleic acid (TNA) [39]. NMR exchange and single molecule FRET experiments could be used to study the protonation/deprotonation of adenine bases and formation of A\(^+\)·C wobble pair [40–44]. In addition, hydrogen exchange data can also be used to probe how intermolecular interactions stabilize nucleic acid duplexes. For example, imino proton exchange studies of a DNA/RNA-protein complex by NMR spectroscopy showed that the protein substantially changes the equilibrium constant for base-pair opening [45–49].

In this review, we discuss several examples of how hydrogen exchange data provide insight into the biological function of interesting nucleic acids. Studies of base-pair opening kinetics have been used to propose mechanisms by which DNA is selectively recognized by its target proteins. Selective recognition is used by seqA to distinguish the hemimethylated GATC from the corresponding fully methylated complex [20], and the higher binding affinity of XPC-hHR23B for a double mismatched cyclobutane pyrimidine dimer (CPD) helps it distinguish the double mismatch from the matched or single mismatched CPD species [22]. Base-pair opening kinetics studies can also suggest the mechanisms that explain how RNA cleavage can be regulated by site-specific mutations, e.g., in the case of the biogenesis of miRNA156a by DICER-like 1 protein (DCL1) [31] and self-cleavage of the Tetrahymena group I ribozyme [28].

2. Hydrogen Exchange Theory

2.1. Hydrogen Exchange of the Base-Paired Imino Protons

Imino proton exchange from a base-pair consists of a two-step process requiring base-pair opening followed by proton transfer to a base catalyst (Fig. 1). The rate constant for imino proton exchange \( k_{\text{ex}} \) is given by Eq. (1):

\[
k_{\text{ex}} = \frac{k_{\text{op}} \times k_{\text{id}}}{k_{\text{cl}} + k_{\text{id}}}
\]

where \( k_{\text{op}} \) and \( k_{\text{id}} \) are the rate constants for opening and closing of the base-pair, respectively, and \( k_{\text{id}} \) is the rate constant for proton exchange by base catalyst in the opening state. In the base-pair, the exchange is
catalyzed by both the added base catalyst and the nitrogen of the complementary base, which acts as an intrinsic catalyst [1,2]. The $k_r$ value is calculated as:

$$k_r = k_B[B] + k_{int} = \frac{k_{coll}}{1 + 10^{\Delta pK_a}}[B] + k_{int}$$

(2)

where $k_B$ is the rate constant for imino proton transfer by a base catalyst, $k_{int}$ is the exchange rate constant catalyzed by an intrinsic base, $k_{coll}$ is the collision rate constant, $[B]$ is the concentration of the externally added base catalyst such as ammonia, Tris, phosphate, and difluoroethylamine (base form) and $\Delta pK_a$ is the $pK_a$ difference between the imino proton and the base catalyst. The $[B]$ values are calculated as $[B] = [B]_{total}/(1 + 10^{(pK_a – pH)})$, where $[B]_{total}$ is the total concentration of the added base catalyst [28,38]. Thus, the $k_{ex}$ for the base-paired imino proton is represented by Eq. (3):

$$k_{ex} = \frac{k_{op}(k_B[B] + k_{int})}{k_{cl} + (k_B[B] + k_{int})} = \frac{k_{op}(k_B[B] + k_{int})}{k_B[B] + k_{int} + k_{op}/k_{cl}}$$

(3)

where $K_{op} (= k_{op}/k_{cl})$ is the equilibrium constant for base-pair opening. $Re$-organization of Eq. (3) yields the following equation:

$$\tau_{ex} = \frac{1}{k_{ex}} = \frac{1}{k_{op}} + \frac{1}{k_{op}[B] + k_{int}} = \tau_0 + \frac{k_{op}[B] + k_{int}}{\tau_0 k_{op}}$$

(4)

where $\tau_{ex}$ is the exchange time ($= 1/k_{ex}$) and $\tau_0$ is the lifetime for closed state of the base-pair ($= 1/k_{op}$). Interestingly, curve fitting the $\tau_{ex}$ of the imino protons as a function of the concentration (base form) of the added base catalyst ($[B]$) within Eq. (4) gives not only the thermodynamic parameter, $K_{op}$, but also kinetic parameter, $\tau_0 (= 1/k_{op})$ value (Fig. 2A) [31,32]. As $[B]$ increases, the $\tau_{ex}$ values converge to the base-pair lifetime, $\tau_0$ in these plots. For example, the U16-A97 base-pair in the wild-type (WT) primary miRNA156a model RNA is expected to have a longer $\tau_0$ than that of the A9C and A10CG mutants ($\tau_0$ of WT, 5.0 ms; A9C, 1.0 ms; A10CG, 1.6 ms) (Fig. 2A) [31]. The lifetime for open state of the base-pair ($\tau_{open} = 1/k_{cl}$) is calculated using the relation $\tau_{open} = K_{op} \times \tau_0$.

Fig. 2. Hydrogen exchange data of the (A) primary miRNA156a modeled RNA (pri-miR156a) [33], (B) DNA dodecamer duplex containing hemimethylated GATC site (HMe-GATC) [20], (C) DNA 13mer duplex (bzDNA13) complexed with ZnADAR1 [39], and (D) (-1C)-mutant P1 RNA duplex ((-1C)-P1) [30]. The solid lines are the best fits to Eqs. (4), (5), (6), and (7), respectively, and the error bars represent the fitting errors during determination of $K_{ex} (= R_0 + k_{ex})$ or $\tau_{ex} (= 1/k_{ex})$ values. [Tris] is the concentration of Tris (base form) which is used for the base catalyst. Secondary structures of the wild-type (WT) and A9C and A10CG mutant pri-miR156a (in (A)), HMe-GATC (in (B)), bzDNA13 (in (C)), and (-1C)-P1 (in (D)) are shown on top of each hydrogen exchange data.
The apparent relaxation rate constant \((k_{\text{ex}})\) determined by curve fitting the exchange data using Eq. (5) (Fig. 2B) [20, 21]. The apparent relaxation rate constant \((R_1)\) for an imino proton determined by NMR experiments is the sum of the \(k_{\text{ex}}\) value and the \(R_1\) relaxation rate constant. As \([B]\) increased, the \(k_{\text{ex}}\) values converge to the \(k_{\text{op}}\) value in these plots. For example, in duplex DNA containing a hemimethylated GATC site, the T7-1A8 and G3-C22 base-pairs are expected to have larger \(k_{\text{ex}}\) (that is, shorter \(R_1\)) than the G5-C20 base-pair \((\tau_0 = 2.4\;\text{ms};\;G3-C22, 2.0\;\text{ms};\;G5-C20, 24.4\;\text{ms})\) (Fig. 2B) [20]. Similarly, under these conditions \((k_{\text{ex}})\) of the imino protons to:

\[
\frac{1}{k_{\text{ex}}} = \frac{1}{k_{\text{op}}} + \frac{1}{k_{\text{ex}}^{\text{obs}}}
\]

where the subscripts, wt and mod, indicate the thermodynamic parameters of the wild-type and modified nucleic acids, respectively [20, 31].

2.2. NMR Measurement of Hydrogen Exchange Rates

The hydrogen exchange rates of the imino protons were determined by a water magnetization transfer experiment, where a selective 180° pulse for water was applied, followed by a variable delay \(t\), and then a Watergate acquisition pulse was used to suppress the water signal [27, 28, 52]. During the delay times between selective water inversion and acquisition pulses, a weak gradient (0.02 G/cm) was applied to prevent the radiation damping of the water signal [27, 28]. The exchange rate constants \((k_{\text{ex}})\) were determined by fitting the relative peak intensities, \(I(t)/I_0\), of the imino protons to:

\[
I(t) = 1 - 2k_{\text{ex}}(R_{1w} - R_{1x})e^{-R_{1w}t} - e^{-R_{1x}t}
\]

where \(I(t)\) and \(I_0\) are the peak intensities of the imino proton at delay times \(t\) and zero, respectively, \(R_{1w}\) and \(R_{1x}\) are the apparent relaxation rate constants for the imino protons and water, respectively, which were determined by inversion recovery experiments [28].

The water magnetization transfer method is prone to some artifacts such as exchange-relayed NOE from rapidly exchanging protons in nucleic acids [53]. To suppress efficiently this artifact, a phase-modulated CLEAN chemical exchange spectroscopy (CLEANEX-PM) was applied to the mixing period of a water-selective pulse sequence [53]. The hydrogen exchange data for various nucleic acids using CLEANEX-PM have been reported [53–56].

Generally, the imino protons in double-helical regions have relatively fast exchange kinetics \((k_{\text{ex}} = 0.1–100\;\text{s}^{-1}\) at 25 °C). Interestingly, very slowly exchanging imino protons have been observed for modified tRNAs [57–59]. Hydrogen-deuterium exchange experiments were used to probe the dynamics and flexibility of the slowly exchanging imino protons in various nucleic acid systems [57–62].

3. Implications for Specific DNA Recognition

3.1. Recognition of Hemimethylated GATC Site

Many DNA binding proteins recognize their particular DNA sequences in a highly selective manner. The DNA-protein interactions require sequence-specific hydrogen bonding, van der Waals interactions, sequence-dependent structural changes. In addition, the flexibility of a DNA duplex to adopt the unique structure in complex is important in sequence-specific recognition. Enzymatic methylation of DNA occurs abundantly in most living organisms and regulates a variety of cellular processes. *Escherichia coli* (E. coli) DNA adenine methyltransferase (dam) methylates the N6 of adenosines, to form the N6-methylated A (m6A), within 5′-GATC-3′ sites at the replication origin oriC [63]. The E. coli seqA protein prefers to bind to newly synthesized, hemimethylated, rather than fully methylated, GATC sites to inhibit the initiation of the second round of chromosomal replication [64]. It was reported that the m6A modification destabilizes the base-pairing of RNA duplexes, because the m6A exhibited high energy anti conformation to maintain the Watson-Crick m6A·T base-pair [65–67]. However, in the GATC-containing DNA duplexes, the m6A methylation at the GATC site stabilized the base-pairs at the GATC site [20]. The crystal structure of the seqA protein complexed with a hemimethylated GATC sequence revealed that the two G-C base-pairs exhibited longer heavy atom distances between G-O6 and C-N4 than that of a Watson-Crick base-pair (see Fig. 1), indicating the a partially opened G·C base-pair [68]. However, in the solution structure of the DNA duplex containing hemimethylated GATC, these two base-pairs formed the stable Watson-Crick base-pairs [69]. Interestingly, NMR hydrogen exchange studies revealed that the \(\Delta \Delta G_{\text{cl}}\) value, calculated using Eq. (11), of the 3′-neighboring
G·C base-pair of the N6-methylated adenine between the DNA duplexes containing the hemimethylated and fully methylated GATC sites is 1.42 kcal/mol, although the ΔΔG^o_bp value, calculated using Eq. (9), is only 0.17 kcal/mol [Fig. 3A] [20]. Using free energy differences calculated from these hydrogen exchange data as shown in Fig. 3A, it was shown that the partial opening of the G·C base pairs in the hemimethylated GATC sequence required a much smaller amount of energy than fully methylated GATC [Fig. 3A] [20]. Thus, it was concluded that the hemimethylated GATC site is energetically more favorable for complex formation with seqA than the corresponding fully methylated complex [20].

3.2. Recognition of Cyclobutane Pyrimidine Dimer

The CPD is one of the major types of cytotoxic, mutagenic and carcinogenic UV-induced DNA photoproducts [70,71]. In mammalian cells, CPD-damaged DNA is repaired by nucleotide excision repair [71,72], which is initiated by the binding of XPC-hHR23B to the site of DNA damage [73,74]. Although the CPD lesions are recognized poorly by XPC-hHR23B, when CPD lesions have double T·G mismatches, the binding affinity of XPC-hHR23B is dramatically increased [74]. A structural study of CPD-containing DNA duplexes suggested that, during nucleotide excision repair, the XPC-hHR23B complex recognizes DNA damage by directly searching for conformational distortions, such as a flexible backbone, a bent helix, or an unusual groove width [75]. NMR hydrogen exchange studies found that the two thymine bases of a CPD formed stable Watson-Crick base-pairs with the two opposite adenine bases, evident by a ΔΔG^o_bp ~ 0.2 kcal/mol relative to normal Watson-Crick T·A base-pairs [21]. When the CPD forms double T·G base-pairs, the ΔΔG^o_bp values are ~1.9 kcal/mol [21]. Interestingly, these base-pair instabilities extended to the two base-pair neighbors, which had ΔΔG^o_bp values ~1.4 kcal/mol [21]. Thus, this study concluded that a double mismatch at the CPD lesion facilitates the opening of the six base-pairs including the CPD, forming a small bubble structure that can be easily recognized by XPC-hHR23B [21].

4. Implications for Sequence-Specific RNA Cleavage

4.1. Biogenesis of miRNA156a

MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate expression of their target genes [76]. In plants, primary miRNAs are sequentially cleaved by DCL1 to make mature miRNA. MiRNA156 plays an important role in the temperature-responsive flowering of plants [77,78]. Plants overexpressing miRNA156 produced more leaves than wild-type plants before flowering by regulating the expression of the SQUAMOSA promoter binding protein-like (SPL) gene family [79,80]. The point mutations which stabilize the B5 bulge of primary miRNA156a affected the mature 156 levels as well as the leaf numbers at flowering of miRNA156 overexpressing plants [31,32]. NMR hydrogen exchange studies revealed that the C·G and U-A base-pairs at the DCL1 cleavage site exhibited unique base-pair stability and opening dynamics, which correlated with the biogenesis of miRNA156a [31,32]. For example, the C15·G98 base-pair in the A9C mutant, which decreased mature miRNA156 levels but decreased the leaf number at flowering compared to wild-type primary miRNA156a, was more stable with a ΔΔG^o_bp of ~0.57 kcal/mol, and showed more dynamic opening/closing, with a ΔΔG‡ of ~0.94 kcal/mol (Fig. 3B) [31]. Similar results were observed for the U16·A97 base-pair [31]. However, the A10G mutant, in which the C15·G98 base-pair had a ΔΔG^o_bp of 0.04 kcal/mol and a ΔΔG‡ of 0.30 kcal/mol (Fig. 3B), did not affect the mature miRNA156 levels or the flowering time of the plants [31]. Thus, it was concluded that precisely tuned base-pair stability/flexibility at the DCL1 cleavage site is critical for the biogenesis of miRNA156a.

Fig. 3. Schematic representations of the Gibbs free energy diagram of the base-pair opening and closing for (A) the G·C base-pair adjacent to the N6-methylated adenine (Am) residue in DNA duplexes containing unmethylated (UMe, black), hemimethylated (HMe, red), or fully methylated (FMe, blue) GATC sites [20] and (B) the C15·G98 base-pair in the WT (black), A9C (red) and A10G (blue) primary miRNA156a [33]. Secondary structures of the UMe-, HMe-, and FMe-GATC DNA in (A) and the WT, A9C, and A10G pri-miRNA156a in (B) are shown on top of each figure.
DNA and then converts it to left-handed Z-DNA, a conformation that destabilizes AT-rich regions [49,98]. However, Z

5. Implications for DNA/RNA-Protein Interactions

5.1. B-Z Transition of DNA by Z-DNA Binding Proteins

ZBPs play important roles in RNA editing, innate immune response and viral infection [86–88]. The crystal structures of ZBPs in complex with a 6-base-pair DNA duplex revealed that two molecules of ZBPs bind to each strand of double-stranded Z-DNA, yielding 2-fold symmetry with respect to the DNA helical axis [89–92]. ADAR1 deaminates adenine in pre-mRNA to yield inosine [89], ADAR1 has two ZBPs, Zα and Zβ, at its NH2-terminus [89]. Hydrogen exchange measurements on the duplex DNA complexed with Zα domain of human ADAR1 (ZαADAR1) revealed that the kex of the G imino protons in the left-handed Z-form helix decreased from 11.1 to 4.8 s⁻¹ as the protein-to-DNA (P/N) molar ratio increased from 0.7 to 2.5 [50]. This observation indicates the possible presence of a mixture of two complex state: DNA-ZαADAR1 and DNA-(ZαADAR1)₂ [50]. These results support an active B-Z transition mechanism in which the ZαADAR1 protein first binds to DNA and then converts it to left-handed Z-DNA, a conformation that is then stabilized by the additional binding of a second ZαADAR1 molecule [50]. Similar hydrogen exchange studies were performed for DNA duplexes complexed with other ZBPs [93–96].

5.2. B-Z Junction Formation in DNA by Z-DNA Binding Proteins

In order for ZBPs to produce Z-DNA in a section of long genomic DNA, two B-Z junctions must be formed at each end of the Z-DNA segment. A crystal structural study of a DNA duplex complexed with ZαADAR1 showed that bases between B- and Z-DNA are almost continuously stacked, with the extrusion of one base-pair at the B-Z junction [97]. Hydrogen exchange studies of the DNA-ZαADAR1 complexes showed that the kex values of the A-T base-pairs in AT-rich regions increased as the P/N ratio increased, indicating that ZαADAR1 significantly destabilizes AT-rich regions [49,98]. However, ZαADAR1 had little effect on the kex values of the G-C base-pairs in GC-rich regions [49,98]. In addition, the base-pair opening kinetics indicated that, in the complex, all G-C base-pairs have larger kop values (smaller slopes in Fig. 2C) and longer τo values (larger y-intercepts in Fig. 2C) than those of free DNA [49]. Thus, it was proposed that an intermediate structure exists during B-Z junction formation by ZαADAR1, in which the DNA duplex displays unique dynamic features: (i) instability of the AT-rich region and (ii) a longer lifetime for the open state of the GC-rich region [49].

5.3. Target Recognition of RNA Aptamer

Macugen is the first modified RNA aptamer to be employed as a human therapeutic and was derived from an in vitro selection against the key angiogenic regulator protein, vascular endothelial growth factor, VEGF165 [99]. VEGF consists of two independent domains, a receptor-binding domain and a 55-amino acid heparin-binding domain (HBD) [100]. A photo-cross-linking study indicated that Macugen specifically recognizes VEGF by targeting the HBD [101]. NMRI studies showed very similar secondary structure for Macugen, whether it is bound to the HBD or to VEGF165 [102]. These studies also found that the aptamer is stabilized by complex formation with either the HBD or VEGF165 [102]. Hydrogen exchange studies showed that many imino protons in the internal loop and neighboring base-pairs exhibit fast exchange in the free aptamer with very large kex values [51]. However, the kex values for many of these imino protons became much smaller upon binding of the HBD or VEGF165 [51]. These hydrogen exchange data support an induced-fit type mechanism in which RNAs with dynamic features in the free state can bind their target protein with extremely high affinity [51].

6. Implications for DNA-PNA Hybrid Duplex Formation

PNAs are one of the most widely used synthetic DNA mimics where the four bases are attached to a N-(2-aminoethyl)glycine (aeg) backbone [103,104]. Chimeric PNA (chipNA), in which a chiral glycerol nucleic acid-like γT monomer is incorporated into the aegPNA backbone, displays excellent RNA selectivity as well as antiparallel selectivity toward non-chimeric PNA [105]. Hydrogen exchange studies revealed that a aegPNA:DNA hybrid is a much more stable duplex (smaller kex) and is less dynamic compared to the corresponding DNA duplex (longer τo and τopen) as [38]. The γT residue in the chiPNA:DNA hybrid destabilizes a specific base-pair (much larger kex) and its neighbors (3- to 60-fold larger kex) compared to the non-chimeric PNA:DNA hybrid, while maintaining the thermal stabilities and dynamic properties of all base pairs [38]. In addition, the two neighboring base-pairs becomes more dynamic than in either the non-chimeric PNA:DNA hybrid or the corresponding DNA duplex (much shorter τo and τopen), meaning that these base-pairs open and reclose much more rapidly [38].

7. Conclusion

Base-pair opening in nucleic acids is a conformational transition that is required for their biological function. Hydrogen exchange is one of the widely used methods to study the thermodynamics and kinetics of base-pair opening in nucleic acids. The hydrogen exchange data of imino protons are analyzed based on a two-state (open/closed) model, where exchange only occurs from the open state. In this review, we discussed several examples of how hydrogen exchange data provide insight into the functional interactions of nucleic acids: 1) selective recognition of DNA by its target proteins; 2) regulation of RNA cleavage by site-specific mutations; 3) intermolecular interaction of proteins with their target DNA or RNA; 4) formation of PNA:DNA hybrid duplexes.

Declarations of Competing Interest

None.

Acknowledgements

This work was supported by the National Research Foundation of Korea [2017R1A2B2A001832], the Samsung Science and Technology Foundation [SSRF-BA1701-10], and the KBSI grant [D39700]. We
thank M. Stauffer, of Scientific Editing Solutions, for editing the manuscript.

References

[1] Leroy JL, Balo N, Figueroa N, Plateau P, Guérin M. Internal motions of transfer RNA: a study of exchanging protons by magnetic resonance. J Biol Mol Struct Dyn 1985;2:915–39.

[2] Guérin M, Leroy JL. Studies of base pair kinetics by NMR measurement of proton exchange. Methods Enzymol 1995;201:383–413.

[3] Lerjon M, Leroy JL. Internal motions of nucleic acid structures and the determination of base-pair lifetimes. Biochimie 1997;79:779–9.

[4] Moe JG, Russu IM. Proton exchange and base-pair opening kinetics in 5′-d(CGCGATTCGGGGGG)-3′ and related oligonucleotides. Nucleic Acids Res 1990;18:8176–85.

[5] Lejon M, Gräslund A. Studies of sequence and length on imino proton exchange and base pair opening kinetics in DNA oligonucleotide duplexes. Nucleic Acids Res 1992;20:5339–43.

[6] Moe JG, Russu IM. Kinetics and energetics of base-pair opening in 5′-d(CGCGATTTCGGGGGG)-3′ and a substituted deoxycytidine containing G-T mismatches. Biochemistry 1992;31:8421–8.

[7] Lejon M, Zdunek J, Fritzsche H, Slézer H, Gräslund A. NMR studies and restrained-molecular-dynamics calculations of a long a+T-rich stretch in DNA. Effects of phosphate charge and solvent approximations. Eur J Biochem 1995;234:832–42.

[8] Folta-Stogniew E, Russu IM. Base-catalysis of imino proton exchange in DNA: effects of catalyst upon DNA structure and dynamics. Biochimie 1996;35:439–49.

[9] Nonin S, Jiang F, Patel DJ. Imino proton exchange and base pair kinetics in the A-form. Nucleic Acids Res 1998;26:3579–87.

[10] Döbnerberg U, Lejon M, Fritzsche H. High base pair opening rates in tracts of GC base pairs. J Biol Chem 1999;274:6957–62.

[11] Wärmländer S, Sandström K, Leijon M, Gräslund A. Base-pair dynamics in an antisense triplex modified by the A-form. RNA 2001;7:2037–48.

[12] Vojvodic P, Canali M, Leroy JL. Opening mechanism of G-T/U-pairs in DNA and RNA duplexes: a combined study of imino proton exchange and molecular dynamics simulation. J Am Chem Soc 2002;124:14659–67.

[13] Coman D, Russu IM. A magnetic nuclear resonance investigation of the energetics of basepair opening pathways in DNA. Biophys J 2005;89:3285–92.

[14] Every AE, Russu IM. Influence of magnesium ions on spontaneous opening of DNA base pairs. J Phys Chem B 2008;112:7689–95.

[15] Cho SJ, Bang J, Lee JH, Choi BS. Base pair opening kinetics and dynamics in double-stranded DNA duplexes that specifically recognize by very short patch repair protein (Vsr). J Biol Chem 2010;285:201–6.

[16] Parker JB, Stivers JT. Dynamics of uracil and 5′-fluorouracil in DNA. Biochemistry 2006;45:13606–15.

[17] Coman D, Russu IM. A nuclear magnetic resonance investigation of the energetics of basepair opening pathways in DNA. Biophys J 2005;89:3285–92.

[18] Vojvodic P, Canali M, Leroy JL. Opening mechanism of G-T/U-pairs in DNA and RNA duplexes: a combined study of imino proton exchange and molecular dynamics simulation. J Am Chem Soc 2002;124:14659–67.

[19] Vojvodic P, Canali M, Leroy JL. Opening mechanism of G-T/U-pairs in DNA and RNA duplexes: a combined study of imino proton exchange and molecular dynamics simulation. J Am Chem Soc 2002;124:14659–67.

[20] Bang J, Bae SH, Park CJ, Lee JH, Choi BS. Structural and dynamics study of DNA dodecamer duplexes that contain un-, hemi- or fully-methylated GATC sites. J Am Chem Soc 2008;130:17888–96.

[21] Saadah MW, Voelhmann MP, Garguly M, Gold B, Stone MP. Site-specific stabilization of DNA by a tethered major groove amine, 7-amino-7-deaza-2′-deoxyguanosine. Biochemistry 2013;52:7659–68.

[22] Bang J, Kang YM, Park CJ, Lee JH, Choi BS. Thermodynamics and kinetics for base pair opening in the decamer DNA duplexes containing cytosidine pyrimidine dimers. FEBS Lett 2009;583:2037–41.

[23] Dhavan GM, Lapham J, Yang S, Crothers DM. Decreased imino proton exchange and base-pair opening in the IFH-DNA complex measured by NMR. J Mol Biol 1999;286:569–71.

[24] Friedman JL, Jiang YL, Stivers JT. Unique dynamic properties of DNA duplexes containing G-T/U-mismatches. J Biol Chem 1999;274:6957–62.

[25] Lejon M, Zdunek J, Fritzsche H, Slézer H, Gräslund A. Studies of sequence and length on imino proton exchange and base pair opening kinetics in DNA oligonucleotide duplexes. Nucleic Acids Res 1992;20:5339–43.

[26] Moe JG, Russu IM. Kinetics and energetics of base-pair opening in 5′-d(CGCGATTTCGGGGGG)-3′ and a substituted deoxycytidine containing G-T mismatches. Biochemistry 1992;31:8421–8.

[27] Lejon M, Zdunek J, Fritzsche H, Slézer H, Gräslund A. NMR studies and restrained-molecular-dynamics calculations of a long a+T-rich stretch in DNA. Effects of phosphate charge and solvent approximations. Eur J Biochem 1995;234:832–42.

[28] Folta-Stogniew E, Russu IM. Base-catalysis of imino proton exchange in DNA: effects of catalyst upon DNA structure and dynamics. Biochimie 1996;35:439–49.

[29] Nonin S, Jiang F, Patel DJ. Imino proton exchange and base pair kinetics in the A-form. Nucleic Acids Res 1998;26:3579–87.

[30] Döbnerberg U, Lejon M, Fritzsche H. High base pair opening rates in tracts of GC base pairs. J Biol Chem 1999;274:6957–62.

[31] Wärmländer S, Sandström K, Leijon M, Gräslund A. Base-pair dynamics in an antisense triplex modified by the A-form. RNA 2001;7:2037–48.

[32] Vojvodic P, Canali M, Leroy JL. Opening mechanism of G-T/U-pairs in DNA and RNA duplexes: a combined study of imino proton exchange and molecular dynamics simulation. J Am Chem Soc 2002;124:14659–67.

[33] Coman D, Russu IM. A nuclear magnetic resonance investigation of the energetics of basepair opening pathways in DNA. Biophys J 2005;89:3285–92.

[34] Every AE, Russu IM. Influence of magnesium ions on spontaneous opening of DNA base pairs. J Phys Chem B 2008;112:7689–95.

[35] Cho SJ, Bang J, Lee JH, Choi BS. Base pair opening kinetics and dynamics in double-stranded DNA duplexes that specifically recognize by very short patch repair protein (Vsr). J Biol Chem 2010;285:201–6.

[36] Parker JB, Stivers JT. Dynamics of uracil and 5′-fluorouracil in DNA. Biochemistry 2006;45:13606–15.

[37] Canali M, Leroy JL. Structure, internal motions and association-dissociation kinetics of the i-motif dimer of (SmCCCTACCT). Nucleic Acids Res 2005;33:5471–81.

[38] Snoussi K, Leroy JL. Imino proton exchange and base-pair kinetics in RNA duplexes. FEBS Lett 2001;509:598–604.

[39] Lee JH, Pardi A. Thermodynamics and kinetics for base pair opening in the P1 duplex of Tetrahymena group I ribozyme. Nucleic Acids Res 2005;33:2853–74.

[40] Hao XZ, Tan M, Liu CD, Feng R, Wang ED, Zhu G. Studying base pair opening close kinematics of RNA47 using TROSY-proton exchange NMR spectroscopy. FEBS Lett 2010;584:444–52.

[41] Chen C, Jiang L, Michalczuk R, Russu IM. Structural energetics and base-pair opening dynamics in sarcin-rinic domain RNA. Biochemistry 2006;45:12606–13.

[42] Kim W, Kim HE, Jung HN, Jun AR, Jang WC, Ahn JH, Park CJ. Base-pair opening dynamics of primary miR156a using NMR elucidates structural determinants important for its processing level and leaf number phenotype in Arabidopsis. Nucleic Acids Res 2017;45:8755–64.
Kim W, Ahn JH. MicroRNA-target interactions: important signaling modules regulating time in diverse plant species. Crit Rev Plant Sci 2014;33:225–37.

Kim D, Hur J, Park K, Bae S, Shin D, Ha SC, et al. Distinct Z-DNA binding mode of a PKR-like protein kinase containing a Z-DNA binding domain (PKZ). Nucleic Acids Res 2014;42:5937–48.

Kim HE, Ahn HC, Lee YM, Kim HE, Lee YM, et al. The biology of left-handed Z-DNA. Nat Med 2003;9:284–95.

Kim HE, Ahn HC, Lee YM, Kim HE, Lee YM, et al. The biology of left-handed Z-DNA. Nat Med 2003;9:284–95.

Kim HE, Ahn HC, Lee YM, Kim HE, Lee YM, et al. The biology of left-handed Z-DNA. Nat Med 2003;9:284–95.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2010;584:4344–50.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Letl 2011;585:772–8.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2011;585:772–8.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2011;585:772–8.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Lett 2011;585:772–8.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Letl 2011;585:772–8.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Letl 2011;585:772–8.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Letl 2011;585:772–8.

Lee AR, Park CJ, Cheong HK, Ryu KS, Park JW, Kwon MY, et al. Sequence discrimination of the Z-DNA domain of human ADAR1 during B-Z transition of DNA duplexes. FEBS Letl 2011;585:772–8.