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## Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

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### Drastic Difference in G:A Base Pairing Between Two Consecutive G:A Mismatches and a Single G:A Mismatch in DNA

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**To cite this Article** Katahira, Masato , Kanagawa, Mayumi and Uesugi, Seiichi(1994) 'Drastic Difference in G:A Base Pairing Between Two Consecutive G:A Mismatches and a Single G:A Mismatch in DNA', *Nucleosides, Nucleotides and Nucleic Acids*, 13: 6, 1507 – 1515

**To link to this Article:** DOI: 10.1080/15257779408012167

**URL:** <http://dx.doi.org/10.1080/15257779408012167>

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**DRASTIC DIFFERENCE IN G:A BASE PAIRING  
BETWEEN TWO CONSECUTIVE G:A MISMATCHES AND A SINGLE G:A MISMATCH IN DNA<sup>#</sup>**

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**ABSTRACT:** Our previous study has demonstrated that "sheared" G:A base pairs are formed for two consecutive G:A mismatches of d(GGACGACATC):d(GATGGAGTCC) [Katahira et al. (1993) *Nucleic Acids Res.*, **21**, 5418-5424]. We report NMR studies of d(GGACGACATC):d(GATGAGTCC) where the central two consecutive G:A mismatches are replaced by a single G:A mismatch. A drastic change was found on the mode of the G:A base pairing. Another kind of G:A base pairing, presumably a "head to head" G:A base pair, is formed in the single G:A mismatch. Additionally, asymmetric thermal stabilities of the base pairs along the duplex were observed.

**INTRODUCTION**

G:A mismatch base pairing is drawing great attention because the formation of G:A base pairs is suggested in some ribozymes and the structure derived from this base pair could play a crucial role in their enzymatic activities.<sup>1,2</sup> In DNA, several different types of G:A base pairs have been observed: "Head to head" G(anti):A(anti),<sup>3-6</sup> G(anti):A(syn),<sup>7</sup> G(syn):A(anti),<sup>6,8</sup> and "sheared" G(anti):A(anti)<sup>1,9-13</sup> base pairs.

Our previous study by NMR<sup>13</sup> has demonstrated that sheared G:A base pairs are formed in both d(GGACGAGTCC)<sub>2</sub> (duplex 1) and d(GGACGACATC):d(GATGGAGTCC) (duplex 2), the base sequences of which are modeled after a hammerhead ribozyme and a small metalloribozyme.<sup>14,15</sup> Base sequence dependency of G:A base pairing has been discussed, and it

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<sup>#</sup> This paper is dedicated to Dr. Morio Ikehara on the occasion of his 70th birthday.

has been concluded that the preferable sequence for the formation of the sheared base pairs is a PyGA sequence.<sup>13</sup>

It has been suggested that the interstrand stacking interaction in the GA:GA segment stabilizes the sheared G:A base pairs. Here we have studied G:A base pairing of a DNA duplex, where the central two consecutive G:A mismatches are replaced by a single G:A mismatch: d(GGACG-CATC):d(GATGAGTCC) (duplex **3**). It was found that a drastic change of G:A base pairing is caused by this replacement. In duplex **3**, the sheared G:A base pair is not formed. Another kind of G:A base pairing, presumably the "head to head" G:A base pair, is formed. Additionally, asymmetric thermal stabilities of base pairs along the duplex is found.

The numbering for the residues of duplex **3** is as follows.

1	2	3	4	5	6	7	8	9	
G	G	A	C	G	C	A	T	C	Scheme I
C	C	T	G	A	G	T	A	G	
18	17	16	15	14	13	12	11	10	

## MATERIALS AND METHODS

The oligomers for duplex **3** were synthesized with a DNA synthesizer (Applied Biosystems Co.), and purified as described previously.<sup>16</sup> For the NMR measurement of non-exchangeable protons, a lyophilized sample was dissolved in 20 mM phosphate buffer (pH 6.0-7.0) containing 0.15 M NaCl. The solution was lyophilized again and then dissolved in 0.4 ml of D<sub>2</sub>O (99.96%). The duplex concentration was 2 mM. For measurement of exchangeable proton spectra, a H<sub>2</sub>O:D<sub>2</sub>O mixture (19:1) was substituted for D<sub>2</sub>O. DSS was used as an internal chemical shift reference.

NMR spectra were all recorded at 285-305 K with a Bruker AM-400 NMR spectrometer. Phase-sensitive NOESY<sup>17</sup> and HOHAHA<sup>18</sup> spectra were recorded by the time-proportional phase-increment method.<sup>19</sup> The mixing times for NOESY were 80, 100, 200 and 300 ms, and mixing time for HOHAHA was 40 ms. The repetition delay was 2.0 s. Two-dimensional spectra were recorded with 450  $t_1$  increment; 160 free induction decays of 2 K data points per increment were collected. The  $t_1$  and  $t_2$  data were apodized with a  $\pi/3$ -shifted sine-bell function. The  $t_1$  data were zero-filled to 1 K points. One-dimensional spectra in H<sub>2</sub>O were accumulated with a 1-1 pulses sequence,<sup>20</sup> and NOE difference spectra were obtained as described previously<sup>16</sup> with irradiation times of 100 and 300 ms.

## RESULTS

FIG. 1 shows the one-dimensional  $^1\text{H}$  NMR spectrum of duplex 3 in  $\text{H}_2\text{O}$  (pH 6.5) at 285 K. The resonances of imino protons have been assigned sequentially by the use of NOE experiments, as described previously.<sup>16,21</sup> It is notable that no imino proton resonance is observed in the region around 10 ppm, while the two imino proton resonances of the G residues involved in the sheared G:A base pairs have been observed at 9.89 ppm and 10.19 ppm in the case of duplex 2.<sup>13</sup>

The resonances of non-exchangeable protons of duplex 3 have been assigned sequentially by analysis of two-dimensional NOESY and HOHAHA spectra in  $\text{D}_2\text{O}$  in the same way as reported for other DNA duplexes,<sup>13,16,21-23</sup> using previously established methods.<sup>24-30</sup> As an example, FIG. 2 shows an expansion of the NOESY spectrum, indicating the sequential assignments of H1' and H6/H8 through the H1'(i-1)-H6/H8(i)-H1'(i) connectivities. In the same way, H2', H2'', H3' and H4' were assigned; the assignments were all self-consistent and confirmed by HOHAHA spectra (data not shown). The assignments are summarized in TABLE 1.

It should be noted that the H8(i)-H1'(i) NOEs for G5 and A14 are much weaker than the CH5(i)-CH6(i) NOEs, indicating that the both residues take on anti conformations. Another point is no remarkable inter-strand NOE cross peak is observed in FIG.2. This will be discussed later.

## DISCUSSIONS

**Difference in G:A base pairing between duplex 2 and duplex 3; the effect of the replacement of two consecutive G:A mismatches by a single G:A mismatch.**

Four different types of base pairing have been observed for G:A mismatches in both the crystal and solution states; (a) "head to head" G(anti):A(anti), (b) G(anti):A(syn), (c) G(syn):A(anti), and (d) "sheared" or "side by side" G(anti):A(anti). In the syn conformation the H8(i)-H1'(i) distance is  $\sim 2.6 \text{ \AA}$ , which is comparable to the CH5(i)-CH6(i) distance ( $2.46 \text{ \AA}$ ), while in the anti conformation this distance is  $\sim 3.8 \text{ \AA}$ , which is greater than the CH5(i)-CH6(i) distance.<sup>31</sup> The H8(i)-H1'(i) NOEs for G5 and A14 are much weaker than the CH5(i)-CH6(i) NOEs (FIG. 2). The NOESY spectrum shown in FIG. 2 is recorded with a

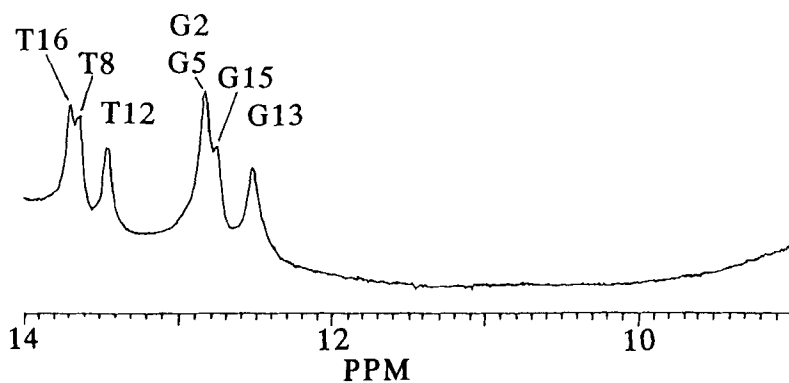


FIG. 1.  $^1\text{H}$  NMR spectrum of the duplex **3** at 285 K (pH 6.5) with the assignments of the imino proton signals indicated by the residue numbers. For the G5 assignment, see the text.

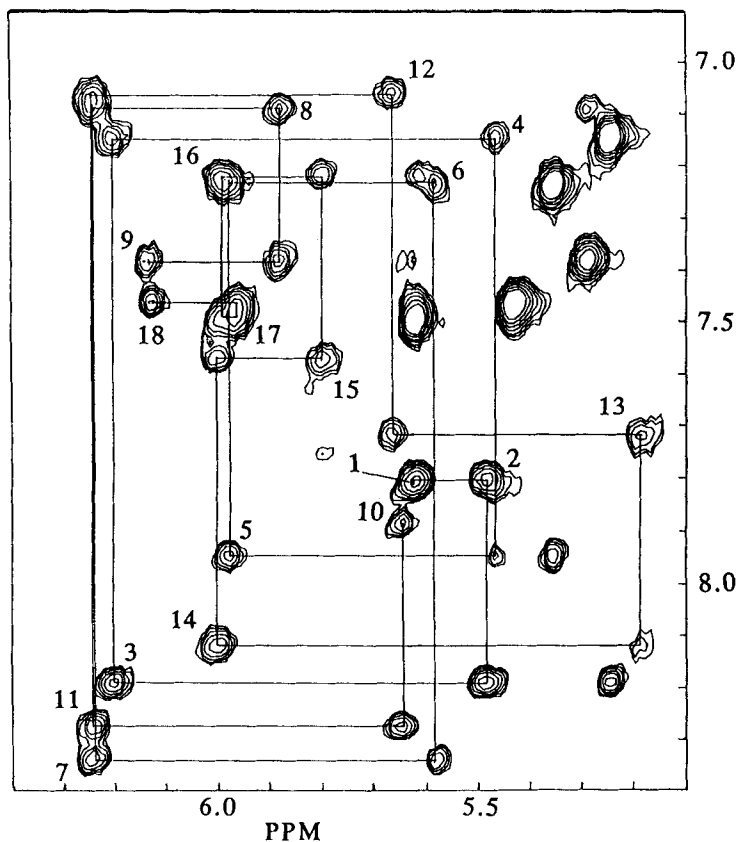


FIG. 2. Expansion of the NOESY spectrum of duplex **3** obtained with a mixing time of 300 ms in  $\text{D}_2\text{O}$  solution at 293 K (pH 7.0). The lines show the  $\text{H1}'(i-1)\text{-H6/H8}(i)\text{-H1}'(i)$  connectivities. The intraresidue cross peaks are labeled.

TABLE 1.  $^1\text{H}$  chemical shifts for duplex 3 at 293 K (pH 7.0).<sup>a</sup>

RESIDUE	H6/H8	H2/H5/CH <sub>3</sub>	H1'	H2'	H2''	H3'	H4'	IMINO/AMINO
G1	7.80		5.62	2.48	2.67	4.80	4.19	
G2	7.81		5.48	2.68	2.77	4.99	4.32	12.83
A3	8.19	7.83	6.21	2.68	2.88	5.04	4.46	
C4	7.15	5.24	5.47	1.58	2.03	4.77	4.01	6.61 8.03
G5	7.95		5.98	2.78	2.78	4.98	4.38	12.83 <sup>b</sup>
C6	7.23	5.36	5.58	1.82	2.31	4.77	4.15	
A7	8.35	7.60	6.24	2.72	2.92	5.00	4.44	
T8	7.09	1.42	5.88	1.90	2.39	4.77	4.08	13.63
C9	7.38	5.28	6.13	2.27	2.27	4.53	3.93	
G10	7.88		5.65	2.56	2.75	4.87	4.22	
A11	8.28	7.83	6.25	2.76	2.94	5.03	4.44	
T12	7.07	1.33	5.66	2.27	1.89	4.81	4.12	13.45
G13	7.72		5.19	2.45	2.59	4.93	4.22	12.52
A14	8.12	7.76	6.00	2.86	2.86	5.03	4.38	
G15	7.57		5.80	2.48	2.68	4.89	4.37	12.77
T16	7.22	1.29	5.99	2.12	2.48	4.82	4.18	13.69
C17	7.49	5.62	5.96	2.18	2.47	4.78	4.11	6.94 8.47
C18	7.47	5.42	6.12	2.27	2.27	4.53	3.93	

<sup>a</sup> At 285 K (pH 6.5) for imino protons.<sup>b</sup> See the text for detail.

relatively long mixing time of 300 ms in order to help the assignment procedure. The intensities of cross peaks in FIG 2 might not reflect the corresponding distances due to a spin diffusion effect caused by the long mixing time. Therefore the NOESY spectrum with the short mixing time of 80 ms was examined, and it was confirmed that H8-H1' NOEs for G5 and A14 are definitely much weaker than the CH5-CH6 NOEs (data not shown). In fact, the H8-H1' distances calculated on the basis of the intensities of the cross peaks in the NOESY spectrum with the mixing time of 80 ms by using geometrically fixed CH5-CH6 distance as the internal reference distance are 3.7 Å for both G5 and A14. Thus (b) and (c) are excluded as the G:A base pair of duplex 3.

Our previous study has demonstrated that sheared G:A base pairs are formed in duplex 2.<sup>13</sup> Because of the "side by side" arrangement of G and A bases, characteristic interstrand NOEs have been observed in the central G:A mismatches. These NOE cross peaks have appeared in the same area as FIG. 2. However, these interstrand NOEs are not observed in

duplex 3. Thus (d) is excluded as the G:A base pair of duplex 3. In the case of duplex 2, the imino proton resonances of G of the G:A mismatches have appeared in the high field region, 9.89 ppm and 10.19 ppm, which has indicated that the imino protons of G are not involved in the hydrogen bonding. Appearance of the imino proton resonances in the high field region is another characteristic feature of (d), where an amino proton instead of an imino proton of G is involved in the hydrogen bonding. Disappearance of resonances in the high field region (FIG. 1) supports the idea that (d) can be excluded for duplex 3. Thus (a) remained an only possible G:A base pair which is consistent with the experimental data.

The only difference of the base sequences between duplex 2 and duplex 3 is the replacement of two consecutive G:A mismatches in duplex 2 by a single G:A mismatch in duplex 3. This difference gives a drastic change in base pairing. The sheared G:A base pairing in duplex 2 is destroyed by the replacement, and another kind of G:A base pairing, presumably the "head to head" G:A base pair, is formed. This drastic change can be rationalized. It has been proposed that the sheared G:A base pairs in the GA:GA segment are stabilized by the interstrand stacking interaction.<sup>1,9,10,13</sup> Because of the "side by side" arrangement of the G and A bases in the GA:GA segment, one G stacks over the other G in the opposite strand and one A stacks over the other A in the opposite strand at the cost of ordinary intrastrand stacking between G and A. This is a key factor for the stabilization of the sheared G:A base pairing. When there is only one G:A mismatch, this interstrand stacking is impossible even if the sheared base pair is formed for the single G:A mismatch. The key factor for the formation of the sheared base pairing is lost in duplex 3. Therefore drastic change of base pairing is inevitable upon the replacement of two consecutive G:A mismatches by a single G:A mismatch.

Observation of the complete  $H1'(i-1)-H6/H8(i)-H1'(i)$  (FIG. 2) and  $H2'/H2''(i-1)-H6/H8(i)-H2'/H2''(i)$  (data not shown) connectivities along the duplex indicates that a "bulged-out" structure is not likely for the G:A mismatch in duplex 3. Although the "head to head" base pairing seems to be only possible base pairing of duplex 3, it is hard to identify base pairing unambiguously due to the thermally unstable character of duplex 3. The imino proton resonances shown in FIG. 1 have been

assigned on the basis of sequential NOEs except for G5. Judging from peak intensities in FIG. 1, it is apparent that another resonance is overlapping on G2 resonance. This overlapping resonance is tentatively assigned to G5. The resonance position, 12.83 ppm, is similar to those observed for the imino proton resonances in the "head to head" G:A base pairs.<sup>3,5,11</sup> The overlapping resonance is thermally unstable. When the temperature is raised, this resonance disappears first due to the proton exchange with water. The unstable character of the resonance makes it difficult to observe the NOEs involving this resonance, which hinders the decisive assignment of this resonance and unambiguous identification of the base pairing.

In spite of this ambiguity, it is still clearly demonstrated that the replacement of two consecutive G:A base pairs by a single G:A base pair causes the drastic change of G:A base pairing from the sheared base pairing to another base pairing. It is very interesting that the conformation of the G:A mismatch is highly sequence dependent.

#### **Asymmetric thermal stabilities of base pairs in duplex 3.**

Unexpected feature of duplex 3 is found on the thermal stability of each base pair in the duplex. The stability of each base pair was examined by monitoring disappearance of imino proton resonances on raising the temperature. The stabilities of base pairs decrease in the following order: 15G, 16T > 2G > 12T, 13G > 5G, 8T. This result indicates that the base pairs of the left segment of duplex 3 in Scheme I (G1-C4:G15-C18) are more stable than those of the right segment (G5-C9:G10-A14). Thus the thermal stability is asymmetric in duplex 3. Usually the stability of a base pair depends on the distance from the termini of the duplex. An inner base pair is more stable than an outer base pair. Thus the stability is almost symmetric even in a non-self-complementary sequence. In fact the nearly symmetric stabilities of the base pairs are observed in the case of duplex 2 (unpublished results). What is observed for duplex 3 is rather exceptional. It is supposed that the unstable central G:A base pair in duplex 3 causes the asymmetric thermal stabilities of the base pairs.

#### **ACKNOWLEDGEMENTS**

We wish to thank Professor H. Akutsu (Yokohama Natl. Univ.) for giving us the opportunity to use his NMR machine. We also thank Mr. H.

Sato for his help on preparing DNAs. M.K. was supported by grants from the Ministry of Education, Science and Culture of Japan (#05780473), the CIBA-GEIGY Foundation for the Promotion of Science, Takarazuka, Japan, the Kihara Yokohama Life Science Foundation, and the Kanagawa Science Technology Academy.

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Received 11/30/93

Accepted 1/14/94