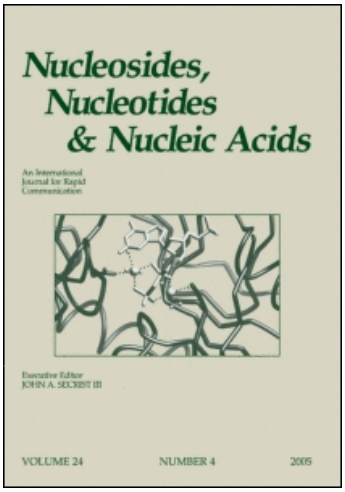

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Nucleosides and Nucleotides. 105. DNA Bending in $d(A)_4$ - $d(T)_4$ TRACTS Containing 3-Deazaadenine or 7-Deazaadenine Substituted for Adenine[§,1]

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NUCLEOSIDES AND NUCLEOTIDES. 105. DNA BENDING IN $d(A)_4$ - $d(T)_4$ TRACTS CONTAINING 3-DEAZAADENINE OR 7-DEAZAADENINE SUBSTITUTED FOR ADENINE§,[1]

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ABSTRACT: Self-complementary decadeoxyribonucleotides containing 3-deazaadenine or 7-deazaadenine in place of one of the adenine moieties in a parent sequence, $d(GAAAATTTTC)_n$, have been synthesized. After phosphorylation at their 5'-ends, the decamers were ligated to form multimers, which were analyzed by polyacrylamide gel electrophoresis. The multimers of the decamer containing 3-deazaadenine (**3**), $d(GAA\mathbf{3}ATTTTC)_n$, showed decreased bending compared with the multimers of $d(GAAAATTTTC)_n$, while the decamer containing 3-deazaadenine at a different position, $d(GAAA\mathbf{3}TTTTC)_n$, did not show any degree of bending. Also, the properties of migration of the multimers containing 7-deazaadenine in the gel will be discussed.

INTRODUCTION

Naturally occurring DNA fragments that display anomalously low mobilities in polyacrylamide gel electrophoresis have been found.[2,3] This property has been generally accepted as diagnostic for the presence of curved structure in the DNA fragments.[4,5] The characteristic feature of these DNA fragments is repeated $d(A)_n : d(T)_n$ ($n > 3$) tracts in phase with the DNA helical repeat (10.4 base-pairs/turn).[6,7] In addition, there is a sequence dependency. Multimers prepared from d(GAAAATTTTC) show bending (curvature) while the multimers having an inverted sequence of the adenine tract-thymine tract, $d(GTTTTAAAAC)_n$, do not.[8-10] Several hypotheses[11-16] and studies on the molecular basis of DNA bending have been reported.[3,17-19] The $d(A)_n : d(T)_n$ tracts appeared to have a particular structure with respect to the ordinary B-DNA helix.[20,21] The hypothesis that this structure is caused by a unique mode of hydration on the $d(A)_n : d(T)_n$

§ *This paper is dedicated to the memory of Professor Tohru Ueda.*

tracts has been proposed.[22-24] The presence of the spine of hydration along the minor groove of the DNA helix was first reported by Dickerson and co-workers in crystals of a duplex consisting of a d(CGCGAATTCGCG) sequence,[25] and this type of hydration was expected in the $d(A)_n : d(T)_n$ tracts in DNA.[26] The spine of hydration is formed by water molecules hydrogen-bonded with thymine *O*-2 atoms and adenine *N*-3 atoms, thus bridging adjacent bases pertaining to different chains. The spine of hydration narrows of the minor groove.[26]

Although no particular hydrations in the major groove have been reported, the formation of bifurcated (three-center) hydrogen bonds on the floor of the major groove, involving an *N*-6 amino group of an adenine hydrogen bonding to two *O*-4 carbonyl groups of the adjacent thymines in the opposite strand, has been reported.[27-31] These hydrogen bondings would also narrow the minor groove.

Approaches using oligonucleotide analogues should efficiently identify the factors that cause DNA bending.[32-35] In this report, we used synthetic decadeoxyribo-nucleotide analogues containing 3-deazaadenine (**3**)[36] or 7-deazaadenine (**7**)[37] in place of adenine to evaluate the hypothesis that hydration involving adenine moieties causes DNA bending. 3-Deazaadenine is an adenine analogue in which an azomethine at *N*-3 is replaced by a methine function (Fig.1). 7-Deazaadenine is likewise the 7-methine analogue of adenine (Fig.1). While an azomethine function is a proton accepter in hydrogen bond formation, a methine function is not. Therefore, multimers containing 3-deazaadenine in place of adenine (Fig. 2, II and III) lack hydrations in the minor groove at 3-deazaadenine sites, while the formation of bifurcated hydrogen bonds is possible at the 3-deazaadenine sites. Therefore, if the formation of bifurcated hydrogen bonds is the only requirement for the DNA bending, replacement of adenine moieties by 3-deazaadenine moieties may not reduce bending strongly. If the hydrations in the minor groove are essential for DNA bending, replacement of adenine moieties by 3-deazaadenine moieties may reduce bending strongly. Because both the formation of the minor groove spine of hydration and the formation of the bifurcated hydrogen bonds on the floor of the major groove are possible at 7-deazaadenine sites, replacement of adenine moieties by 7-deazaadenine moieties may not reduce bending strongly.

MATERIALS AND METHODS

Synthesis of decadeoxyribonucleotides.

An unmodified oligomer, d(GAAAATTTTC), and oligomers containing 3-deazaadenine (**3**) or 7-deazaadenine (**7**), d(GAA**3**ATTTTC), d(GAAA**3**TTTTC), d(GAA**7**ATTTTC), and d(GAAAA**7**TTTTC), were prepared by the phosphoramidite triester method[38] on a DNA synthesizer (Applied Biosystems model 381A). The amidite

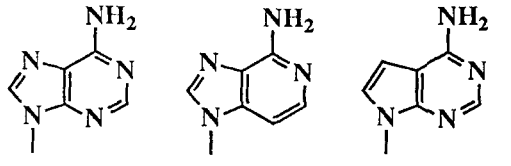

Fig. 1. Structures of deazaadenines.

I	d(GAAAATTTTC)$_n$
II	d(GAA**3**ATTTTC)$_n$
III	d(GAAA**3**TTTTC)$_n$
IV	d(GAA**7**ATTTTC)$_n$
V	d(GAAA**7**TTTTC)$_n$

Fig. 2. Sequences examined.

synthons of 2'-deoxy-3-deazaadenosine and 2'-deoxy-7-deazaadenosine were synthesized by the reported method[39] starting from 5'-*O*-dimethoxytrityl-*N*^{6}-dimethylaminomethylene-2'-deoxy-3-deazaadenosine[36] and 5'-*O*-dimethoxytrityl-*N*^{6}-benzoyl-2'-deoxy-7-deaza-adenosine,[37] respectively. After enzymatic ^{32}P labelling of the 5'-ends of the oligomers, the oligomers were partially digested by snake venom phosphodiesterase, and the sequences of these oligomers were confirmed by mobility shift analysis of the digests[40] (data not shown).

Preparation of multimers of the decadeoxyribonucleotides.

The preparation of multimers was done by a method essentially similar to that described before.[34] A mixture of a decamer (6 μg, 0.2 OD units), T4 polynucleotide kinase (purchased from Takara Shuzo, Japan, 1 μL, 10 units/1 μL), ATP (25 μL, 1.0 mM), 5 x kination buffer (10 μL, 250 mM Tris-HCl, 50 mM $MgCl_2$, 25 mM DTT, pH 8.0), and H_2O (15 μL) was incubated at 37 °C for 2 h, then cooled slowly. This phosphorylation reaction was done in duplicate. T4 DNA ligase (purchased from Takara Shuzo, 1 μL or 3 μL, 350 units/μL) was added to each kination mixture and the mixtures were incubated at 16 °C for 1 h, then 25 μL of the incubated solution was taken out from each mixture and the multimers were precipitated by addition of EtOH and incubation at -70 °C for 2 h. The precipitates were collected by centrifugation (15 K rpm) at -10 °C for 15 min, then washed with 70% EtOH and dried. The rest of the incubation mixtures were further incubated at 37 °C for 1 h, and the multimers were precipitated and dried similarly.

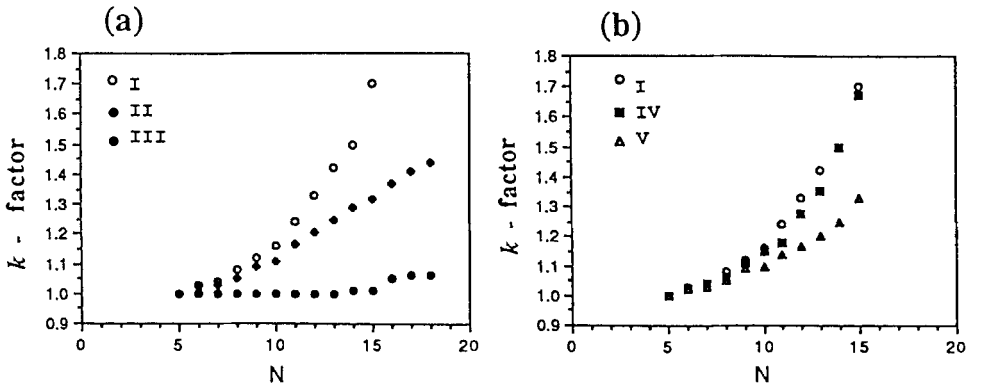

Fig. 3. Plots of *k*-factor (apparent length/sequence length) versus number of repeats for multimers composed of decadeoxyribonucleotides (N).

These four precipitates were combined in H_2O (150 μL), then the solution was concentrated. The residue was taken up in a loading buffer (0.04% bromophenol blue, 0.04% xylene cyanol, 7% (w/v) sucrose in H_2O, 20 μL), and electrophoresed.

Polyacrylamide gel electrophoresis of multimers

A portion of the multimers (2.5 μL of the above mixture) was put on a 12% polyacrylamide gel (17.5 cm x 36.5 cm or 27.5 cm x 36.5 cm) with a thickness of 0.5 mm. Electrophoresis was run at 1000 volts (5-10 mA) for 8 h at 4 °C in TBE Buffer (45 mM Tris-borate, 1.25 mM EDTA, pH 8.6). The bands were stained with ethidium bromide and photographed.

RESULTS

We synthesized decadeoxyribonucleotides containing 3-deazaadenine or 7-deaza-adenine in place of adenine in the parent sequence of d(GAAAATTTTC) as shown in Fig. 2. The decamers containing the adenine analogues (Fig. 2, II-V) were synthesized in good yields similar to the synthesis of the unmodified decamer. Each decamer, consisting of a self-complementary sequence, formed a duplex by itself in solution. Each decamer (duplex) was phosphorylated and ligated to form multimers (Fig. 2, I-V), which were analyzed by polyacrylamide gel electrophoresis. The degree of bending, based upon the migration anomaly, was obtained by plotting the "*k* -factor" (apparent length / sequence length) versus the number of repeats for multimers (N) (Fig. 3). Multimer I is a positive marker for the bending (Fig. 3a and b). Replacement of the third adenine from the 5'-end by 3-deazaadenine decreased the bending to some extent (Fig. 3a, multimer II).

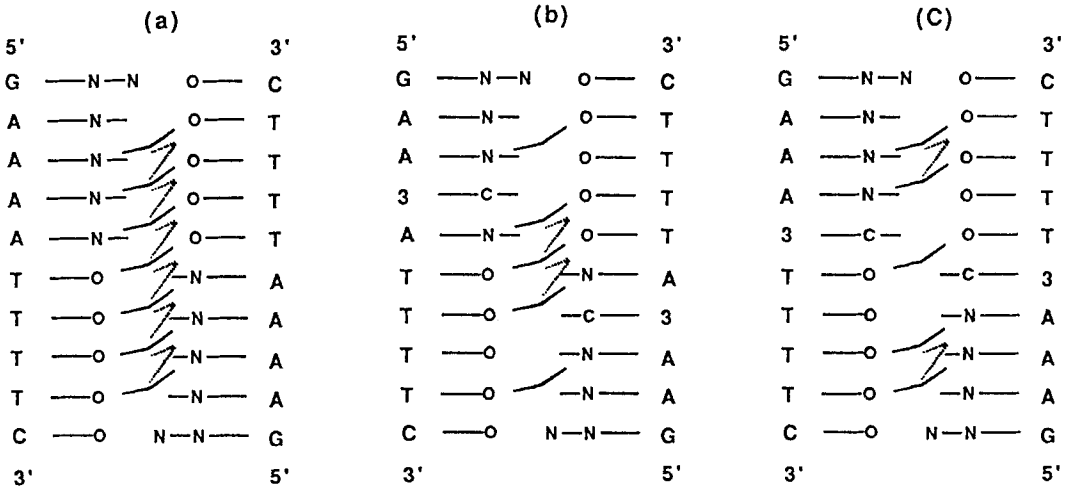

Fig. 4. Unwound minor groove diagrams of d(GAAAATTTTC)-(a), d(GAA3ATTTTC)-(b) and d(GAAA3TTTTC)-(c) showing the potential hydrations formed by water molecules hydrogen-bounded with thymine *O*-2 and adenine *N*-3 atoms (solid wedges) and second layer water molecules (broken wedges).

Replacement of the fourth adenine by 3-deazaadenine completely abolished the bending (Fig. 3a, multimer III). On the other hand, replacement of the third adenine by 7-deazaadenine caused no reduction of bending (Fig. 3b, multimer IV). However, replacement of the fourth adenine decreased bending to some extent (Fig. 3b, multimer V).

DISCUSSIONS

3-Deazaadenine is an adenine analogue in which an azomethine at *N*-3 is replaced by a methine function. 7-Deazaadenine is likewise a 7-methine analogue of adenine (Fig.1). The several oligodeoxyribonucleotides containing 3-deazaadenine or 7-deazaadenine so far synthesized showed similar properties to those of the parent oligomers as follows. After they were introduced into oligomers, both analogues form Watson-Crick type hydrogen bondings with thymines in duplexes.[35-37,41] The CD spectral patterns of duplexes containing these analogues are similar to those of B-DNA helix.[36,37,41] The distinct difference is that while the azomethine function is a proton acceptor in the hydrogen bond formation, the methine function is not. Therefore, replacement of an adenine with a 3-deazaadenine in the pertinent position of the $d(A)_n : d(T)_n$ tract would disrupt hydration in the minor groove at a 3-deazaadenine site. The schemes of potential hydrations in the minor groove of the decamers, I, II, and III are shown in diagrams (Fig. 4). Since a hydration between thymine *O*-2 atoms in different chains at a d(AT) step has been found in

the same crystal in which the minor groove hydration has been found,[25] formation of seven consecutive hydrations is possible along the minor groove of the duplex consisting of decamer I (Fig. 4a), which showed the largest bending among the decamers (Fig. 3a, multimer I). A hydration between adenine *N*-3 atom and cytosine *O*-2 atom at both ends of the duplex may be impossible owing to a steric hindrance from the 2-amino group of the guanine. Three consecutive hydrations and two isolated hydrations can occur along the minor groove of the duplex of decamer II (Fig. 4b), which showed a moderate degree of bending (Fig. 3a, multimer II). In contrast, a couple of consecutive hydrations and a isolated hydration can occur along the minor groove of decamer III (Fig. 4c), which did not show any degree of bending (Fig. 3a, multimer III). Interestingly, the total number of water molecules that are involved in the potential hydrations in the minor groove of the duplex of decamer II (4 water molecules) is the same as that of the duplex of decamer III (4 water molecules). Therefore, the mode of connectivity of hydrations may be important for DNA bending. The connectivity of the potential hydration in the minor groove of decamer II may be similar to that of a decamer consisting of a d(CGGAATTCCG) (three consecutive hydrations can occur at a AATT sequence[25]), and multimers of $d(CGGAATTCCG)_n$ also showed some degree of bending.[34] In contrast, the connectivity of the potential hydrations of decamer III may be similar to that of a decamer consisting of a d(NAAANNAAAN) sequence (N = C, T or G), and multimers of $d(NAAANNAAAN)_n$ may not show obvious bending since this sequence does not contain either $d(A)_n : d(T)_n$ tracts ($n > 3$) or the $d(A)_n$ - $d(T)_n$ sequence ($n > 1$) that seems to be indispensable to DNA bending.[6,7,34] Therefore, the consecutiveness of the spine of hydration along the minor groove of the $d(A)_n : d(T)_n$ tract is possibly one of the factors that cause DNA bending. It is interesting that at least three consecutive minor groove hydrations are also possible for the smallest elements requested for DNA bending, a $d(A)_4 : d(T)_4$ tract and a $d(A)_2$ - $d(T)_2$ sequence.

These results may not contradict the results of studies using oligodeoxyribonucleotides containing hypoxanthine (**H**).[32-34] Hypoxanthine can form a Watson-Crick type base pair with cytosine in a duplex like guanine (G), but not like G, **H** contains no 2-amino group that interferes with formation of the minor groove hydration. Therefore, a minor groove hydration can occur at a hypoxanthine site, however a bifurcated hydrogen bond on the floor of the major groove cannot form at the hypoxanthine site since **H** contains no amino group at its 6-position. Multimers containing d(AA**H**AA) : d(TTCTT) tracts maintained a fairly large degree of bending while multimers containing d(AAGAA) : d(TTCTT) tracts did not show any degree of bending.[32] Since both **H** and G cannot form bifurcated hydrogen bonds, differences in numbers and conectivities of hydrations along the minor grooves of the multimers may cause the difference of the degrees of bending.

These results together with our study indicate the importance of the connectivity of minor groove hydrations. Dickerson *et al.*[25] reported a double layer of water molecules binding along the minor groove at the d(AATT) sequence. The first layer of water molecules forms a continuous spin of hydration bridging adenine *N*-3 atoms and thymine *O*-2 atoms, and is augmented by a second layer of water molecules that serves to complete the tetrahedral environment bridging the water molecules of the first layer. The second layer hydration may fix some conformation (B'-conformation)[20,26] and may require the consecutive first layer hydrations to form. A double layer of hydrations similar to those found by Dickerson *et al.* may occur along the minor groove of the d(**3**ATT) site in decamer II (Fig. 4b), which showed some degree of bending, and the second layer would contain two water molecules binding consecutively. On the other hand, there is a possible second water layer containing two isolated water molecules along the minor groove of decamer III (Fig. 4c), which did not show any degree of bending. Furthermore, the highly organized hydrations found by Dickerson *et al.* may occur not only in decamer II but also in the $d(A)_n : d(T)_n$ tracts ($n > 3$) and in the $d(A)_n$ - $d(T)_n$ sequences ($n > 1$) and may contribute to DNA bending.

These results and discussions also do not contradict results of imino-proton-exchange NMR experiments[19] and premelting behaviours of duplexes containing the $d(A)_n : d(T)_n$ tracts.[17,18] However, the minor groove hydration may not be the only cause of DNA bending. For instance, multimers containing $d(\mathbf{H})_5 : d(C)_5$ tracts did not show any serious degrees of bending,[33] even though highly consecutive minor groove hydrations can form at the tracts. Stacking interactions also may drive the A-tract base pairs into a conformation in which the minor groove of the helix is narrowed.[42] There may be a difference in the delocalization of the π-electron system between the purine moiety and the 3-deazapurine moiety, which will eventually affect the hydration in the minor groove as well as the base stacking interaction within the duplexes and result in alteration of the overall conformation.

Replacement of the third adenine moiety from the 5'-end of the parent sequence by 7-deazaadenine did not reduce bending (Fig. 3b, multimer IV). However, replacement of the fourth adenine by 7-deazaadenine reduced bending to some extent (Fig. 3b, multimer V). Similar results were recently reported.[35] Although we have no explanation for this phenomenon at present, some possible factors may be pointed out. Hydration to the 7-azomethine of the adenine portion was found in crystals of d(CGCGAATTCGCG).[25] This hydration was not as tight as the hydrations in the minor groove, but the presence or absence of this hydration may alter the conformation of the duplex. Or there may be a difference in the delocalization of the π-electron system between the purine moiety and the 7-deazapurine moiety[41] as discussed for 3-deazaadenine, which will eventually affect the hydration in the minor groove or the formation of bifurcated hydrogen bonds as well as the

base-stacking interaction within the duplex and result in alteration of the overall conformation.

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