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The synthesis and properties of oligodeoxyribonucleotides containing N^6-methoxyadenine

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ABSTRACT

Oligodeoxyribonucleotides containing N^6-methoxyadenine (M) have been synthesized. The order of stability of duplexes consisting of synthesized oligodeoxyribonucleotides, 5′d(CCTGGTAXCAGGTCC)3′-5′d(GGACCTGNTACCAGG)3′ (X = M, A, G. N = A, G, T, C), was M: A (Tm = 52°C) >M: T (50°C) >M: G (48°C) >M: C (46°C) observed by thermal denaturation in a buffer of 0.01 M Na cacodylate, and 0.1 M NaCl at pH 7.0. The Tms are within a range of 6 degrees of difference, which is smaller than those of Tms of the duplexes containing A:N pairs (11 degrees) and G:N pairs (11 degrees). DNA replication study on a template-primer system,

5′d(^{32}p-CAGCTTTCGC)3′
3′d(GTCGAAAGCGMAGTCG)5′,

showed that TTP and dCTP were incorporated into DNA strands at a site opposite to M by Klenow DNA polymerase, but dATP and dGTP were not.

INTRODUCTION

Methoxyamine and hydroxylamine (a presumed intermediate in nitrate reduction *in vivo*) are potent mutagens (1). The reaction of cytosine or adenine at pH 5–6 with methoxyamine or hydroxylamine leads to replacement of the amino group by a methoxyamino group (-NHOCH$_3$) or a hydroxylamino group (-NHOH) (2,3). Although the reaction of adenine with these reagents is slower than that of cytosine, formation of N^6-methoxyadenine (M) and N^6-hydroxyladenine (as well as corresponding cytosine derivatives) in DNA strands may contribute to mutation (4,5).

The substitution by electronegative groups such as amino, hydroxyl, and methoxy on the exocyclic amino group of cytosine increases the ratio of the imino tautomer (6,7). Similar tautomerism of 9-substituted-N^6-methoxyadenine derivatives (Fig. 1a) has been demonstrated by proton nuclear magnetic resonance (^{1}H NMR) (8–11), ultraviolet (11), and infrared spectroscopies (11). These studies have shown that the tautomerism is markedly dependent on solvent polarity and the imino tautomer is more stable than the amino tautomer in polar solvents such as water or dimethylsulfoxide (8,11). Also, the equilibrium between two orientations of the exocyclic *N*-methoxy group which, may be oriented *syn* or *anti* with respect to *N*-1 of the adenine ring has been anticipated. In crystals of 2′,3′,5′-*O*-trimethyl-N^6-methoxyadenosine and N^9-benzyl-N^6-methoxyadenine, the N^6-methoxyadenine moiety exists in the imino form and the methoxy group is in the *syn* orientation (12,13). Intramolecular hydrogen bonding between N^1-H and N^6-OMe stabilizes the *syn* conformation in the crystals. However, the conformation of the methoxy group in solutions has not been clear.

Stolarski *et al.* reported that the tautomeric equilibrium of N^6-methoxyadenosine was appreciably shifted to the amino or the imino forms on addition of uridine or cytidine at the nucleoside level, respectively (9,10). Therefore, changing tautomeric forms, N^6-substituted adenines may form stable base pairs with thymine (T) or cytosine (C) in DNA duplexes (14). Schemes for possible hydrogen bonding in a (N^6-substituted adenine): T and a (N^6-substituted adenine): C pairs are shown in Fig. 1b. In the amino form, the N^6-substituted adenines can form a base pair with T in Watson–Crick geometry and the orientation of the methoxy group should be *anti*. On the other hand, the N^6-substituted adenines should be in the imino form where the methoxy group may be in both or one of *syn* and *anti* conformations to form the (N^6-substituted adenine): C pair.

The stabilization of the imino tautomer by the substitutions at exocyclic amino groups may be a cause of mutation induced by *N*-substituted nucleosides. For instance, 2′-deoxy-N^4-aminocytidine and 2-amino-N^6-hydroxyladenine are potent mutagens and the anticipated mechanism for induction of mutation by these compounds is that these compounds are metabolized to the corresponding 2′-deoxynucleoside 5′-triphosphates, and then they are incorporated into DNA strands in place of TTP and dCTP, or dATP and dGTP, respectively (6,15). Similar mechanisms have been proposed for N^6-hydroxyladenine or N^6-methoxyadenine which are promutagens and may be generated in cells by treatment with hydroxylamine or methoxyamine. It has been demonstrated that N^6-methoxyadenosine triphosphate and 2′-deoxy-N^6-hydroxyladenosine triphosphate are incorporated

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into RNA or DNA strands by a RNA polymerase or DNA polymerases in place of GTP and ATP or dGTP and dATP, respectively (14,16).

When 2'-deoxy-N^6-hydroxyladenosine and 2'-deoxy-N^6-methoxyadenosine (dM) are incorporated into DNA or when adenine moieties are directly converted to the N^6-substituted adenines in DNA by treatment with hydroxylamine or methoxyamine, the base pair formation of the N^6-substituted adenines in DNA strands with incoming dNTPs (N = A, G, T, C) during replication seems to be one of the important steps for mutation. It has been anticipated that incorporation of dCTP into DNA strands at the site opposite to N^6-substituted adenines induces an A to G transition (4). However, base pair formation of N^6-substituted adenines during replication has not been examined well. Also, detail of formation and stability of base pairs of N^6-substituted adenines with other bases in DNA duplexes have not been examined sufficiently.

In this report, we describe the synthesis of oligonucleotides containing N^6-methoxyadenine (M) (17,18). Stabilities of duplexes containing the M:N pairs (N = A, G, T, C) were studied by thermal denaturation. Also, incorporation of dNTPs by Klenow DNA polymerase into DNA strands at the site opposite to M is examined using a template-primer system consisting of synthesized oligomers.

MATERIALS AND METHODS

General

Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus (Yanagimoto, Japan) and are uncorrected. UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer (Shimadzu Corporation, Japan). Mass spectra (MS) were measured on a Jeol JMX-DX303 spectrometer (JEOL, Japan). CD spectra were measured by a Jasco 500A CD spectrometer (Jasco, Japan). TLC was done on Merck Kieselgel F254 precoated plates (Merck, Germany). The silica gel used for column chromatography was YMC gel 60A (70-230 mesh) (YMC Co., Ltd., Japan). The ^{1}H NMR spectra were recorded on a JEOL JNM-GX 270 (270 MHz) spectrometer (JEOL) with tetramethylsilane as internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. Peaks for the amino or the imino tautomers are indicated by H_a or H_i, respectively.

2'-Deoxy-N^6-methoxyadenosine (**1**). This nucleoside was synthesized from 2'-deoxyadenosine according to reported methods (11,19) with minor modifications (83% as crystals from H_2O): mp 121–123.5°C. MS *m/z* 281 (M^+); ^{1}H NMR (DMSO-d_6) 11.20 (br s, 0.85 H, H_i-N^1), 10.90 (br s, 0.15 H, H_a-N^6), 8.40 (s, 0.15 H, H_a-2), 8.28 (s, 0.15 H, H_a-8), 8.05 (s, 0.85 H, H_i-8), 7.56 (br s, 0.85 H, H_i-2), 6.19 (t, 1H, H-1'), 5.26 (d, 1H, 3'-OH), 4.96 (t, 1H, 5'-OH), 4.35 (m, 1H, H-3'), 3.85 (m, 1H, H-4'), 3.76 (s, 3H, OMe), 3.54 (m, 2H, H-5'), 2.63–2.15 (m, 2H, H-2'). *Anal.* Calcd for $C_{11}H_{15}N_5O_4$ 1/2 H_2O: C, 45.55; H, 5.56; N, 24.15. Found: C, 45.45; H, 5.57; N, 24.28.

5'-O-(4,4'-Dimethoxytrityl)-2'-deoxy-N^6-methoxyadenosine (**4**). According to the reported methods (18,20), **1** was converted to **4** (85% as a foam): EI-MS *m/z* 583 (M^+); ^{1}H NMR ($CDCl_3$)

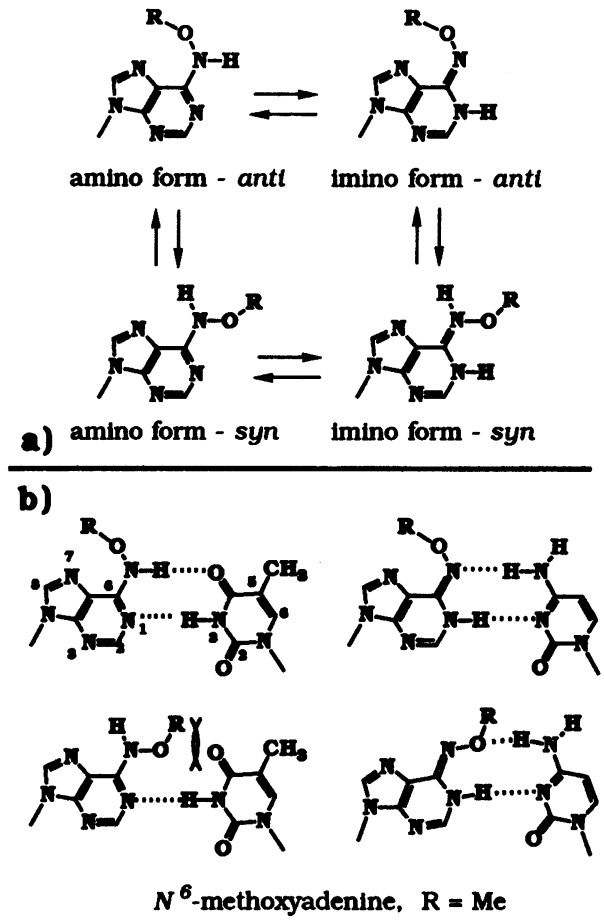

Figure 1. Amino-imino tautomerism of N^6-substituted adenine.

Table 1. Tm (°C) of the Helix-to-Coil transition of 5'd(CCTGGTAXCAGGTCC)3'—5'd(GGACCATNGTCCAGG)3' (X = M, A, G. N = T, C, A, G) at 6.7 μM.

	N = T	N = C	N = A	N = G
X = M	50	46	52	48
X = A	54	44	45	53
X = G	48	59	54	52

9.01 (br s, 0.4 H, H_i-N^1), 8.72 (br s, 0.6 H, H_a-N^6), 8.45 (s, 0.6 H, H_a-2), 8.01 (s, 0.6 H, H_a-8), 7.67 (s, 0.4 H, H_i-8), 7.43–7.37 (m, 2.4 H, H_i-2, ArH), 7.29–7.10 (m, 7 H, ArH), 6.84–6.78 (m, 4 H, ArH), 6.45 (t, 0.6 H, H_a-1'), 6.27 (t, 0.4 H, H_i-1'), 5.35 (m, 1 H, 3'-OH), 4.69 (m, 0.6 H, H_a-3'), 4.61 (m, 0.4 H, H_i-3'), 4.14–4.09 (m, 1H, H-4'), 3.97 (s, 1.8 H, N_aOMe), 3.89 (m, 1.2 H, N_iOMe), 3.78 (s, 6H, ArOMe), 3.52–3.41 (m, 2H, H-5'), 2.88–2.46 (m, 2H, H-2'). *Anal.* Calcd for $C_{32}H_{33}N_5O_6\beta4/3$ H_2O: C, 63.14; H, 5.79; N, 11.50. Found: C, 63.26; H, 5.70; N, 11.48.

3'-O-2-Cyanoethyl(N,N-diisopropylamino)phosphinyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-N^6-methoxyadenosine (**5**). According to the reported methods (18,20), **4** was converted to **5** (78% as a foam): EI-MS *m/z* 790 (M^+); ^{1}H NMR ($CDCl_3$) 9.00 (br s, 0.4 H, H_i-N^1), 8.64 (br s, 0.6 H, H_a-N^6), 8.45 (s, 0.6 H, H_a-2), 8.06–8.00 (m, 1 H, Hi-2, Ha-8), 7.73–7.69

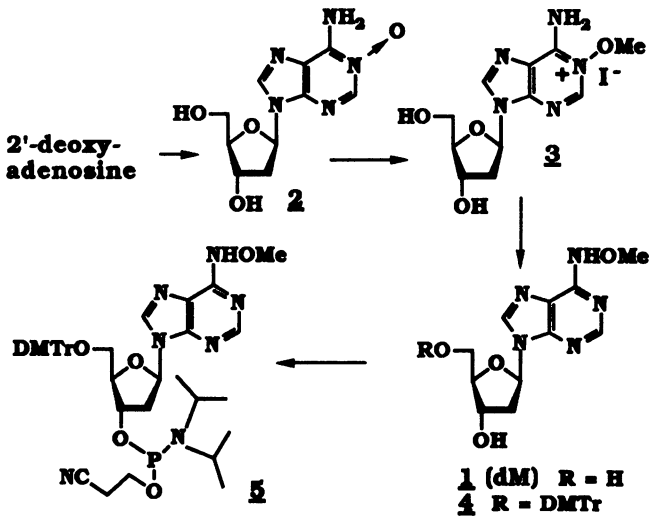

Figure 2. Synthesis of an amidite synthon of N^6-methoxyadenine.

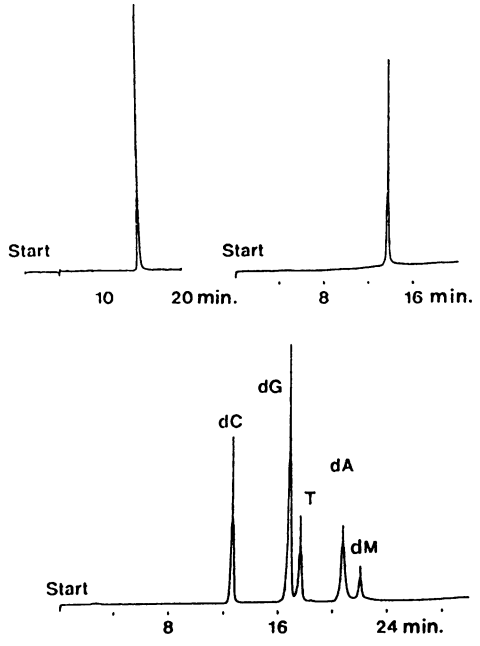

Figure 3. Profiles for HPLC analysis of d(CCTGGTAMCAGGTCC) with C-18 (upper left) and ion exchange (upper right) columns, and profile for HPLC analysis of the mixture of nucleosides obtained by complete hydrolysis of the nucleotide (lower).

(m, 0.4 H, H_i-8), 7.42−7.39 (m, 2H, ArH), 7.32−7.16 (m, 7H, ArH), 6.81−6.76 (m, 4 H, ArH), 6.45 (t, 0.6 H, H_a-1'), 6.28 (t, 0.4 H, H_i-1'), 4.74 (m, 1 H, H-3'), 4.33 (m, 1 H, H-4'), 3.97 (s, 1.8 H, N_a-OMe), 3.90 (m, 1.2 H, N_i-OMe), 3.77 (s, 6 H, ArOMe), 3.63−3.51 (m, 2 H, H-5'), 3.45−3.27 (m, 4 H, CH_2CH_2CN), 2.88−2.59 (m, 2 H, H-2'), 1.37−1.08 (m, 14 H, isoPr).

Synthesis of oligonucleotides

The oligonucleotides were synthesized on a DNA synthesizer (Applied Biosystem Model 381A, CA, U.S.A.) by the reported method (20). Then, fully protected oligonucleotides containing **M** were deblocked and purified by the same procedure as for the purification of natural oligonucleotides. Each oligonucleotide in this preparation showed single peak by HPLC analysis with a C-18 column (Chemcosorb 5-ODS-H) and an ion exchange column (TSKgel DEAE-2SW, Toso, Japan).

After complete hydrolysis of the oligonucleotides to nucleosides by snake venom phosphodiesterase (Boehringer Mannheim, Germany) and bacterial alkaline phosphatase (Takara Shuzo Co., Ltd., Japan), the nucleoside composition of each oligonucleotide was confirmed by HPLC (ϕ 4.6×250 mm, Chemcosorb 5-ODS-H), eluted with a linear gradient from 0% to 50% MeOH in water in 30 minutes. Extinction coefficients of the oligomers were calculated by the reported method (21).

Thermal denaturation and CD spectroscopy

Each sample contained appropriate oligonucleotides in a buffer of 0.01 M Na cacodylate (pH 7.0, 3 mL) containing 0.1 M NaCl. The solution was heated at 70°C for 20 minutes, then cooled gradually to an appropriate temperature and used for thermal denaturation study. A thermally induced transition of each mixture of oligomers was monitored at 254 nm by UV-260 (Shimadzu) with a temperature controller 140-SPR-5 (Shimadzu). Sample temperature was monitored by Model-2542 pocket thermometer (Yokogawa Hokushin Electric, Japan). Sample temperature was increased one degree per one minute. Each Tm is given an average of 3 measurements. Thermodynamic parameters are derived by the reported method (22). Samples for CD spectroscopy were prepared by the same procedure used in the thermal denaturation study and spectra were measured at room temperature.

Incorporation of TTP and dCTP into DNA strands by Klenow DNA polymerase

Solutions (50 μL) of a template [3'(GTCGAAAGCGXAGTCG)5' (**X** = **M** or A) (0.83 pmol)], a primer [5'(^{32}p-CAGCTTTCGC)3' (0.83 pmol)], dNTP (50 pmol) (23,24), and Klenow DNA polymerase (0.1 units) (Takara Shuzo, Co., Ltd.) in a buffer containing 67 mM potassium phosphate (pH 7.6), 6.7 mM $MgCl_2$ and 1 mM mercaptoethanol were incubated at 20°C. After 0, 5, 10, 20 minutes, aliquots (10 μL) of the reaction were added to 50 mM EDTA (10 μL). The whole was heated on a boiling water bath, then cooled and concentrated *in vacuo*.

DNA loading solution (95% formamide, 0.1% xylene cyanol, 0.1% bromophenol blue, 5 μL) was added to each sample and the mixtures were analyzed by electrophoresis at room temperature for 5 h on 15% polyacrylamide gels (25 cm wide, 40 cm long, and 0.5 mm thickness, 750 V) containing 8 M urea (23). After gels were autoradiogramed, spots were cut and their radioactivities were measured on a liquid scintillation counter (Tri-Carb 460C, Packard, U.S.A.). The ratio (%) of incorporation of dNTPs into the 3'-end of the primer can be calculated as: Ratio = (radioactivity of newly generated spots)/(radioactivity of the remaining primer + radioactivity of newly generated spots)×100.

RESULTS AND DISCUSSIONS

Synthesis and properties of oligonucleotides

2'-Deoxy-N^6-methoxyadenosine (**1**) was synthesized from 2'-deoxyadenosine (dA) by the reported methods (11,19) with minor modifications, then **1** was converted to the amidite synthon

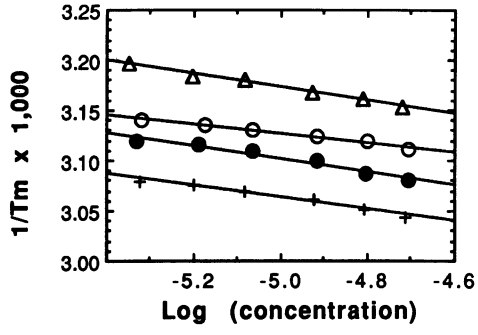

Figure 4. Plots of Tm^{-1} vs. log (concentration) in 0.01 M Na cacodylate buffer, 0.1 M NaCl at pH 7.0 for 5'd(CCTGGTAXCAGGTCC)3'-5'd(GGACCTGNTA-CCAGG)3' [X = M, N = T (●); X = M, N = C (○); X = A, N = T (+); X = A, N = C (△)] over a concentration range of 4.5 μM−20 μM.

Table 2. ΔG° (Kcal/mol at 298 K), ΔH° (Kcal/mol) and ΔS° (Kcal/mol/K) for the Helix-to-Coil transition of 5'd(CCTGGTAXCAGGTCC)3'—5'd(GGACC-ATNGTCCAGG)3' (X = M, A. N = T, C, A, G) derived from the plots in Fig. 4.

	ΔG°	ΔH°	ΔS°
M:T	-6.2	-91.2	-0.285
M:C	-6.4	-93.8	-0.293
A:T	-8.3	-101.9	-0.314
A:C	-2.5	-56.7	-0.182

5 (18,20) (Fig. 2). The amino-imino tautomerisms of **1**, **4**, and **5** were observed by NMR study. Similarly to the other N^6-methoxyadenine derivatives (8,11), the tautomerism was dependent on solvent polarity. The imino tautomer of **1** was more stable than the amino tautomer in dimethylsulfoxide, a polar solvent [the ratio of amino tautomer (a) and the imino tautomer (i), (a:i) = 0.15:0.85], but the amino tautomers of **4** and **5** were more stable than corresponding imino tautomers in $CHCl_3$, a less polar solvent (a:i = 0.6:0.4). The oligonucleotides containing **M** have been synthesized and purified as same as normal oligonucleotides (20). Each oligonucleotide in this preparation showed a single peak by HPLC analysis with C-18 reverse phase and DEAE ion exchange columns (Fig. 3, upper). The nucleoside composition of each oligonucleotide was confirmed by HPLC analysis of a mixture of nucleosides obtained by enzymatic complete hydrolysis of the oligomer (Fig. 3, lower).

Thermal denaturation of duplexes

Each profile of thermal denaturation of the duplexes consisting of 5'd(CCTGGTAXCAGGTCC)3' (X = M, A, G) and 5'd(GG-ACCTGNTACCAGG) (N = T, C, A, G) showed a single transition corresponding to a Helix-to-Coil transition (data not shown). Tms of the transitions are listed in Table 1.

In contrast to the large differences between Tms of the (A:T)-duplex [the duplex containing the A:T pair] and the (A:C)-duplex (10 degrees) and between Tms of the (G:C)-duplex and the (G:T)-duplex (11 degrees), the difference between Tms of the (**M**:T)-duplex and the (**M**:C)-duplex (4 degrees) was rather small. Namely the (**M**:T)-duplex and the (**M**:C)-duplex were less stable than the (A:T)-duplex and the (G:C)-duplex, but were more stable than the (A:C)-duplex and the (G:T) duplex. The imino form of **M**, the predominant form in polar solvents such as water, is

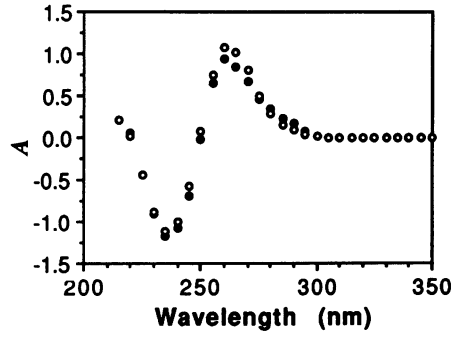

Figure 5. CD spectra for 5'd(CCTGGTAXCAGGTCC)3'-5'd(GGACCTGNTA-CCAGG)3'. [a], X = M, N = T (●); X = A, N = T (○). $A = [\theta] \times 100{,}000$, expressed per oligonucleotide.

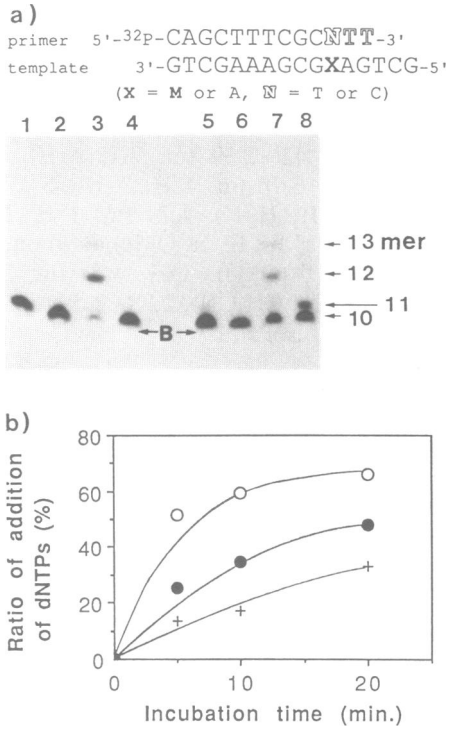

Figure 6. a). Autoradiogram of a polyacrylamide gel showing incorporation of dNTPs into the primer at the site opposite to **X** (indicated by at the 3'-end of the primer), A and G (indicated by); the natural template (X = A, lane 1−4) or the modified template (X = **M**, lane 5−8) and the primer were incubated with Klenow DNA polymerase in the presence of dATP (lane 1 and 5), dGTP (lane 2 and 5), TTP (lane 3 and 7) and dCTP (lane 4 and 8). B indicates bromophenol blue. 10 mer indicates unextended primer and 11 mer, 12 mer, and 13 mer indicate extended primers. b). Ratio of addition of dNTPs to the 3'-end of the primer: X = A, TTP (○); X = **M**, TTP (●); X = **M**, dCTP (+).

not suitable to form a base pair with T (Fig. 1b). Also, a steric hindrance (25) between the methoxy group and the *N*-7 of **M**, which are close to each other in the **M**:T pair (Fig. 1b) may destabilize the base pair. On the other hand, the favorable imino tautomer can stabilize the **M**:C pair (Fig. 1b) and may make amends for the unfavorable steric effects of the methoxy group.

Recently, Brown and Lin (18) reported similar observations. However, they reported that a duplex containing the **M**:C pair

is not more stable than a duplex containing the A:C pair. The observation is not same as our observation that the M:C pair is more stable than the A:C pair. Therefore, to clarify this point, we measured the Tms of the duplexes containing the M:T, the M:C, the A:T, and the A:C pairs over a range of concentrations. The resulting data are shown in Fig. 4 as a plot of the inverse melting temperature, Tm^{-1}, against the logarithm of the concentration (22). Such a plot allows comparison of the melting temperatures of different molecules at the same concentration. Thermodynamic values (22) derived from the plots are listed in Table 2. These values indicated that the (M:T)-duplex and the (M:C)-duplex are similarly stable and are much more stable than the (A:C)-duplex but less stable than the (A:T)-duplex.

Additionally, we found that M formed fairly stable base pairs with A and G with similar stabilities to the M:T and the M:C pairs as shown in Table 1. Therefore, Tms of the duplexes containing the M:N pairs were within the range of 6 degrees of difference (from 46°C to 52°C) which was smaller than those of Tms of the duplexes containing the A:N pairs (11 degrees) and the G:N pairs (11 degrees).

To examine conformations of the duplexes, we measured CD spectra of the duplexes of 5'd(CCTGGTAXCAGGTCC)3'-5'd(GGACCTGNTACCAGG)3' (X = M, A. N = T, C, A, G). An example of spactra is shown in Fig. 5. All spectra showed a positive (transition point at 260 nm) to negative (transition point at 235 nm) splitting which was attributable to B-like right-handed DNA conformation. Values of $[\theta]/100,000$ (per oligonucleotide) of the duplexes were 0.94 at 260 nm, −1.2 at 235 nm (M:T); 1.2, −1.1 (M:C); 0.89, −0.91 (M:A); 0.99, −1.2 (M:G); 1.1, −1.1 (A:T); 1.0, 1.2 (A:C); 0.98, −0.99 (A:A); 0.94, −1.0 (A:G). No obvious difference in spectra was observed. The result indicates that the differences in Tm values of the duplexes are mainly represent the differences in thermal stabilities and stacking abilities of the M:N pairs and the A:N pairs.

dNTP incorporation by Klenow DNA polymerase

The base-pairing properties of M in DNA strands with incoming dNTP substrates during replication of DNA are biologically important since formation of M in DNA is expected to contribute to mutation (4,5). The occurrence of the M:C mispairs may lead to A:T to G:C transitions and the occurrence of the M:A or the M:G mispairs may lead to A:T to T:A or A:T to C:G transversion mutations. When the template, 3'(GTCGAAAGCGXAGTCG)5' (X = M or A), and the primer, 5'(^{32}P-CAGCTTTCGC)3', are incubated with Klenow DNA polymerase in the presence of each dNTP, spots for a 11 mer [5'(^{32}P-CAGCTTTCGCN)3', in the case of the presence of dCTP or dATP or dGTP] or spots for a 12 mer [5'(^{32}P-CAGCTTTCGCTT)3', in the case of the presence of TTP] will be observed by polyacrylamide gel analysis if each dNTP is incorporated into the 3'-end of the primer at the sites opposite to M or A. In the presence of the M-containing template (X = M), newly generated spots for the 11 mer or the 12 mer were observed in reactions containing dCTP (Fig. 6a, lane 8) or TTP (lane 7), respectively. In contrast, only TTP was incorporated into the primer in the presence of the normal template (X = A) (lane 3). In the reactions containing TTP, faint spots corresponding to a 13 mer, 5'(^{32}P-CAGCTTTCGCTTT)3', which might be generated by formation of a G:T mispair, were observed beside the main spots of the 12 mer and faint spots of unelongated 11 mer, 5'(^{32}P-CAGCTTTCGCT)3' (lane 3 and 7). In contrast to the results of reactions containing pyrimidine nucleoside triphosphates, no obvious spot for 11 mer was observed in the reactions containing dATP (lane 1) or dGTP (lane 2).

The result indicated that the formation of the M:N base pairs by the DNA polymerase do not correlate directly with the thermal stabilities of the duplexes (Table 1). Similar result was observed in a study by Eritaja *et al.* using a different base analog (26) and they expected that not only stability of base pairs but also the conformation of a sugar-phosphate backbone is important for the formation of base pairs by the DNA polymerase. In the CD study, we observed that the M:N pairs in the middle of duplexes do not disorder whole duplex conformations largely, but conformations of the M:N pairs at the end of duplexes could be more flexible than those in middle of duplexes. Therefore, according to their hypothesis, our observation can be explained as follows. The favoured M:C and M:T pairs can occur with Watson−Crick geometry without disordering the backbone conformation but the unfavored M:A and M:G pairs may disorder a normal duplex conformation owing to the fact that the purine-purine type of base pairs are larger than the purine-pyrimidine type of base pairs in size and/or the purine-purine base pairs are not in a Watson−Crick geometry.

We studied rate of incorporation of TTP and dCTP with respect to incubation time (Fig. 6b). The rates of incorporation of TTP and dCTP at the site opposite to M were approximately 1/2 and 1/3 of the rate of incorporation of TTP at the site opposite to A, respectively. These rates are not small as indicated by the result that formation of the M:C pair was obviously faster than formation of the G:T mispair which was observed as formation of the 13 mer in Fig. 6a (lanes 3 and 7). The results suggest that DNAs containing M can be replicated and the M:C pair occurs in rather high frequency. After introduced into DNA, the M:T and the M:C pairs are stable and they do not disorder duplex conformation largely. Therefore, they can be exist in DNA and finally can cause A:T to G:C transitions in cells.

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