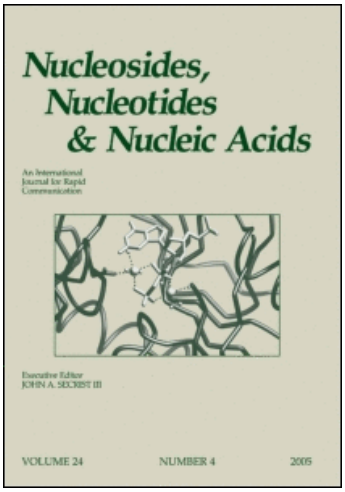

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T. Shida[a]; M. Arakawa[a]; J. Sekiguchi[a]
[a] Department of Applied Biology, Faculty of Textile Science and Technology, Shinshu University, Nagano, Japan

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PROPERTIES OF DNA DUPLEXES CONTAINING AN ABASIC SITE: EFFECT OF NUCLEOTIDE RESIDUE OF THE OPPOSITE PART OF A 2-DEOXYRIBOSYLFORMAMIDE RESIDUE

Toshio Shida*, Masahiko Arakawa, and Junichi Sekiguchi
Department of Applied Biology, Faculty of Textile Science and Technology, Shinshu University, 3-15-1 Tokida, Ueda, Nagano 386, Japan

Abstract: To elucidate the conformational properties of a DNA duplex with a 2-deoxyribosylformamide residue (dF), oligomers containing this abasic residue and a propanediol residue in the center of the helix were synthesized and characterized by UV absorption and CD. By a comparison of the Tms of the duplexes containing a dF residue and a propanediol residue (P), the stability of the duplex containing the abasic damage was judged to depend on the kind of nucleotide residue on the opposite side of the abasic residue rather than the kind of abasic residue.

Introduction

Abasic sites in DNA are created by the loss of a purine or pyrimidine residue and are considered to be important intermediates in mutagenesis and carcinogenesis.[1,2] These lesions, which are produced by chemical fragmentation of bases[3] as well as hydrolysis of the *N*-glycosidic bond,[4,5] lack primary coding information. Unless lesions are repaired prior to DNA replication, these non-coding lesions promote misincorporation of nucleotides and mutagenesis.[6] Base fragmentation by reactions of free radicals and oxidizing agents are common non-coding lesions in DNA. In the case of the thymine residue, hydroxy radicals preferentially attack the C(5) position in a neutral solution and at the C(6) in an acidic solution. By the subsequent reactions, thymine residues in DNA are converted to radiation products such as urea, formylurea, and formamide, which attach to the DNA chain.[3] 2-Deoxy-β-*D*-ribofuranosyl-*N*-formamide (2-deoxyribosyl-formamide, dF) is a major oxidative product formed from T in the DNA by ionizing radiation.[7] The formation of dF may involve the attack of the hydroxy radical on the C5-C6 double bond of the pyrimidine ring, followed by hydrolytic cleavage of the hydroxy-hydroperoxy intermediates. A similar oxidative product was found among the degradation products of d(CpG) by a 1,10-phenanthroline-copper ion complex.[8] Since the degradation was

This paper is dedicated to the 70th birthday of Dr. Morio Ikehara.

derived from the dG residue, the first step in the production of dF may be oxidation of the guanine residue at the C4-C5 double bond.

We are interested in the influence of the dF residue in the DNA chain and its structural and biochemical consequences. In our previous studies,[9,10] an oligodeoxyribonucleotide containing a dF residue was synthesized and the presence of a dF residue in the oligomer was confirmed by ion-spray mass spectrometry. Furthermore, the dF residue was found to affect considerably the stability of the DNA duplex. Herein, we report the effects of a nucleotide residue on the opposite side of a dF residue on the structural properties of DNA. We also prepared a modified oligodeoxyribonucleotide lacking the furanose ring structure but retaining the carbon atoms of the sugar-phosphate backbone.

Materials and Methods

Materials and general procedures

Silica gel column chromatography was conducted with a Merck Silica gel 60. Reverse-phase silica gel chromatography was conducted with a Waters preparative C18 (mesh 55-105 μm). Thin layer chromatography (TLC) was performed on a Merck Silica gel 60 F_{254} using a $CHCl_3$-MeOH mixture. Nuclease P1 was obtained from Yamasa Shoyu Co. and incubated in 0.05 M ammonium acetate (pH 5) at 37°C for 2 h with the enzyme (5 μg/ml).

UV absorption spectra were recorded on a Shimadzu UV-250 spectrophotometer. For melting temperature measurement, a Shimadzu UV-3100 spectrophotometer equipped with a SPR-5 Shimadzu temperature programmer was used. Circular dichroism (CD) spectra were recorded on a JASCO J-600 spectropolarimeter. For temperature control, a thermo-jacket cell and a circulating bath were used. The molar absorption coefficient, ε, and the molar ellipticity, [θ], are presented in terms of per base residue values. HPLC was conducted with a Shimadzu LC-6A pump and on a SPD-6A UV monitor. The reverse-phase column used was a Wakopac WS-DNA (250 x 4.6 mm i.d.).

Oligonucleotide synthesis and purification

For the incorporation of dF into oligodeoxyribonucleotide via the standard solid-phase phosphotriester method, triethylammonium [2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-β-*D*-ribofuranosyl-*N*-formamide-3-*O*-(2-chlorophenyl phosphate)] was synthesized from dF according to the published procedure.[10] Similarly for the incorporation of propanediol residue (P) into oligomer, triethylammonium [3-(4,4'-dimethoxytrityloxy)-1-propanol-*O*-(2-chlorophenyl phosphate)] was synthesized from 3-(4,4-dimethoxytrityloxy)-1-propanol.[11] Oligodeoxyribonucleotides were synthesized on a manual synthesizer which was charged with nucleoside-loaded 1% polystyrene resin (10 μmol). Then, fully

protected oligonucleotides containing dF and P were deblocked and purified by the same procedure as for the purification of natural oligonucleotides.

Results and Discussion

Solid-phase synthesis of the oligodeoxyribonucleotides (Fig. 1), was performed by the phosphotriester method.[12] The condensation reactions were carried out with 2,4,6-trimethylbenzenesulfonyl-3-nitrotriazolide (MSNT). The dF and the P residues were inserted with 93% and 86% coupling yields respectively, as calculated from the amount of released trityl cation. The purity of the final products was checked by HPLC and found to be greater than 97%. Figure 2 shows the reverse-phase HPLC elution profiles of d(GATACGFATCCAG) (A) and d(GATACGPATCCAG) (B). Because of the *cis-trans* conformers of the dF residue in the oligomer, the top of the peak of this oligomer was divided into two (Fig. 2, A).[7,10] The oligomer containing the P residue,[11,13,14] corresponding to C-3, C-4, and C-5 of 2-deoxyribose, was used as an acyclic abasic model to compare the oligomer containing the dF residue.

The stability of the duplexes containing a dF residue or a P residue was examined by thermal denaturation. The profiles of thermal denaturation of the duplexes consisting of d(GATACGXATCCAG) (X=A, G, C, T, F, P) and d(CTGGATYCGTATC) (Y=A, G, C, T)
showed a single transition corresponding to a helix-to-coil transition. Fig. 3 shows the melting profiles of the duplexes consisting of d(GATACGXATCCAG) (X=T, C, F, P) and d(CTGGATACGTATC) (Y=A). The difference in melting temperature (Tm) among the four duplexes was extremely large. The Tm of the duplex containing the C-A mispair (CA duplex) was 11 degrees lower than that of the duplex containing the T:A pair (TA duplex). On the other hand, the melting profiles of the duplexes containing a F-A mispair (FA duplex) and a P-A mispair (PA duplex) were similar and they had Tms lower than that of the CA duplex. This tendency of the stability of the FA duplex is identical to that of the duplex containing the dF residue we reported previously.[10]

To compare the stability of the duplexes accurately, the Tms of the duplexes was measured over a range of oligomer concentrations. Fig. 4 shows the resulting data as a plot of the inverse melting temperature, Tm^{-1}, against the logarithm of the oligomer concentration.[15] Such a plot allows comparison of the Tms of different duplexes at the same concentration. Table 1 shows the thermodynamic parameters derived from the plots.[16] The average Tm of the duplexes containing the normal A:T, T:A, G:C, and C:G pair is 13 degrees higher than that of the duplexes containing the A-C mispair (AC duplex and CA duplex). In addition, the average Tm of the duplexes containing the A-C mispair is approximately 6 degrees higher than that of the duplexes containing the abasic residue (FY

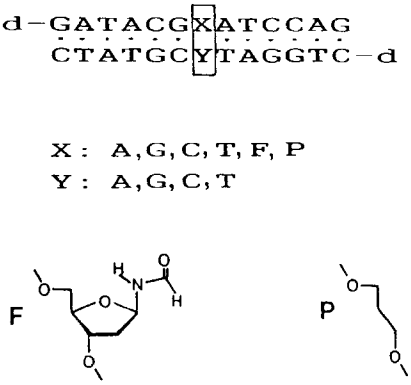

FIG. 1. Oligonucleotide Duplexes Used in This Study and Structural Formula for Abasic Site. F, 2-deoxyribosylformamide residue; P, propanediol residue. The boxed part indicates the difference among the duplexes.

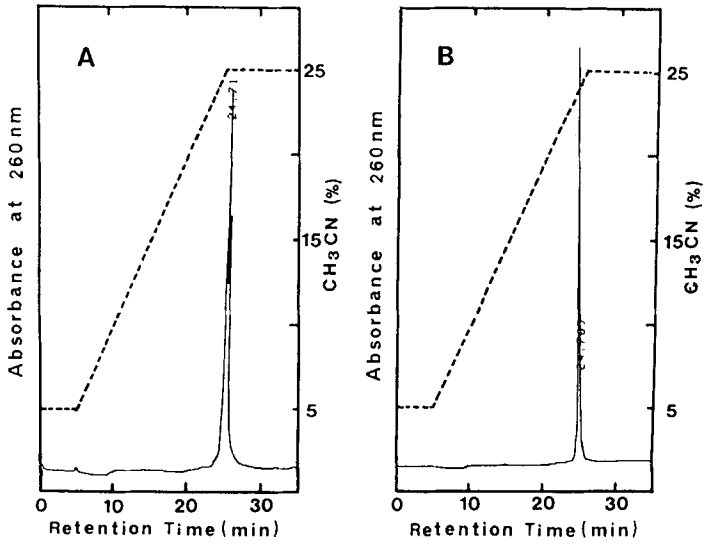

FIG. 2. HPLC Elution Profiles of the Purified d(GATACGFATCCAG) (A) and d(GATACGPATCCAG) (B). A linear gradient of acetonitrile from 5% to 25% in 0.1 M triethylammonium acetate (pH 7.0) at room temperature was used. Wavelength of detection: 260 nm.

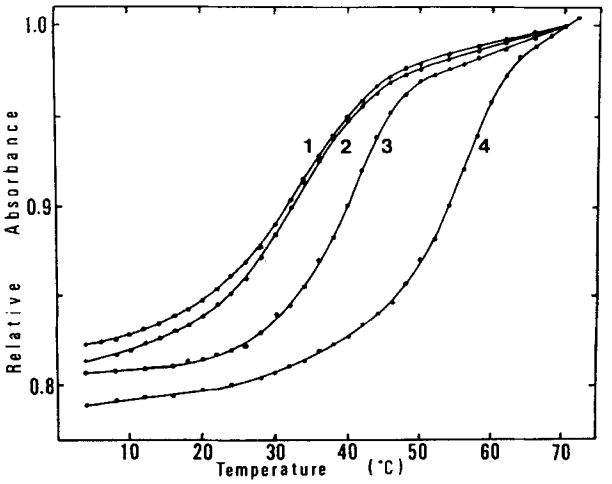

FIG. 3. Normalized Melting Curves for d(GATACGXATCCAG)/d(CTGGATACGTATC) Duplexes. 1, X=P; 2, X=F; 3, X=C; 4, X=T. Melting profiles of the duplexes (about 1 *A* unit/ml) were measured in 0.1 M NaCl-0.01 M sodium cacodylate (pH 7.0) at 260 nm.

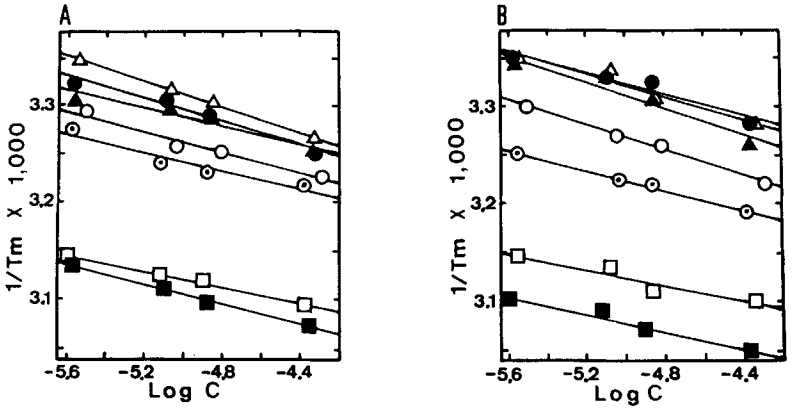

FIG. 4. Plots of Tm^{-1} vs log (concentration) for d(GATACGXATCCAG)/d(CTGGATYCGTATC) Duplexes (XY duplex). Measured in 0.1 M NaCl-0.01 M sodium cacodylate buffer (pH 7.0). A: FA duplex (○); FG duplex (▲); FC duplex (●); FT duplex (△); AC duplex (◎); AT duplex (□); GC duplex (■). B: PA duplex (○); PG duplex (▲); PC duplex (●); PT duplex (△); CA duplex (◎); TA duplex (□); CG duplex (■).

TABLE 1: Thermodynamic parameters for double helix formation of the tridecamers[a)]

duplex(X:Y)[b)]	Tm (°C)[c)]	ΔG° (kcal/mol)[d)]	ΔH° (kcal/mol)	ΔS° (kcal/mol K)
A:T	47	-15	-110	-0.32
T:A	47	-15	-114	-0.33
G:C	49	-14	-90	-0.26
C:G	52	-15	-102	-0.29
A:C	35	-10	-95	-0.28
C:A	37	-10	-88	-0.26
F:A	33	-9	-87	-0.26
F:G	31	-9	-99	-0.30
F:C	30	-8	-73	-0.22
F:T	29	-8	-68	-0.20
P:A	33	-9	-70	-0.21
P:G	29	-8	-68	-0.20
P:C	28	-8	-84	-0.26
P:T	28	-8	-76	-0.23

a) Measured in 0.1 M NaCl, 0.01M sodium cacodylate (pH 7.0).
b) Duplex (X:Y): d-GATACGXATCCAG
CTATGCYTAGGTC-d
c) Calculated for 10 μM oligomer concentration.
d) Calculated for 25°C.

duplex and PY duplex, Y=A, G, C, T). These differences in Tm values of the duplexes indicate that the stability of the duplex is due to both complementary hydrogen bonding and base-stacking interactions with adjacent base pairs. On the other hand, the difference in the average Tms between the FY duplexes (Y=A, G, C, T) and PY duplexes (Y=A, G, C, T) is small. The FY duplexes had slightly higher Tms than the PY duplexes. The difference in Tms between the FY duplexes and PY duplexes (FA duplex-PA duplex, FG duplex-PG duplex, FC duplex-PC duplex, and FT duplex-FT duplex) was in the range of 0 to 3 degrees. This indicates that the difference in the kind of abasic and/or acyclic lesions lacking the abilities of base-pairing and base-stacking does not appreciably affect the stability of the DNA duplex. The thermodynamic impact of these lesions primarily results from removal of the base rather than the sugar ring.[17] Additionally, we found that the Tms of the FA, FG, FC, and FT duplexes decreased in that order which was essentially the same as that observed for the PY duplexes (Y=A, G, C, T). The difference in the Tms between the XA duplexes (X=F, P) and XT duplexes (X=F, P) is approximately 5 degrees, which is quite large. It is well established that under a given set of solution

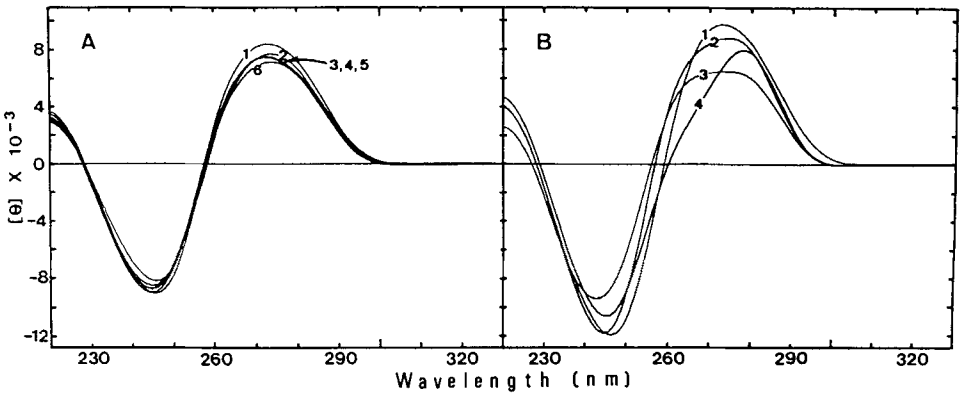

FIG. 5. CD Spectra for d(GATACGXATCCAG)/d(CTGGATYCGTATC) Duplexes (XY duplex). A: PC duplex (1); FC duplex (2); PT duplex (3); FG duplex (4); PG duplex (5); FT duplex (6). B: CG duplex (1); AC duplex (2); GC duplex (3); AT duplex (4).

conditions the relative stability of a DNA duplex structure depends on its base sequence.[18] The stability of a DNA duplex appears to depend primarily on the relationship of the nearest-neighbor bases.[19] The difference and order of stability of duplexes containing the abasic lesion reported here is considered to be attributable to the ability of the base-stacking interaction of the base residues which are on the opposite side of the abasic lesion of the duplex.

To examine the conformation of the duplexes, CD spectra of the duplexes consisting of d(GATACGXATCCAG) (X=A, G, C, T, F, P) and d(CTGGATYCGTATC) (Y=A, G, C, T) were measured in 0.1 M NaCl at pH 7. Fig. 5 shows the spectra. No obvious difference was observed in the spectra of the duplexes containing the abasic lesion. All spectra showed a positive (around 270 nm) to negative (around 245 nm) splitting and the CD magnitudes of both bands were nearly identical. This observation is consistent with the results on essentially the same family of duplexes.[17] In contrast to the CD spectra of the duplexes containing the abasic lesion, the duplexes containing the normal base pair and the mispair showed various CD patterns which were classified into the category of B-like DNA conformations. In spite of not only the kind of the abasic lesion but also the kind of nucleotide residue in the opposite site of an abasic lesion, the global conformations of duplexes as characterized by CD spectroscopy seem to be similar.

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