# Recognition of a Guanine–Cytosine Base Pair by 8-Oxoadenine†

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*Received November 25, 1991; Revised Manuscript Received May 6, 1992*

ABSTRACT: Two oligodeoxyribonucleotides, d-CTTCTTTTTTATTTT, I(A), and d-ATTATTTTTTA-TTTT, II(A), where C is 5-methylcytosine and A is 8-oxoadenine, were prepared and their interactions with the duplex d-GAAGAAAAAAYAAAA/d-TTTTZTTTTTTCTTC, III·IV(Y·Z), were studied. Oligomers I(A) and II(A) each form triplexes with III·IV(G·C) at temperatures below 20 °C as shown by continuous variation experiments, melting experiments, and circular dichroism (CD) spectroscopy. The CD spectra of these triplexes are almost identical to those formed by I(C) and II(C), oligomers which contain cytosine in place of 8-oxoadenine. This suggests that the 8-oxoadenine-containing triplexes have conformations which are very similar to those of the cytosine–containing triplexes. The melting temperature ($T_m$) for dissociation of the third strand of triplex II·III·IV(A·G·C) is 22 °C at pH 7.0 and 8.0, whereas the $T_m$ of the corresponding transition in triplex II·III·IV(C·G·C) decreases from 28 °C at pH 7.0 to 17 °C at pH 8.0. The pH dependence of the $T_m$ in the latter triplex reflects the necessity of protonating the N-3 of cytosine in order for it to form two hydrogen bonds with G of the G·C base pair. It appears that the keto form of 8-oxoadenine can potentially form two hydrogen bonds with the N-7 and O-6 atoms of G of the G·C base pair, when the 8-oxoadenine is in the syn conformation and in contrast to cytosine does not require protonation of the base. Oligomer I(A) does not form triplexes with III·IV(Y·Z) when Y·Z is A·T or T·A. Triplex formation is observed when Y·Z is C·G or U·A with the third strands melting at approximately 9 and 13 °C, respectively. The latter A·U·A triplex may be stabilized by formation of a single hydrogen bond between the N-6 exocyclic amino group of 8-oxoadenine and the O-4 or uracil of the U·A base pair. The ability of 8-oxoadenine to form A·G·C triads suggests that this modified base may be a useful alternative to cytosine or 5-methylcytosine for use in triplex formation at physiological pH.

The possibility of using oligonucleotides to regulate gene expression at the DNA level has recently led to renewed interest in the formation of triple-stranded DNA (Cooney et al., 1988; Strobel et al., 1988; Dervan, 1989; Povsic & Dervan, 1989; Maher et al., 1989; Francois et al., 1989; Pei et al., 1990; Hanvey et al., 1990; Birg et al., 1990; Collier et al., 1991; Strobel & Dervan, 1991; Orson et al., 1991). Triplex formation is most often observed with oligodeoxyribonucleotides which contain thymidine and deoxycytidine or 5-methyldeoxycytidine. These can form T·A·T and C·G·C triads via Hoogsteen hydrogen bonding with purine bases in a homopurine tract in a double-stranded DNA target. In the latter triad, protonation at N-3 of C is required in order to form two hydrogen bonds with G of the target strand. Because of this requirement, triplex formation by oligopyrimidines which contain multiple C residues is sensitive to pH and is usually restricted to pH values of 7 or less. This limits the potential use of such oligomers as anticode inhibitors under physiological conditions.

Recent studies have shown that the requirement for base protonation can be eliminated by using bases or base analogs which possess suitably arranged hydrogen-bond-donating groups which are capable of interacting with guanine of G·C base pairs. For example, Ono et al. (1991, 1992) have shown that deoxypyrimidine oligomers which contain pseudoisocytosine in place of cytosine form triplexes with an oligodeoxyribonucleotide duplex at pH 7.0. Koh and Dervan (1992) have shown that a base analog, 1-(2-deoxy-β-D-ribofuranosyl)-3-methyl-5-amino-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-one, can interact with G·C base pairs in duplex DNA over an extended range of pH. Oligodeoxyribopurines have also been shown to participate in triplex formation (Orson et al., 1991; Postel et al., 1991; Beal & Dervan, 1991; Radharkrishnan et al., 1991). Hydrogen-bonding schemes which do not require base protonation have been proposed for these purine·purine strand interactions. In this communication, we report that pyrimidine oligomers which contain the modified purine 8-oxoadenine are capable of forming stable triplexes with double-stranded oligodeoxyribonucleotides at neutral and basic pH.

## MATERIALS AND METHODS

*Synthesis of 5′-O-(4,4′-Dimethoxytrityl)-$N^6$-benzoyl-8-methoxy-2′-deoxyadenosine.* 8-Bromo-2′-deoxyadenosine was prepared by a procedure analogous to that used to prepare 8-bromoadenosine (Ikehara & Kaneko, 1970). A solution of sodium methoxide was prepared by dissolving 0.4 g of sodium metal in 14 mL of dry methanol. One gram of 8-bromodeoxyadenosine was added and the mixture was stirred at 65 °C for 18 h. After cooling, the solution was diluted with 3 mL of glacial acetic acid and the solvents were then removed by evaporation. The crude 8-methoxy-2′-deoxyadenosine was purified by flash column chromatography on 60 g of silica gel using methanol/chloroform (1:9 v/v) as solvent, which yielded 0.8 g of pure nucleoside. $^1$H-NMR (300 MHz, DMSO-$d_6$): δ 2.07, 2.99 (m, H-2′), 3.40–3.81 (m, H-3′, H-5′) 4.08 (s, $OCH_3$), 4.38 (m, H-4′), 6.16 (t, H-1′), 6.91 (s, 6-$NH_2$), 8.0 (s, H-2).

8-Methoxy-2′-deoxyadenosine was converted to its $N^6$-benzoyl derivative by reaction of 0.9 g of the nucleoside with 2 mL of benzoyl chloride and 2 mL of trimethylsilyl chloride in 20 mL of dry pyridine for 2 h following the transient protection procedure of Gao et al. (1986). The reaction

† These studies were supported by a grant from the National Institutes of Health, GM 45012.

0006-2960/92/0431-6788$03.00/0 

mixture was cooled to 0 °C, diluted with 7.5 mL of water, and treated with 5 mL of 30% ammonium hydroxide solution for 30 min. The solvents were evaporated and the crude nucleoside was purified by flash column chromatography on 60 g of silica gel using methanol/chloroform (1:9 v/v) as solvent, which yielded 0.92 g (77%) of $N^6$-benzoyl-8-methoxy-2′-deoxyadenosine.

The partially protected nucleoside was dissolved in 20 mL of dry pyridine and the solution was treated with 0.91 g of dimethoxytrityl chloride for 4 h at room temperature. Pyridine was evaporated, the residue was dissolved in 10 mL of ethyl acetate, and the solution was extracted with saturated sodium bicarbonate solution. The aqueous phase was extracted with two 10-mL portions of ethyl acetate and the combined organic extracts were dried over anhydrous sodium sulfate. Pure 5′-*O*-(4,4′-dimethoxytrityl)-$N^6$-benzoyl-8-methoxy-2′-deoxyadenosine (0.98 g) was obtained in 54% yield after flash column chromatography on 60 g of silica gel using chloroform/methanol/triethylamine (98:1:1 v/v/v) as solvent.

*Synthesis of 5′-O-(4,4′-Dimethoxytrityl)-$N^6$-benzoyl-8-methoxy-2′-deoxyadenosine 3′-O-(2-Cyanoethyl N,N′-diisopropylphosphoramidite).* A solution of 0.8 g of 5′-dimethoxytrityl-$N^6$-benzoyl-8-methoxy-2′-deoxyadenosine in 10 mL of dry methylene chloride was treated with 0.44 mL of 2-cyanoethyl chloro-*N,N′*-diisopropylphosphoramidite in the presence of 0.72 mL of diisopropylamine for 1 h at room temperature following the procedure of Sinha et al. (1983). The solution was diluted with 3 mL of methanol and, after 15 min of stirring, further diluted with 50 mL of saturated sodium bicarbonate. The aqueous mixture was extracted with two 25-mL portions of methylene chloride and the organic solution was dried over anhydrous sodium sulfate. The nucleotide was obtained in 89% yield (0.85 g) after purification by silica gel column chromatography using methylene chloride/hexane/triethylamine (50:45:5 v/v/v) as solvent.

*Synthesis of Oligodeoxyribonucleotides.* Oligodeoxyribonucleotides were synthesized on a Biosearch 8700 DNA synthesizer on 1-μmol scales on controlled pore glass supports using base-protected 5′-*O*-dimethoxytrityl-2′-deoxyribonucleoside 3′-*O*-(2-cyanoethyl *N,N′*-diisopropylphosphoramidite) synthons. Each support was then treated with 1 mL of a solution containing pyridine/concentrated ammonium hydroxide (1:1 v/v) for 6 h at 55 °C. The support was removed by filtration and the solvents were evaporated. In the case of the 8-oxoadenine-containing oligomers, the residue was further treated with 1 mL of a solution containing thiophenol/triethylamine/dioxide (1:2:2 v/v/v) under argon for 48 or 96 h at 37 °C. After evaporation, the residue was dissolved in water and the solution was loaded onto a Bio-Gel TSK-DEAE-5-PW HPLC ion-exchange column (7.5 × 75 mm). The column, which was monitored at 254 nm, was eluted with a 50-mL linear gradient of 0.1–0.5 M sodium chloride in 10 mM Tris hydrochloride at pH 7.0 at a flow rate of 0.8 mL/min. The 8-oxoadenine-containing oligomers were purified on a semipreparative, RAC-II, C-18 reversed-phase HPLC column (Whatmann Inc.) using a 30-mL linear gradient of 5–15% acetonitrile in 0.1 M sodium phosphate buffer, pH 5.8, at a flow rate of 1.5 mL/min. Each oligomer was desalted on a Sep-Pak cartridge which had been previously equilibrated with the following solutions: 10 mL of acetonitrile, 10 mL of acetonitrile/water (1:1 v/v), and 10 mL of 0.1 M sodium phosphate buffered at pH 5.8. The Sep-Pak cartridge was washed with 10 mL of water and the oligomer was eluted with 3 mL of acetonitrile/water (1:1 v/v).

*Characterization of Oligodeoxyribonucleotides.* The purity of each oligomer was checked by gel electrophoresis on a 15% denaturing gel after the oligomer had been phosphorylated using [$\gamma$-$^{32}$P]ATP and polynucleotide kinase. Each oligomer migrated as a single band on the gel.

The base ratios of the 8-oxoadenine-containing oligomers were determined according to the procedure of Miller and Cushman (1992). A sample containing 0.05–0.10 $A_{260}$ unit of oligomer was incubated with 5 ng of snake venom phosphodiesterase and 0.4 unit of bacterial alkaline phosphatase in 48 μL of a solution containing 10 mM Tris and 2 mM magnesium chloride at 37 °C for 16 h. The digest was subjected to reversed-phase HPLC on a C-18 Microsorb column (0.46 × 15 cm, Rainin, Inc.) using a linear gradient of 2–3% acetonitrile in 0.1 M sodium phosphate (pH 5.8) for 12 min, followed by a linear gradient of 3–20% acetonitrile in 0.1 M sodium phosphate (pH 5.8) for 8 min at a flow rate of 1.0 mL/min. For d-CTTCTTTTTTATTTT, the base ratio was 1.00:4.63:0.46 d-C:d-T:d-A, and for d-ATTA-TTTTTTATTTT, the base ratio was 3.89:1.00 d-T:d-A.

The extinction coefficients of the oligomers were determined as previously described (Miller & Cushman, 1992). A sample containing 0.2–0.5 $A_{260}$ unit of each oligomer was incubated with 5 ng of snake venom phosphodiesterase in 48 μL of 2 mM magnesium chloride/10 mM Tris hydrochloride, pH 8.2, for 16 h at 37 °C. The $A_{260}$ of the resulting solution of mononucleotides was then used to calculate the molar extinction coefficient of the oligomer. The following molar extinction coefficients of the monomers at 260 nm were used: d-T, 8700; d-C, 7600; d-A, 15 340, d-G, 12 160; d-C, 4110; and d-A, 13 000.

*Melting Experiments.* Triplexes were formed by mixing 0.5 mL of a 1.0 or 2.0 μM solution of the oligomer with 0.5 mL of a 1.0 or 2.0 μM solution of the preformed target duplex at room temperature. The solutions were then stored overnight at 4 °C. All experiments were carried out in melt buffer, which consisted of 0.1 M sodium chloride, 20 mM magnesium chloride, and 50 mM Tris hydrochloride buffered at pH 7.0 or 8.0. The $A_{260}$ profiles of the solutions were recorded as a function of temperature using a Varian 219 spectrophotometer fitted with a thermostated cell compartment connected to a Neslab RTE-100 programmable, circulating temperature bath. The solutions were heated from 0 to 60 °C at a rate of either 0.5 or 1 °C/min. No differences in the melting curves were observed for samples heated at either of these rates. In one experiment, triplex I·III·IV(C·G·C) was heated at 0.2 °C/min. The transition for melting of the third strand was identical to that observed when the triplex was heated at 0.5 °C/min. The melting temperatures were determined from the midpoint of the transition curves. Experiments employing duplicate samples gave identical melting curves and melting temperatures.

*Circular Dichroism Spectroscopy.* Samples were prepared in the same buffer as used in the melting experiments at a total oligomer concentration of 4.8 μM. Therefore, duplex solutions contained 2.4 μM each of strands III and IV, and triplex solutions contained 1.6 μM each of strands I or II and III and IV. Circular dichroism spectra were measured at 1-nm increments with a constant bandwidth of 0.8 nm on an AVIV 60 DS spectropolarimeter (AVIV Associates, Lakewood, NJ) and are the average of three scans. $\Delta\epsilon$ per residue ($M^{-1}\cdot cm^{-1}$) was calibrated using 1*S*-(+)-10-camphorsulfonic acid (Johnson, 1985). Sample temperature was regulated by circulating fluid from a Neslab RTE-4DD constant-temperature bath through the cuvette holder. Dry nitrogen gas was

D-CTTCTTTTTTTXTTTT (I)

D-XTTXTTTTTTTXTTTT (II)

D-GAAGAAAAAAAYAAAA (III)
CTTCTTTTTTZTTTT-D (IV) | TARGET DUPLEX

FIGURE 1: Oligonucleotides used in triplex formation experiments. C is 5-methyldeoxycytidine.

flushed over the cuvette windows to prevent condensation at low temperatures. Baseline correction and data calculations were performed using the AVIV plotting software.

## RESULTS AND DISCUSSION

Triplex formation by 8-oxoadenine-containing oligomers was studied using the oligomer systems shown in Figure 1. The first system consists of a "third strand" oligomer, I(X), and a Watson–Crick base-paired "target" duplex, III·IV(Y·Z). This system probes the ability of a single 8-oxoadenine, A, at position X in oligomer I to support triplex formation with various base pairs at position Y·Z in the target duplex III·IV. Upon formation of a pyrimidine·purine·pyrimidine triplex, the sugar–phosphate backbone of oligomer I will have the same polarity as the purine strand, III, of the duplex (Moser & Dervan, 1987). 5-Methylcytosines, C, were substituted for cytosine residues in oligomer I in order to enhance the stability of the triplexes at pH 7 (Povsic & Dervan, 1989).

The second system consists of oligomer II(X), which contains three noncontiguous 8-oxoadenine or cytosine residues, and target duplex III·IV(G·C). This system tests the ability of a third strand containing multiple 8-oxoadenine residues to participate in triplex formation.

The oligonucleotides were synthesized by standard procedures using nucleoside phosphoramidite synthons and controlled pore glass supports. Oligonucleotides I(A) and II(A) were prepared using the synthon 5′-*O*-(4,4′-dimethoxytrityl)-*N*$^6$-benzoyl-8-methoxy-2′-deoxyadenosine 3′-*O*-(2-cyanoethyl *N*,*N*′-diisopropylphosphoramidite). The 8-methoxy group serves as a "masked" form of the 8-oxo group in this synthon. Treatment of the protected oligomers with the standard ammonium hydroxide deprotection reagent resulted in only a low conversion of the 8-methoxy group to the 8-oxo group. Complete conversion was effected by treating the partially deprotected oligomers with thiophenol in the presence of triethylamine. In the case of oligomer I(A), conversion was complete after 48 h. Oligomer II(A), however, required treatment for 96 h. This synthetic method is an attractive alternative to the previously reported method of Guy et al. (1988), which employed 5′-*O*-(4,4′-dimethoxytrityl)-*N*$^6$-phenoxyacetyl-8-oxo-2′-deoxyadenosine 3′-*O*-(2-cyanoethyl *N*,*N*′-diisopropylphosphoramidite). Although conversion of the 8-methoxy group to the 8-oxo group requires an additional treatment with thiophenol, the presence of the 8-methoxy group reduces the possibility of side reactions between the phosphoramidite reagent and the 8-oxo group which could occur during the tetrazole-activated coupling reactions.

The 8-oxoadenine-containing oligomers were characterized by digestion with a combination of snake venom phosphodiesterase and bacterial alkaline phosphatase followed by separation of the component nucleosides by reversed-phase HPLC. The expected nucleoside ratios were observed for each oligomer. Because 8-methoxydeoxyadenosine and 8-oxodeoxyadenosine have retention times of 19.2 and 16.8 min, respectively, this procedure clearly demonstrated that the 8-methoxyadenine had been completely converted to 8-oxoadenine by the thiophenol treatment.

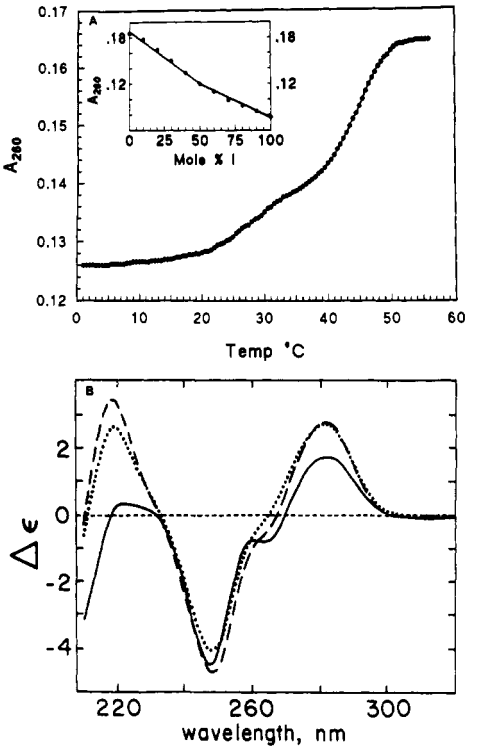


FIGURE 2: (A) Absorbance vs temperature profile of a solution containing 0.5 μM I(A) and 0.5 μM III·IV(G·C). The inset shows a continuous variation titration of I(A) and III·IV(G·C) at 1.5 μM total strand at 7 °C. (B) Observed circular dichroism spectra of a solution containing 1.6 μM I(A) and 1.6 μM III·IV(G·C) at 10 °C (—) and 35 °C (···) and the calculated weight-averaged spectrum of 1.6 μM I(A) and 1.6 μM III·IV(G·C) at 10 °C (- - -). All experiments were performed in standard buffer at pH 7.0.

Ultraviolet mixing and melting experiments were used to study the stoichiometries and stabilities of the complexes formed by the oligomers shown in Figure 1. As is shown by the inset in Figure 2A, solutions containing varying proportions of I(A) and the target duplex III·IV(G·C) gave maximum hypochromicity at 260 nm at a stoichiometry of duplex to third strand of 1:1. This behavior is consistent with the formation of a triplex, I·III·IV(A·G·C). Similar results were obtained with oligomer II(A) and target duplex III·IV(G·C), as is shown by the inset in Figure 3A. Again maximum hypochromicity was observed at a 1:1 stoichiometry of II(A) to III·IV(G·C).

The effect of temperature on the $A_{260}$ of triplex I·III·IV (A·G·C) is shown in Figure 2A. The rate of heating in this experiment was 1 °C/min. An identical curve was obtained at a heating rate of 0.5 °C/min. In addition, curves identical to the melting curves were obtained when the solutions were cooled from 60 to 0 °C at either 0.5 or 1 °C/min. These observations suggest that the system was at thermal equilibrium under the conditions of the melting experiments.

The first transition, whose midpoint occurs at 29 °C and which accounts for 33% of the total observed hypochromicity, corresponds to dissociation of the third strand, I(A), from the duplex. The second transition, whose midpoint occurs at 45 °C, corresponds to melting of the target duplex, III·IV(G·C). A separate experiment in which duplex III·IV(G·C) was melted alone confirmed that the melting temperature of the duplex is 45 °C. Similar melting behavior and hypochromicity were observed for the triplex formed by I(C) and III·IV(G·C), except the first transition occurred at 32 °C.

Oligomer II(A) also formed a triplex with III·IV(G·C) as indicated by the mixing experiment and melting curve shown

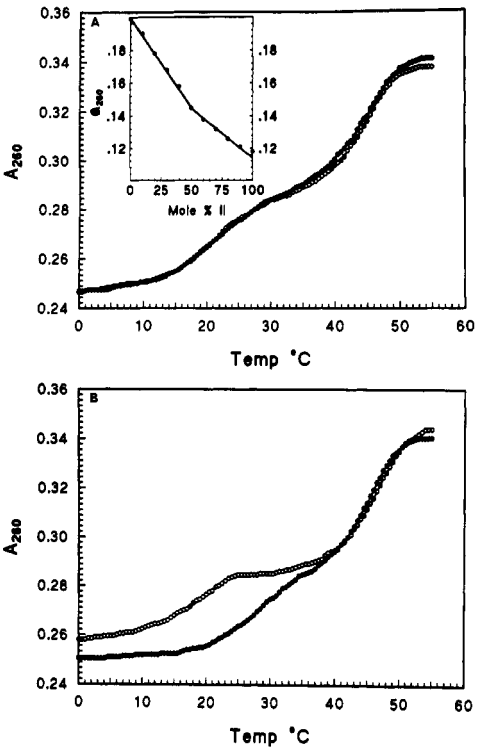


FIGURE 3: (A) Absorbance vs temperature profile of a solution containing 1.0 $\mu$M II(A) and 1.0 $\mu$M III·IV(G·C) at pH 7.0 (●) or pH 8.0 (○). The inset shows a continuous variation titration at pH 7.0 of II(A) and III·IV(G·C) at 1.5 $\mu$M total strand at 10 °C. (B) Absorbance vs temperature profile of a solution containing 1.0 $\mu$M II(C) and 1.0 $\mu$M III·IV(G·C) at pH 7.0 (●) or pH 8.0 (○).

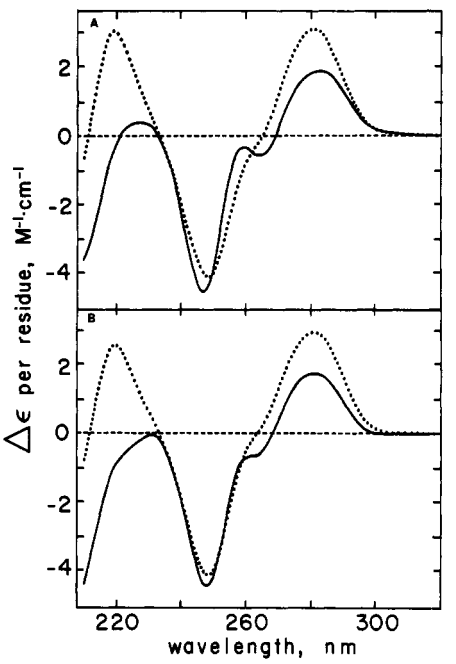


FIGURE 4: (A) Observed circular dichroism spectra of a solution containing 1.6 $\mu$M II(A) and 1.6 $\mu$M III·IV(G·C) at 10 °C (—) and 35 °C (···). (B) Observed circular dichroism spectra of a solution containing 1.6 $\mu$M II(C) and 1.6 $\mu$M III·IV(G·C) at 10 °C (—) and 35 °C (···). The experiments were performed in standard buffer at pH 7.0.

in Figure 3A. An identical curve was obtained when the solution was cooled. At pH 7.0, the third strand of this triplex melted at 22 °C. This melting curve may be compared to that of the triplex formed by oligomer II(C), which contains three cytosine residues, and duplex III·IV(G·C). As shown in Figure 3B, the third strand of this triplex melts at 28 °C. Interestingly, a hysteresis effect was observed when the solution containing II(C), III(G), and IV(C) was cooled at 0.5 °C/min from 60 to 0 °C, although the final absorbance of the solution was identical to that of the initial triplex solution. The shape of this annealing curve between 35 and 0 °C suggested that triplex formation occurred in a less cooperative manner than did melting of the third strand.

Formation of triplex I·III·IV(A·G·C) was further confirmed by the circular dichroism (CD) spectra, as shown in Figure 2B. At 10 °C, the CD spectrum of I(A)·III·IV(G·C) is quite different from that of a calculated spectrum of the target duplex and a noninteracting third strand. The observed spectrum shows reduced $\Delta\epsilon$ at 220 and 280 nm, behavior which is characteristic of triplex formation for sequences of this type having predominantly T·A·T base triads (Pilch et al., 1990). At 35 °C, a temperature above the $T_m$ of the third strand, the CD spectrum was essentially identical to the sum of the spectra of I(A) and III·IV(G·C). The CD spectra of the triplex I·III·IV(C·G·C) behaved in a similar manner at 10 and 40 °C, and the spectrum of this triplex was virtually identical to that of I·III·IV(A·G·C) at 10 °C (data not shown).

The CD spectrum of triplex II·III·IV(A·G·C) at 10 °C is shown in Figure 4A. For comparison, the CD spectrum of the triplex formed by II(C) and III·IV(G·C) is shown in Figure 4B. The spectra of both triplexes are very similar at 10 °C. At 35 °C, a temperature above the melting temperature of the triplexes, the CD spectra are identical to the sum of the CD of duplex, III·IV(G·C), and the third strand. These results and the result obtained with triplex I·III·IV(A·G·C) suggest that the overall conformation of the triplexes formed by oligomers which contain 8-oxoadenine is very similar, if not identical, to that of the triplexes formed when the third strand contains cytosines.

Triplex formation by I(A) or II(A) with III·IV(G·C) could occur if the keto form of 8-oxoadenine is in the syn conformation. As shown in Figure 5A, the N-6 exocyclic amino group and the N-7 imido group of 8-oxoadenine can potentially form hydrogen bonds with the O-6 and N-7 atoms, respectively, of G. 8-Oxoadenosine has been shown to exist in the keto form with the base in the syn conformation (Cho & Evans, 1991), and recent NMR results suggest that, when incorporated in a DNA duplex, 8-oxoadenine exists in the keto form, although in this case the base appears to adopt an anti conformation in the duplex (Guschlbauer et al., 1991). A similar hydrogen-bonding scheme was recently proposed by Young et al. (1991) for the interaction of $N^6$-methyl-8-oxoadenine with G·C base pairs in duplex DNA.

The proposed hydrogen-bonding scheme is supported by the melting behavior of triplexes formed by strand (IA or C) or strand (IIA or C) with the target duplex III·IV(G·C) at pH 8.0. In the triplexes formed by I(C) or II(C), the cytosines are expected to be protonated and to form two hydrogen bonds with G of the duplex (Rajagopol & Feigon, 1989). Thus, raising the pH should disfavor protonation of cytosine and decrease the stability of the triplex. Because 8-oxoadenine contains two hydrogen-bond donors at positions N-7 and N-6 and the p$K$ of the hydrogen at position 7 is approximately 8.7 (Cho & Evans, 1991), its ability to participate in triplex formation should not be affected by an increase in pH, at least in the physiological pH range.

The I·III·IV(A·G·C) triplex is more stable at pH 8.0 than is the I·III·IV(C·G·C) triplex. Thus, at pH 8.0 the third strand of I·III·IV(A·G·C) melted at 22 °C and the first transition accounted for 32% of the total hypochromicity, whereas the third strand of I·III·IV(C·G·C) melted at 20 °C and the hy-

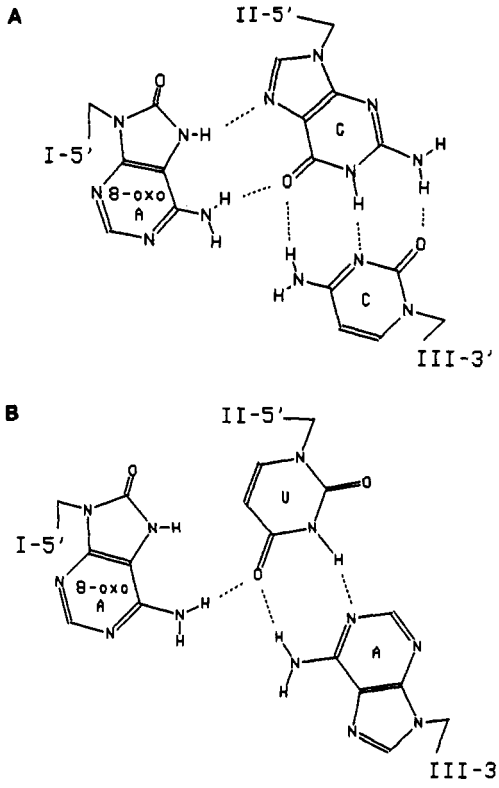


FIGURE 5: (A) Possible hydrogen-bonding scheme for the 8-oxo-A·G·C triad. (B) Possible hydrogen-bonding scheme for the 8-oxo-A·U·A triad.

pochromicity of the transition was only 18% of the total. This behavior is consistent with decreased protonation of both the cytosine and the two 5-methylcytosine bases in I(C), which would be expected to decrease the $T_m$ of the triplex. A smaller reduction in $T_m$ would be expected for the triplex formed by I(A), which contains only two 5-methylcytosines.

This effect is even more striking for the triplexes formed by II(A) or II(C). As shown in Figure 3A, the melting curves for the 8-oxoadenine-containing triplex are identical at pH 7.0 and 8.0. In contrast, as shown in Figure 3B, the first transition in the melting curve for the cytosine-containing triplex formed by II(C) decreases from 28 to 17 °C when the pH is raised from 7.0 to 8.0.

Recent studies have examined the effect of base triad "mismatches" on triplex formation (Plum et al., 1990; Mergny et al., 1991; Roberts & Crothers, 1991). We have studied the ability of 8-oxoadenine to recognize or accommodate other base pairs in the I·III·IV(A·X·Y) system. Examination of molecular models suggests that 8-oxoadenine in the syn conformation can fit at the site opposite the C·G base pair in the triplex I·III·IV(A·C·G). No hydrogen-bonding interactions between 8-oxoadenine and C·G would be expected in this situation. As summarized in Table I, the melting curve of a 1:1 mixture of I(A) and III·IV(C·G) does indeed show two transitions, although the hypochromicity of the first transition is only 6% of the total hypochromicity and the melting temperature of the third strand is approximately 9 °C. A higher melting temperature, 13 °C, and increased hypochromicity, 11% of the total, was observed for the third strand of triplex I·III·IV(A·U·A), as is shown in Figure 6. This increase could be due to the formation of a single hydrogen bond between the N-6 exocyclic amino group of 8-oxoadenine and the O-4 of the uracil of the U·A base pair, as shown in Figure 5B. Although a single hydrogen bond could also be formed by 8-oxoadenine and the O-4 of thymine, no triplex

Table I: Melting Temperatures of Oligodeoxyribonucleotide Complexes

| oligomer | | | $T_m$[a] | |
|---|---|---|---|---|
| I (X) | III (Y) | IV (Z) | *b* | *c* |
| 8-oxo-A | G | C | 29 | 45 |
| | | | 22[d] | 45[d] |
| 8-oxo-A | C | G | 9 | 41 |
| 8-oxo-A | U | A | 13 | 42 |
| 8-oxo-A | T | A | – | 44 |
| 8-oxo-A | A | T | – | 44 |
| C | G | C | 32 | 45 |
| | | | 20[d] | 45[d] |

[a] The melting experiments were carried out at an oligomer concentration of 0.5 μM per strand in standard buffer containing 0.1 M NaCl, 20 mM $MgCl_2$, and 50 mM Tris·HCl, pH 7.0. [b] Transition: triplex → duplex + I (– indicates the transition was not observed). [c] Transition: duplex → single strands. [d] The melting experiments were carried out at pH 8.0.

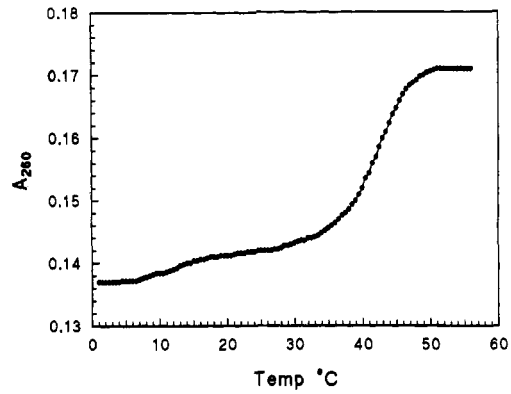


FIGURE 6: Absorbance vs temperature profile of a solution containing 0.5 μM I(A) and 0.5 μM III·IV(U·A) at pH 7.0.

formation was observed for a 1:1 mixture of I(A) and III·IV-(T·A). In this case it appears that triplex formation is prevented by the interaction of the 5-methyl group of the thymine with the five-membered ring of the 8-oxoadenine. Molecular models also suggest that syn 8-oxoadenine could be accommodated opposite an A·T base pair in the triplex; however, under the conditions of our experiments, no triplex formation was observed for a 1:1 mixture of I(A) and III·IV-(A·T).

The results of our studies demonstrate that oligopyrimidines containing 8-oxoadenine can form stable triplexes with homopurine tracts of double-stranded DNA. Recent studies on oligopyrimidines containing a similar base, $N^6$-methyl-8-oxoadenine, suggest that such oligomers can interact with purine tracts of duplex DNA and can prevent in vitro transcription (Young et al., 1991). It appears that 8-oxoadenine interacts in a specific manner with G·C base pairs of the duplex. Because this interaction does not require base protonation, 8-oxoadenine might be a useful alternative to cytosine or 5-methylcytosine for use in triplex formation at physiological pH.

## ACKNOWLEDGMENT

We thank Dr. David Shortle for the use of the CD spectropolarimeter and Mr. Rick Russell for carrying out preliminary studies on the synthesis of oligomer II(A).

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