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PURINE 8-SUBSTITUTION MODULATES THE RECOGNITION BY RESTRICTION ENDODEOXYRIBONUCLEASE *EcoRI* OF OCTADEOXYRIBONUCLEOTIDES (dGGAATTCC)

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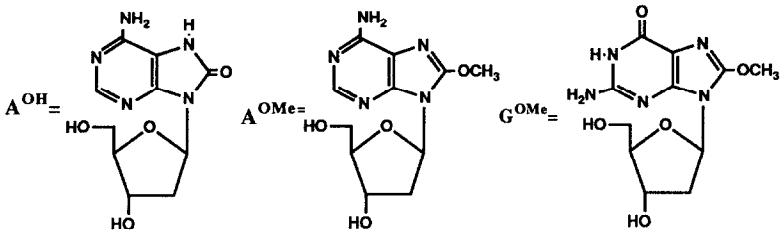
Abstract: Octadeoxyribonucleotides with the sequences d(GGA*ATTCC), d(GGAA*TTCC), and d(GG*AATTCC) containing modified base moieties which have an isosterically altered recognition sequence of the restriction endodeoxyribonuclease EcoRI. The oligomers, with replacement to deoxy-7,8-dihyroadenosine-8-one (dA^{OH}), 8-methoxy-deoxyadenosine (dA^{OMe}) and 8-methoxydeoxyguanosine (dG^{OMe}) from deoxyadenosine or deoxyguanosine were used to study their cleavage by the restriction endodeoxyribo-nuclease EcoRI. The hydrolysis by EcoRI of the modified oligomers were perfectly resisted compared to d(GGTTAACC). These results suggest that changes in the base sugar torsion angles of oligomer may modulate recognition by EcoRI.

Restriction endonucleases catalyze the cleavage of specific sequences in double stranded DNA. Due to their high specificity for DNA sequences and simplicity of the DNA site recognized, they serve as convenient model systems for the investigation of specific protein-DNA interactions.[1] The incorporation modified sugar and base moieties into oligodeoxyribonucleotides have been used to study the interaction of the restriction endonucleases with their substrates.[2-23] We have been interested in a comparison of the structural requirements needed for the recognition of the substrate by enzymes. Such a comparison proves an excellent opportunity to learn something about the fundamental principles governing specific protein/nucleic acid interactions. In order to study the interaction of the restriction endodeoxyribonuclease EcoRI with the nucleotide analogues in the EcoRI recognition sites we have synthesized octadeoxyribonucleotides of $d(GGA^{OH}ATTCC)$ **(1)**, $d(GGAA^{OH}TTCC)$ **(2)**, $d(GGA^{OMe}ATTCC)$ **(3)**, $d(GGAA^{OMe}TTCC)$ **(4)**, $d(GG^{OMe}AATTCC)$ **(5)** by the H-phosphonate approach[24] which contain the EcoRI binding site. Deoxy-7,8-dihydroadenosine-8-one was the major product liberated directly from the DNA chain during irradiation, as it is interesting for the interaction with enzymes.[25] On the other hand, the methoxy group has been shown to occur in the natural purine nucleoside spongosine[26], the isomer 8-methoxyadenosine and guanosine were prepared.[27,28] We have demonstrated that these adenosine and guanosine modified DNA duplexes were highly resistant to hydrolysis by the

dGGA^{OH}ATTCC =1; dGGAA^{OH}TTCC=2; dGGA^{OMe}ATTCC=3; dGGAA^{OMe}TTCC=4; dGG^{OMe}AATTCC=5; dGGAATTCC=6



restriction endodeoxyribonuclease EcoRI. The stabilities of these complementary octadeoxyribonucleotides was investigated by measuring temperature-absorbance profiles and circular dichroism (CD) spectra, since these duplexes had a right handed B-DNA like structure.

Four of these modified self complementary fragments, d(GGA^{OH}ATTCC) (**1**), d(GGAA^{OH}TTCC) (**2**), d(GGA^{OMe}ATTCC) (**3**), and d(GGAA^{OMe}TTCC) (**4**), result in dA^{OH} or dA^{OMe}-dT base pairs in the double stranded form nucleic acid, while the fragment, d(GG^{OMe}AATTCC) (**5**), produces dG^{OMe}-dC base pairs in the DNA duplexes. The thermal denaturation experiments indicated that these derivatives exhibited thermally induced helix coil transition (Table 1).[29] The modified sequences has major influence on the oligomer duplexes. In order to establish that the modified

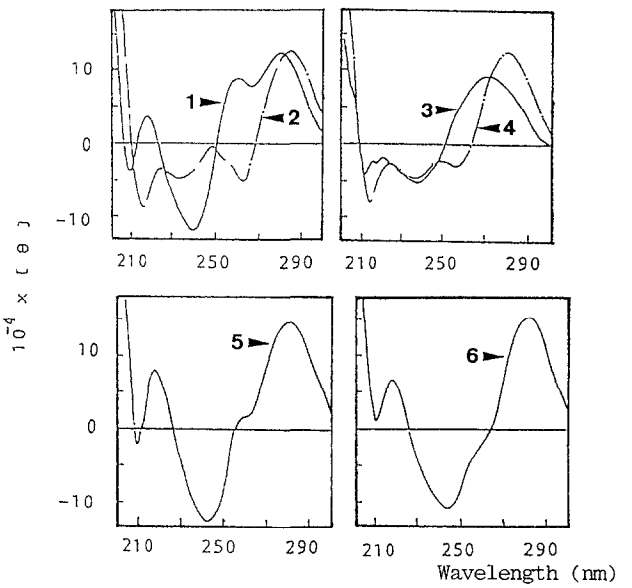


Fig. 1. CD spectra of the oligodeoxyribonucleotides **1-6** in 10 mM TRIS/HCl (pH 7.6) buffer containing 80 mM NaCl and 20 mM $MgCl_2$ at 18°C.

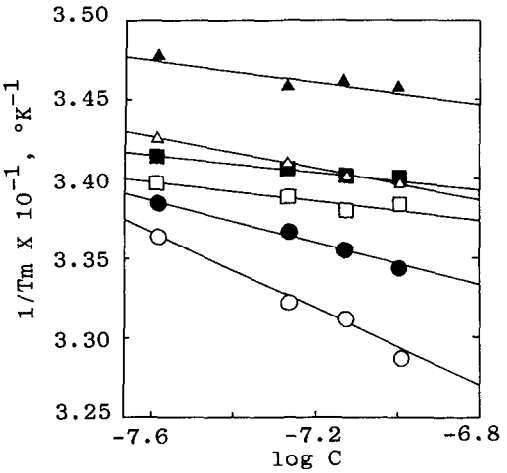


Fig. 2. Plot of 1/Tm vs. log C for the oligomers **1** ( ), **2** ( ), **3** ( ), **4** ( ), **5** ( ) and **6** ( ) in H_2O. All solutions contained 80 mM NaCl, 20 mM Mg_2Cl, and 10 mM TRIS/HCl, pH 7.6.

oligonucleotides adopted a B type helix, their circular dichroism (CD) spectra[30] were recorded in low salt concentration at 18°C (Fig. 1).[31] These includes the sequences, d(GGAA^{OH}TTCC) (**2**), d(GGA^{OMe}ATTCC) (**3**), and d(GGAA^{OMe}TTCC) (**4**) exhibited a thermally induced helix to coil transition under the assay conditions. This suggests that the Tm values for these fragments are near 18°C.

Thermodynamic parameters for the transitions were determined over the concentration range 2-25 µM single strand concentration (C_T) from plots of 1/Tm vs. log C_T (Fig. 2). This graph allows the calculation of the enthalpy (ΔH) from slope and the entropy (ΔS) from the intercept for each particular oligomer.[32-34] The energetic contributions to ΔH from duplex formation comes both from hydrogen bonding and from stacking interactions with adjacent base pairs. Since the difference in Tm values between two different oligomers is proportional to the

Table 1 Enthalpy and entropy change values for double helix formation of the octamers **1-6.**

Compd.	Tm(°C)[a)]	ΔH(KJ/mol)	ΔS(KJ/mol.K)	ΔΔG[b)](KJ/mol)
6	27.1	-154	-0.37	-
1	22.1	-597	-1.89	7.5
2	20.0	-479	-1.49	9.2
3	19.4	-327	-0.98	6.9
4	16.3	-778	-2.56	27.5
5	23.8	-275	-0.79	2.0

a) oligomer concentration was 5 µM; b) the values were calculated at 25 °C.

free energy difference ΔΔG between the two duplexes, the ΔΔG values can be calculated according to equation: $\Delta\Delta G = 2.3\ RT\ \Delta\log(c)$.[35] The principal here is that RT times the difference in the logarithm of total concentration results in the same Tm for the two different oligomers. The free enthalpy change (ΔΔG) between the oligomer **6** and the octamers **1**-5 reflects the stability of these different duplexes in the environment of the aqueous buffer solution and is directly related to the Tm values. Due to slopes of the curves of Fig. 2 the calculated values were all taken at a temperature of 25°C. The entalpy change for the transition of the duplex to the single-stranded form most pronounced for the oligomer **6**. The oligomers **1**-5 containing 8-substituted adenosine or guanosine have a lower ΔH value compared to that of parent oligomer **6**. According to Table 1 some of the enthalpy and entropy value do not correspond to the Tm values. As can be seen, the oligomer **1** containing dA^{OH} in position 3 (from the 5'-end) has a lower ΔH and ΔS value compared to that containing dA^{OH} in position 4, but exhibits a higher Tm value. This suggests that the free enthalpy change values term plays an important part in the process of duplex formation of oligomers **1**,2 containing dA^{OH}. The switch of structure of protonation at 7-nitrogen of adenine base would likely result in incorporation of the 7,8-dihyroadenine-8-one base across from thymine in a DNA helix.

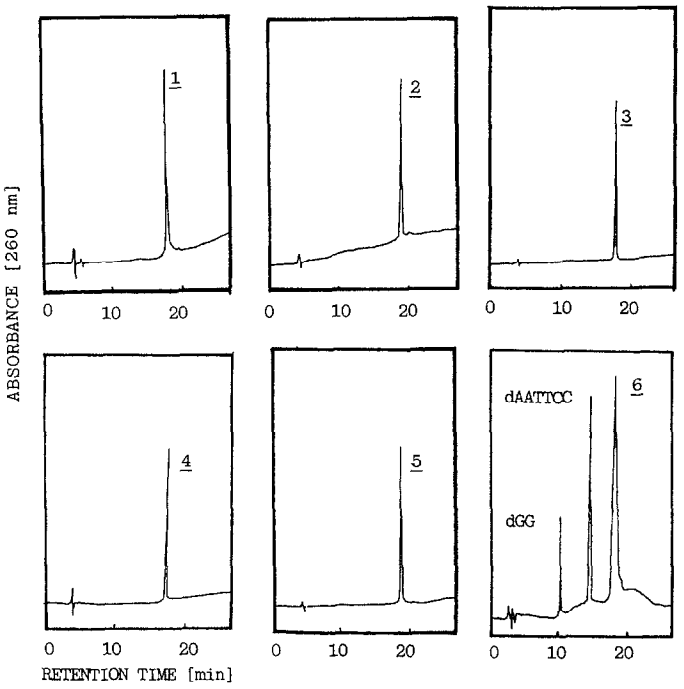


Fig. 3. Reverse phase HPLC elution profile of the oligomers (**1**-**6**) after digestion with the endodeoxyribonuclease EcoRI for 36h.

On the other hand, the enthalpy values for the octamers **3**-5 containing dA^{OMe} and dG^{OMe} corresponds to the melting temperature. From the ΔH values of Table 1, it can be seen that the oligomer **4** which contains the steric and hydrophobic adenosine in position 4 (from the 5'-end) exhibits the lowest ΔH value. The oligomers 3 and 5 which contains 8-methoxydeoxyadenosine or 8-methoxydeoxyguanosine in position 2 or 3 from the 5'-end show higher values. These results show that the oligonucleotides containing 8-substituted nucleosides (dA^{OH}, dA^{OMe}, dG^{OMe}) in the recognition sequence were more sensitive to helix stability. Further, the present of the 8-substituted nucleosides in the center of the recognition sequences clearly results in a less stable structure than observed with oligonucleotides containing 8-substituted nucleosides in the 5'-side of the sequence.

The cleavage by restriction endodeoxyribonuclease EcoRI of the duplexes containing the natural or modified nucleosides were determined by HPLC technique (Fig. 3).[36] As expected, octanucleotides (**1**-5) containing dA^{OH}, dA^{OMe}, and dG^{OMe} in place of dA or dG at the cleavage site within the recognition sequence (dGAATTC) were perfectly resistant to hydrolysis by the octamer. A very important characteristic of those oligomers is their thermal stability, especially as EcoRI requires a double-strand structure as substrate. In addition to its value for the study of nucleic acid structure and protein-nucleic acid interactions, 8-substituted adenosine and guanosine have also generated the attention of some investigators for its mutagenic properties.[26,26]

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