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SYNTHESIS OF IRON-BINDING OLIGONUCLEOTIDES AND THEIR REACTIONS WITH SINGLE-STRANDED DNA

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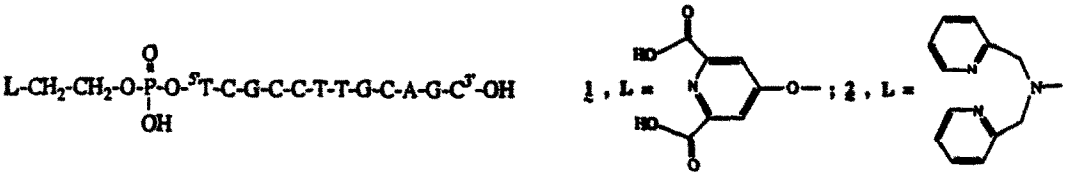
Abstract. Two 12-base oligonucleotides were synthesized and modified by attaching metal-binding ligands (2,6-pyridinedicarboxylate and 2,2'-dipicolylamine) to their 5'-ends . When hybridized to a 135-base single stranded DNA, in the presence of Fe^{2+}, O_2, and a reducing agent, these oligonucleotides cleaved the complementary DNA chain with unique and high base-sequence specificities.

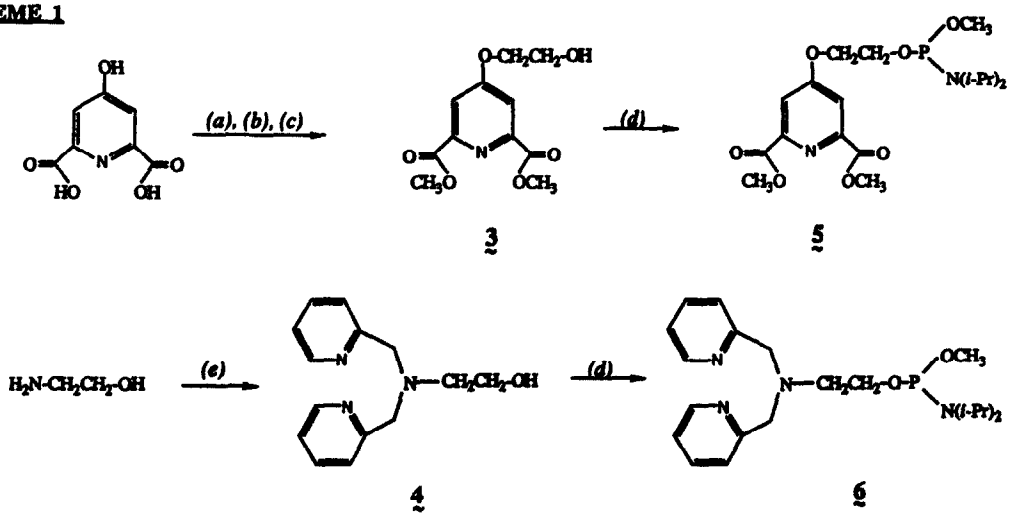
Sequence-specific cleavage of DNA by synthetic probes has been the subject of intensive studies for more than a decade.[1-4] Sequence-specificity in the cleavage of single stranded DNA can be achieved by attaching DNA cleaving groups (e.g., metal complexes) to oligonucleotide probes that have sequences complementary to that of the target DNA.[5-8] Although such modified oligonucleotide probes have shown high DNA binding specificities, they cleave DNA at several bases around the tethered metal complex due to intermediacy of diffusible free radicals. In an attempt to design probes that can cleave DNA via nondiffusible intermediates, and as a part of our investigation of the effect of structural variations of the metal complexes on the specificity of DNA cleavage, we report here the synthesis of two modified oligonucleotides (1 and 2) and the study of their activities toward DNA cleavage. The iron complex of 2 showed an unusually high DNA cleaving activity over a range of proximate bases while that of 1 showed a remarkably high site selectivity.



The synthesis of 1 was achieved via compound 3 which was prepared from dimethyl 4-hydroxy-2,6-pyridinedicarboxylate upon reaction with tetrahydropyranyl (THP) derivative of 2-bromoethanol, followed by deprotection (Scheme 1). Compound 2 was synthesized from 4 which was obtained by reductive amination of 2-pyridinecarboxaldehyde with 2-aminoethanol in the presence of sodium cyanoborohydride. The phosphoramidite derivatives (5 and 6) were obtained upon reacting 3 and 4 with methyl N,N-diisopropylchlorophosphoramidite, respectively. The analytical data for all compounds are consistent with the assigned structures.[9]

The oligonucleotide part of the probes was synthesized by the standard automated solid-phase phosphoramidite method.[10] The phosphoramidites 5 and 6 were coupled to the 5'-hydroxyl groups of the oligonucleotides during the thirteenth addition cycle in the DNA synthesizer. In the case of 1, the final hydrolytic deprotection step was carried out in aqueous NaOH instead of the standard NH_4OH treatment to avoid possible amminolysis of the ester groups. After HPLC purification and 3'-end labeling with ^{32}P, base sequences of the oligonucleotides were verified by Maxam-Gilbert chemical sequencing methods.[11]

SCHEME 1



(a) $MeOH/H_2SO_4$, reflux, 3 h, 95%; (b) t-BuO$^-K^+$/DMSO, $BrCH_2CH_2O$-THP, ambient temperature, 4 h, 75%; (c) MeOH/HCl, 3 h, 80%; (d) $ClP(OMe)(N\text{-}iPr_2)$, CH_2Cl_2, 0.5 h, 85%; (e) 2-Py-CHO, $NaBH_3CN$, 48 h, 45%.

Testing the modified oligonucleotides for their ability to cleave DNA was conducted by using a 135 base-pair (bp) BamHI/PvuI restriction fragment of pUC9 plasmid DNA as a template containing a sequence (A-77 to G-88) which is complementary to that of the probes. Figure 1 shows a high-resolution denaturing polyacrylamide gel analysis of cleavage fragments of the template. A typical cleavage reaction involved hybridization of the denatured template to the probe (500-fold molar excess of the probe relative to the template was used) at 25°C and pH 7.5. The reaction was then initiated by adding Fe^{2+} (10 μM) and dithiothreitol (DTT, 5 mM).

Probe 2 cleaved the complementary DNA template at a single stretch of bases covering eight contiguous nucleotides. The cleavage was centered around A-74 and T-75 of the template, three bases down from the 5'-end of the probe bearing the ligand. This pattern of cleavage and the observed inhibition by catalase, suggest that diffusible species could be responsible for the cleavage of DNA. However, the ability of this probe to cleave DNA at one stretch of bases rather than two could be attributed to the short ethylene bridge between the ligand and the 5'-end of the probe. Clearly, this is different from what was previously observed in the case of EDTA-oligonucleotide probes which, under similar conditions, cleaved the template DNA at two stretches of bases each extending over several nucleotides on both sides of the base carrying the tethered ligand.[12, 13]

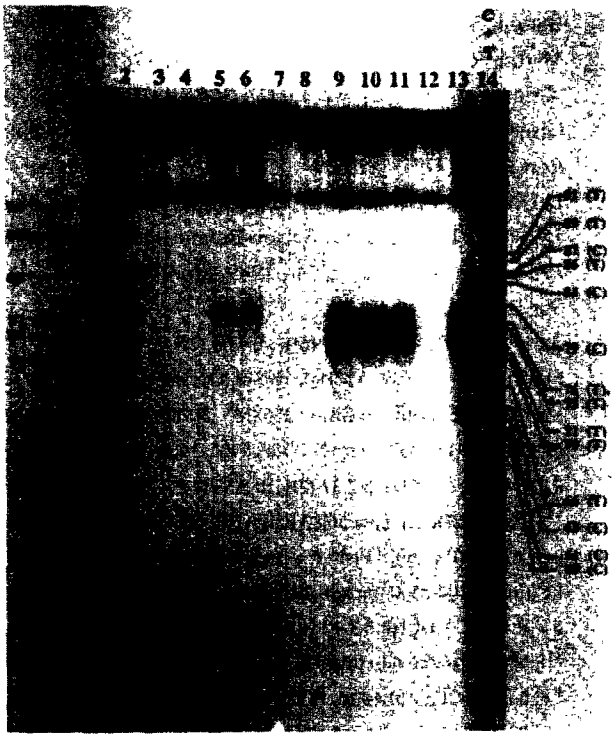


Figure 1: Autoradiogram of denaturing gel showing cleavage of 3'-end labeled 135-bp BamHI/PvuI restriction fragment of pUC9 plasmid DNA by 1 and 2. Lanes 1 and 14 are Maxam-Gilbert G and C+T ladders of the template, respectively. Lanes 2 and 13 represent the cleavage products by 1 and 2, respectively [~2 nM template, 1 μM probe, 10 μM Fe^{2+} and 5 mM DTT]. Lane 3 is a control showing the 3'-end labeled template strand after treatment with 1 μM of unbound ligand (4-hydroxy-2,6-pyridinedicarboxylate), 10 μM Fe^{2+} and 5 mM DTT. Lane 4, as in lane 2 and in the presence of 1 mM ethidium bromide. Lane 5, as in lane 2 and in the presence of catalase [60 μg/mL]. Lane 6, as in lane 2 and in the presence of 100 μM H_2O_2. Lane 7 shows the 5'-end labeled DNA fragment treated as in lane 2. Lane 8 is a control showing the 3'-end labeled fragment after treatment with 1 μM of an unmodified probe, 10 μM Fe^{2+} and 5 mM DTT. Lane 9, as in lane 13 and in the presence of 100 μM H_2O_2. Lane 10, as in lane 13 but using 2 μM of 2. Lane 11, as in lane 13 and in the presence of 1 mM ethidium bromide. Lane 12, as in lane 13 and in the presence of catalase [60 μg/mL].

The unprecedented high cleavage specificity observed in the case of 1, where template cleavage occurred mainly at two bases (A-77, G-78), could be due to intercalation of the planar aromatic portion of the 2,6-pyridinedicarboxylate(Fe^{II}) complex between the last two base-pairs of the template-probe double strand.[14] This may explain why a strong intercalating agent, such as ethidium bromide, totally inhibited the cleavage (Figure 1, lane 4), and indicates that ligand intercalation could be a prerequisite for DNA cleavage. The fact that neither catalase nor H_2O_2, by contrast, has any significant effect on the activity of 1 suggests that the mechanism of DNA cleavage does not involve typical Fenton chemistry. Currently, we are investigating the mechanism of such cleavage and the nature of the active intermediates involved.

Acknowledgement

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9. Spectral data:

3, ^{1}H NMR ($CDCl_3$): δ 3.99 (6H, s), 4.05 (2H, t), 4.25 (2H, t), 7.82 (2H, s); ^{13}C NMR ($CDCl_3$): ppm 53.50 (OCH_3), 61.80 (CH_2OH), 70.60 (OCH_2), 114.40 (C_3- and C_5- py), 149.70 (C_4- py), 165.00 (C_2- and C_6- py), 167.50 (-COO); MS (EI): m/z (relative intensity) 255 (M^+, 2), 223 (11), 196 (100), 164 (43), 152 (35), 120 (38); IR (Nujol): 3175-3500 (broad), 1750, 1700, 1600, 1460, 1350, 1200, 1100, 1050 cm^{-1}.

4, ^{1}H NMR ($CDCl_3$): δ 2.37 (2H, t), 3.58 (2H, t), 3.79 (4H, s), 5.10 (1H, broad), 7.05 (2H, m), 7.25 (2H, m), 8.45 (2H, m); ^{13}C NMR ($CDCl_3$): ppm 56.80 (CH_2OH), 59.70 (CH_2N), 60.20 (CH_2N), 122.00 (C_5- py), 123.00 (C_3- py), 136.50 (C_4- py), 148.90 (C_6- py), 159.40 (C_2- py); MS (EI): m/z (relative intensity) 243 (M^+, 2), 212 (17), 378 (100), 119 (27), 93 (98); IR (film): 3100-3500 (broad), 2950, 2800, 1750, 1675, 1600, 1550, 1450, 1420, 1375, 1150, 1075, 1000 cm^{-1}.

5, ^{1}H NMR ($CDCl_3$): δ 1.12 (12H, d), 3.36 (3H, d), 3.54 (2H, m), 3.93 (6H, s), 4.15 (2H, m), 4.35 (2H, m), 7.82 (2H, s); ^{31}P NMR (d_6-acetone): ppm 151.62; ^{13}C NMR (d_6-acetone): ppm 24.08 (d, *i*-pr CH_3), 42.70 (d, NCH), 49.96 (d, $POCH_3$), 52.10 (ester CH_3), 61.64 (d, $POCH_2$), 69.31 (d, CH_2), 114.40 (C_3- and C_5- py), 150.02 (C_4- py), 164.89 (C_2- and C_6- py), 166.87 (COO); MS (EI): m/z (relative intensity) 416 (M^+, 13), 315 (53), 205 (33), 161 (100); IR (film): 3000, 1750, 1730, 1600, 1430, 1400, 1350, 1250, 1175, 1100, 1030 cm^{-1}.

6, ^{1}H NMR (d_6-acetone): δ 1.15 (12 H, d), 2.80 (2H, m), 3.35 (3H, d), 3.58 (2H, m), 3.76 (2H, m), 3.90 (4H, s), 7.20 (2H, m), 7.60 (2H, m), 7.70 (2H, m), 8.48 (2H, m); ^{31}P NMR (d_6-acetone): ppm 149.36; ^{13}C NMR (d_6-acetone): ppm 24.16 (d, *i*-pr CH_3), 42.60 (d, *i*-pr CH), 50.30 (d, $POCH_3$), 54.95 (d, NCH_2), 60.78 (s, CH_2), 61.75 (d, $POCH_2$), 121.85 (s, C_5-py), 121.90 (s, C_3-py), 122.65 (s, C_4-py), 136.11 (s, C_6-py), 148.83 (s, C_2-py); IR (film): 2950, 1750, 1600, 1550, 1490, 1450, 1400, 1250, 1175, 1050, 975 cm^{-1}.

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