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Synthesis and Characterization of Oligodeoxynucleotides Containing *N*-(deoxyguanosin-8-yl)-1-aminopyrene

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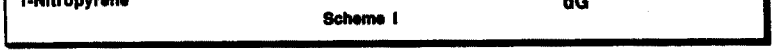
Abstract: Oligodeoxynucleotides containing *N*-(deoxyguanosin-8-yl)-1-aminopyrene, the major DNA adduct formed by 1-nitropyrene *in vivo*, were synthesized in 10-20% yield by reacting *N*-hydroxy-1-aminopyrene with unmodified oligodeoxynucleotides in aqueous DMF. A remarkable feature of this straightforward synthesis is that the covalent adduction was primarily dependent on the existence of double stranded form of the oligonucleotides.

1-Nitropyrene (NP), the predominant nitropolycyclic hydrocarbon found in diesel exhaust[1,2], is an established mutagen and tumorigen.[3-5] Nitroreduction has been demonstrated to be associated with some of the biological effects of NP and the major DNA adduct formed by reductively activated NP both *in vivo* and *in vitro* is *N*-(deoxyguanosin-8-yl)-1-aminopyrene (dG^{AP}) (Scheme I).[4] However, an unambiguous relationship between this DNA adduct and cellular transformation is yet to be established. To elucidate the mechanism of mutagenesis and carcinogenesis, oligodeoxynucleotides containing defined carcinogen-DNA adducts are needed for both physico-chemical and biological studies.[6] Synthesis of oligonucleotides containing dG^{AP}, however, has not been reported. In this communication, we report an easy approach to synthesize such modified oligonucleotides.



Scheme I

Initially, we attempted to synthesize *N*-acetoxy-*N*-(trifluroacetyl)-1-aminopyrene and several related compounds because similar activated derivatives have been used successfully for the synthesis of deoxyguanosine C8-adducts of several aromatic amines.[6-11] Unfortunately, in our hands, all these compounds were too unstable to be used in the chemical adduction step. As a result, we concentrated our efforts to investigate the reaction of *N*-hydroxy-1-aminopyrene, one of the presumed metabolites of NP, with deoxyguanosine and DNA. After screening a variety of solvent systems, we selected DMF-H_2O (1: 9), pH 5.0 - 5.5, which provided the best yield in several reactions, as the solvent system for all subsequent reactions. The reaction with deoxyguanosine was nevertheless unsatisfactory [12] (table 1), although at least an order of magnitude higher yields in the reaction with calf thymus DNA could be achieved. This suggested that the secondary structure of DNA may play an important role in the reaction and, therefore, we used a number of self-complementary oligonucleotides for the adduction reaction.[13] As shown in table 1, there was only a ~2.5% yield in the reaction with d[ATGCAT] whereas 15-20% yield could be achieved with the self-complementary decamer d[AAATGCATTT] containing the same internal sequence as in the hexamer. For the decamer, however, if the

solvent system was changed to a non-aqueous medium, no reaction took place. We presumed that the lack of reaction in the latter case as well as with the hexamer was due to a predominantly single stranded nature of these oligonucleotides under the reaction conditions. To ascertain whether a double stranded region of DNA constitutes a primary requirement, we used another hexamer d[CGCGCG] which remains in duplex form at room temperature but the second G possesses more double stranded character because of fraying at the ends of the duplex oligomer. Indeed, the major product isolated from this reaction involved modification of the second G (charactrization of modified oligomers - vide infra). This product was isolated in 6.5% yield compared to a total yield of ~2% for all the other modified hexamers. To rule out the possibility that hairpin structures are preferred for adduction, we also used a non-self-complementary 13-mer with or without the complementary DNA strand. As expected, significant adduction only took place in the presence of the complementary strand. These experiments suggest that in the reaction of *N*-hydroxy-1-aminopyrene with DNA, intercalation is a necessary prerequisite for covalent bond formation. Since this derivative is believed to be one of the metabolites of NP, this data appears to be relevant to the reaction in cellular systems as well.

Table 1

DNA Fragment	% Yield of Adducted Material*
dG	0.5
d[ATGCAT]	2.5
d[AAATGCATTT]	16
d[CGCGCG]	Major 6.5[†] Minor <2
d[CCCATCGCTACCC]	1.7
d[CCCATCGCTACCC] WITH COMPLEMENTARY 13-MER	9.2

* Yields provided here are an average of 3-5 determinations.

† This product was identified as d[CGCG^{AP}CG]

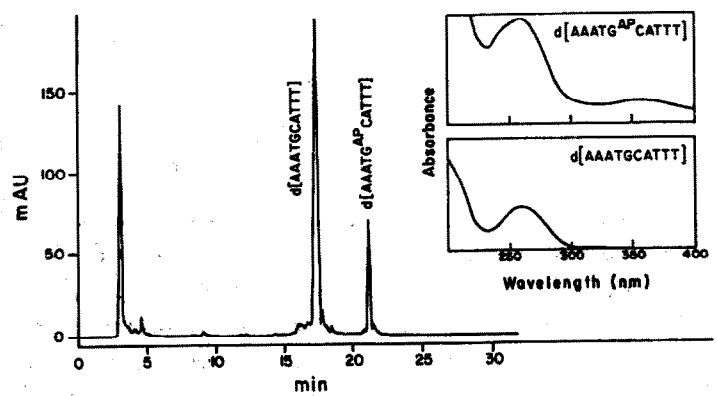


Figure 1. A typical HPLC profile of the reaction of the 10-mer with *N*-hydroxy-1-aminopyrene. The absorption spectra of the unmodified and modified 10-mers are shown in the inset.

Characterization of the modified oligonucleotides was carried out by enzymatic digestion followed by HPLC analysis of component nucleosides. As shown in figure 2, the digested modified decamer displayed the dC, dT, and dA peaks as in the unmodified decamer. However, a conspicuous absence of the dG peak and the presence of a late eluting substance that coeluted with an authentic sample of dG^{AP} was noted.[14] In the case of oligomers containing multiple guanines, the method of digestion to nucleosides cannot provide evidence of the site of adduction. We, therefore, investigated the possibility of inducing a strand cleavage at the adducted guanine as carried out by piperidine treatment on oligonucleotides containing the C8 deoxyguanosine adduct of aminofluorene.[10,15] Since such a cleavage is likely to occur at the 5' C-O bond of the deoxyribose unit

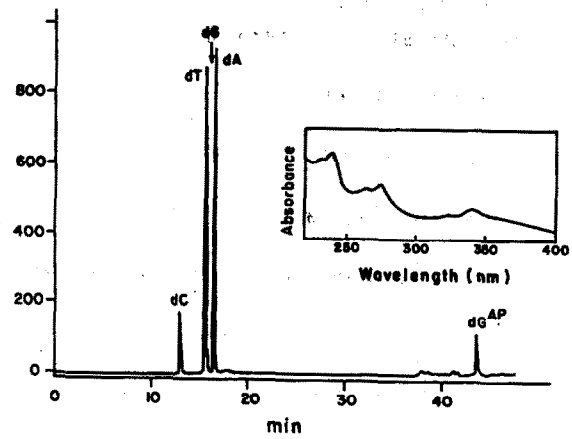


Figure 2. HPLC profile of enzymatically digested d[AAATG^{AP}CATTT]. Absorption spectrum of dG^{AP} is shown in the inset.

of the adducted G, as was demonstrated in an alkali induced cleavage of abasic sites[16] (which is likely to be an intermediate in this process[17]), we needed such fragments as standards. These oligonucleotide 3' phosphates were generated as follows: the decamer and hexamer that contained deoxyuridine in the place of dG^{AP} were synthesized and treated with the enzyme uracil DNA glycosylase to produce abasic sites by excision of the uracil residues;[16] the abasic site containing oligomers were purified, ^{32}P radiolabeled, and subsequently cleaved by piperidine treatment. Comigration of these standards by gel electrophoresis provided evidence that the 5'-^{32}P-labeled decamer generated 5'-end radiolabeled d[AAAT] -3'-phosphate as expected, whereas the major modified hexamer isolated from the reaction with d[CGCGCG] provided d[CGC] -3'-phosphate, as the labeled fragment.

The absorption spectrum of the modified decamer at ~355 nm exhibited ~15 nm red shift with respect to the corresponding absorption maximum of dG^{AP} suggesting that there are considerable interactions of the pyrenyl residue and the DNA bases (insets in Figure 1 & 2). Thermal melting experiments indicated that the presence of two dG^{AP} adducts in the middle of both the hexamer and the decamer have induced significant perturbation in the double stranded character of the oligonucleotides. As shown in Figure 3, no cooperative melting was observed for the modified 10-mer, although the extent of hyperchromicity suggested that at least a portion of this decamer was in duplex form.

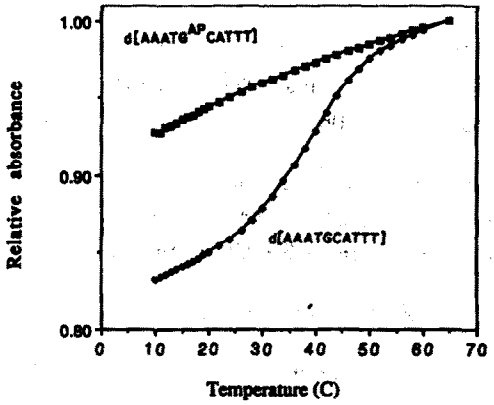


Figure 3. Absorption vs.temperature plots of unmodified and modified 10-mers.

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12. An enhancement of deoxyguanosine concentration increased the yield of dG^{AP}. However, the best yield we could obtain by optimizing these conditions was ~2.5%.

13. Conditions: 20 - 30 nmol of the oligonucleotide was stirred at room temperature with 12 µmol of 1-nitrosopyrene and 3 µmol ascorbic acid in 100 µL DMF - Na acetate - acetic acid buffer (pH 5.0 - 5.5) (1: 9) under nitrogen and protected from light for 14 - 16 hr. Under these conditions 1-nitrosopyrene is reduced by ascorbic acid to *N*-hydroxy-1-aminopyrene.[4]

14. A sample of dG^{AP} was prepared by treating calf thymus DNA with *N*-hydroxy-1-aminopyrene followed by enzymatic digestion of the adducted DNA. dG^{AP} was isolated by butanol extraction and purified by reverse phase HPLC. ^{1}H-NMR, FAB-MS, and absorption spectrum of the purified compounds were in accord with published spectral data of dG^{AP} (ref. 4).

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