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Oligonucleotide model with non-identical complementary strands for chromatographic studies of structure-dependent photosusceptibility☆

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Abstract

In a previous work, we used a quantitative chromatographic analysis of two self-complementary oligonucleotides to correlate the conformational differences between the oligonucleotide duplexes and photochemical susceptibilities of constituent oligomers. In this work we describe a new double-stranded oligonucleotide model with non-identical complementary strands. To separately analyze photoproducts in two strands, one of them is used in a partially protected form (the hydrophobic 5′-dimethoxytrityl group uncleaved). Using a reversed-phase column, the oligomers and products of their UV photomodification are separated into two groups of peaks. This facilitates the quantitation of photoproducts in each of the complementary strands. Three 15-mer oligonucleotides, 5′-TTTTTAT-TAAATATA-3′ (F5), 5′-AAAAATAATTTATAT-3′ (F6) and 5′-TATATTTAATAAAAA-3′ (F7) form the parallel-stranded (*ps*) F5 · F6 and the ordinary antiparallel-stranded (*aps*) F5 · F7 duplexes. For these particular sequences, the rate of cyclobutane thymine dimer formation in the *ps* DNA has been estimated as ca. 1.5–2 times that in the ordinary *aps* DNA.

1. Introduction

We are developing a chromatographic approach to conformational studies of nucleic acids [1,2]. The positions and/or mobilities of adjacent pyrimidines favorable for the photodimerization depend on the structural details and rigidity of nucleic acid conformations as shown for the double-stranded A, B, B′ forms and the triple-stranded helix [1–5]. Self-complementary decanucleotides were previously used for the reversed-phase chromatographic analysis of the B and B′ form double-stranded helices, and a decreased photoproduct yield in the structurally rigid B′ conformation was shown [1,2]. These decamers and the products of their modification were chromatographically resolved enough to provide their quantitative analysis. However, such self-complementary models are not always available. Another oligonucleotide model could consist of the complementary strands with different sequences. In this approach, it is desirable to separately analyze the photoproducts in both

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☆ Part IV in the series High-performance liquid chromatography of the photoproducts of nucleic acid components. For Part III, see Ref. [1].

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5'-DMTr-TTTTTATTAAATATA-3' (F5)  ps duplex
     5'-AAAAATAATTTATAT-3' (F6)

5'-DMTr-TTTTTATTAAATATA-3' (F5)  aps duplex
     3'-AAAAATAATTTATAT-5' (F7)
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strands. In a reversed-phase chromatography this could be achieved by deliberately increasing the hydrophobicity of one strand.

As a new model we chose to study the three 15-mer oligonucleotides, 5′-TTTTTATTAAATATA-3′ (F5), 5′-AAAAATAATTTATAT-3′ (F6) and 5′-TATATTTAATAAAAA-3′ (F7) which form the ordinary antiparallel-stranded (*aps*) F5·F7 and parallel-stranded (*ps*) F5·F6 duplexes [6,7]. They were used to compare the photoproduct yields under UV irradiation. To separately analyze the photoproducts in both duplex strands, one of them was rendered hydrophobic by leaving the 5′-dimethoxytrityl protecting group uncleaved. UV irradiation of oligomers combined into either *aps* or *ps* duplexes resulted in the hydrophobicity changes which were low in comparison with the initial differences in hydrophobicity between complementary (protected and deprotected) oligomers. This allowed the elution of two strands and products of their photomodification out of the reversed-phase column as well separated groups of peaks. Quantitation of the modified oligomers with these specific sequences allowed estimation of the thymine dimerization in the *ps* DNA as ca. 1.5–2 times greater than that in the ordinary *aps* DNA.

2. Experimental

2.1. Materials

Oligonucleotides DMT-d(TTTTTATTAAATATA) (F5), d(AAAAATAATTTATAT) (F6), d(TATATTTAATAAAAA) (F7), d(CCTTTAAAGG) and d(GCGCGCACGTGGGCCCTTGCAGCGCTCTAGACC) were synthesized using standard phosphoramidite chemistry. They were purified by electrophoresis and RP-HPLC. Snake venom phoshodiesterase I (PDE I) was from Pharmacia (Piscataway, NJ, USA). All other chemicals of analytical-reagent grade were from various commercial sources.

2.2. UV Irradiation

Oligonucleotides and their double-stranded complexes were UV irradiated at 300 nm using a Model 1-1430 Foto/Phoresis UV transilluminator (Fotodyne, New Berlin, WI, USA) equipped with four 15 W lamps. The oligonucleotide mixtures of F5·F6 and F5·F7 (1:1 molar ratio chromatographically determined after an exhaustive PDE I digestion) were prepared in 20 m*M* sodium cacodylate, 0.5 *M* NaCl (pH 7.2). The duplexes were annealed from 90°C by a slow cooling (90 min) to −7.5°C. The 1 mm length quartz cells containing oligonucleotide solutions (10 μM strand concentration) were placed just underneath the surface of the partially melted ice prepared with 2.1 *M* NaCl solution (freezing point of −7.5°C [8]) and irradiated from a 1 cm distance for various periods of times.

2.3. Chromatographic conditions

Chromatography was accomplished using a Millipore–Waters system (Milford, MA, USA) consisting of a 600E solvent-delivery unit, a 717 autosampler, and a 996 photodiode array detector, operated under a control of a Millenium 2010 Chromatographic Manager. The products of oligonucleotide photomodification were separated on a NovaPak C_{18} (150 × 3.9 mm) reversed-phase column immersed in a Shel-Lab water bath (Sheldon, Cornelius, OR, USA) using a 0–10% B (10 min), 10–20% B (20 min), 20–50% B (30 min) gradient of 0.1 *M* ammonium acetate, pH 6.8 (eluent A), and acetonitrile–water (1:1, eluent B) at a flow-rate of 1.2 ml/min and 45°C. The chromatographic peak areas were calculated using detector traces at 260 nm, the wavelength for which the extinction coefficients of nucleic acid components are well known.

2.4. Characterization of photoproducts

To localize photoproducts in the oligonucleotide sequences, peaks were collected in Eppendorf tubes; volatile salt was evaporated in a Speed-Vac; the residue was redissolved in a buffer; and then the collected peaks of modified oligomers were subjected to digestion with PDE I which sequentially cleaves 5′-nucleoside monophosphates from the oligomer 3′-end and stops at the photoproduct position. Resulting monucleotides were chromatographically quantitated using a reversed-phase column. Peak areas and molar absorption coefficients were used to calculate the 5′-AMP/5′-TMP ratios that allowed determination of the number of residues between the photoproduct and the 3′-end, that is the sequence position of the photoproducts. Peaks containing cyclobutane thymine dimers were identified using oligonucleotide irradiation at 300 nm in the presence of 5% acetone which promotes the formation of cyclobutane pyrimidine dimers [9,10].

3. Results and discussion

In order to test a new model of nucleic acid structure with the different complementary strands appropriate for chromatographic studies, we chose the partially characterized oligonucleotide system [6,7]. The *ps* F5 · F6 and *aps* F5 · F7 duplexes at 2 m*M* strand concentrations melted at 8°C and 27°C in 10 m*M* cacodylate, 100 m*M* NaCl, pH 7.2 [6]. Under our experimental conditions, the melting temperatures are expected to be several degrees higher due to the increases in strand and salt concentrations [11]. However, to be sure that we irradiated the double-stranded complexes, we used the conditions well below the melting points for F5 · F6 and F5 · F7. The primary photoproducts of UV irradiation under conditions employed are expected to be thymine dimers [9]. To avoid artifacts during the analysis of irradiated samples due to possible on-column association of complementary strands, chromatography was accomplished at the temperature above the melting points for both *ps* and *aps* duplexes.

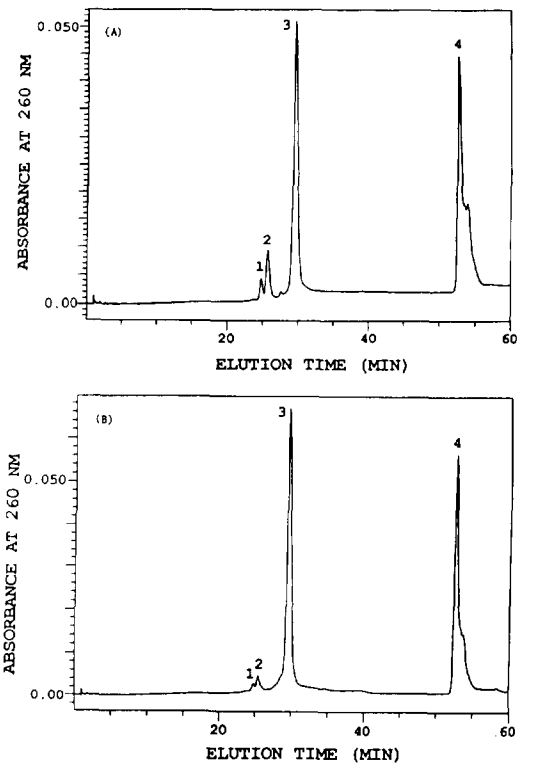

Fig. 1. Chromatograms of the oligonucleotide complexes UV irradiated for 5 min under the conditions in which the double-stranded structures exist (20 m*M* sodium cacodylate, 0.5 *M* NaCl, pH 7.2, −7°C). Column: NovaPak C_{18} (150 × 3.9 mm). Eluents: A, 0.1 *M* ammonium acetate (pH 6.8); B, 50% acetonitrile in water. Gradient: 0–10% B (10 min), 10–20% B (20 min), 20–50% B (30 min) at 45°C. Flow-rate, 1.2 ml/min. (A) *ps* duplex F5 · F6. Peaks: 1 = d(AAAAAT-AAT< >TTATAT); 2 = d(AAAAATAATT< >TAT-AT) and other photoproducts of F6; 3 = F6; 4 = DMT-F5 and its photoproducts. (B) *aps* duplex F5 · F7. Peaks: 1 = d(TATAT< >TTAATAAAAA); 2 = d(TATATT< > TAATAAAAA) and other photoproducts of F7; 3 = F7; 4 = DMT-F5 and its photoproducts.

Fig. 1A and B show the chromatograms of UV-irradiated oligonucleotide complexes F5 · F6 (*ps*) and F5 · F7 (*aps*), respectively. The group of peaks 1–3 in panel A corresponds to the oligomer F6 and its photomodification products which have lower retention times. Digestion of collected fractions with PDE I, which sequentially cleaves 5′-nucleoside monophosphates from the oligomer 3′-end and stops at the photoproduct

position, as well as the acetone-sensitized irradiation were used for peak identification. The acetone-sensitized irradiation producing mostly cyclobutane thymine dimers T< >T resulted in accumulation of modified oligomers in peaks 1 and 2. An HPLC quantitation of enzymatically cleaved mononucleotides for peak 1 (data not shown) gave the 5′-AMP/5′-TMP ratio which within a few percent experimental error was that expected for 5′-AAAAATAAT< >TTATAT-3′. Significantly higher peak 2 is a composite one. Its appearance under acetone-sensitized irradiation shows that it contains a modified oligomer 5′-AAAAATAATT< >TATAT-3′. Yet, the 5′-AMP/5′-TMP ratio derived from quantitation of the PDE I digestion products for peak 2 indicates the presence of other photoproducts. Presumably they result from an accumulation of non-cyclobutane photoproducts, the T[6-4]T photoadducts [9]. This suggestion is consistent with our earlier data according to which non-cyclobutane pyrimidine adducts are slightly more hydrophobic than cyclobutane dimers that results in later off-column elution of T[6-4]T-modified oligomers [1,2,12]. The magnitude of peak 2 is closer to that of peak 1 when a single-stranded oligomer is irradiated and these peaks are approximately equal under acetone-sensitized irradiation. The second important feature of this chromatogram is the poor-resolved group of peaks 4 corresponding to the protected oligomer DMT-F5 and the numerous products which result from its modification. In this case, the number of photoproducts is expected to be large since F5 contains several thymine dinucleotide units. Similarly, the panel B contains peaks 1 (5′-TATAT< >TTAATAAAAA-3′), 2 (5′-TATATT< >TAATAAAAA-3′ and some uncharacterized products), 3(F7) and 4 (F5 and its photoproducts). Note that in the case of DMT-F5, photomodified oligomers have longer retention times relative to the parent compound. The reason for this is not clear. However, it is not an artifact: the chromatogram of UV-irradiated partially deprotected F5 has two groups of photoproduct peaks, one in the front of deprotected oligomer and second at the tail of tritylated oligomer (Fig. 2).

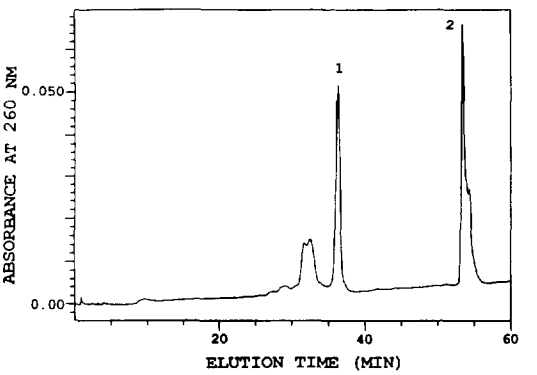

Fig. 2. Chromatogram of the partially protected oligonucleotide F5 UV irradiated for 5 min. Chromatographic conditions as in Fig. 1. Peaks: 1 = F5; 2 = DMT-F5 and its photoproducts.

Thus, it is clear that protected and deprotected oligomers and their photomodification products are eluted out of the reversed-phase column as the separate groups of peaks. This allows a separate analysis of photomodifications in each of the complementary strands. A purposeful optimization may result in the resolution of numerous peaks allowing their total quantitation. At this stage we limited ourselves to the quantitation of only early eluted photoproduct peaks. As mentioned above, these are the homogeneous peaks of primary modification products containing 5′-AAAAATAAT< > TTATAT-3′ and 5′-TATAT< >TTAATAAA-AA-3′. A comparison of the rates of cyclobutane dimer formation revealed that they are approximately equal for the adjacent thymine dinucleotides of the same oligomer (data not shown). Therefore, the peaks 1 in these chromatograms can be used as quantitative markers for the primary photodamages in the *ps* and *aps* duplex DNA. Fig. 3 shows that at low extents of modification (several percent of oligomers contain damaged sites, i.e., oligomers contain single dimers), the photosusceptibility of the *ps* DNA is higher than that of the *aps* DNA. The initial accumulation rate for the cyclobutane thymine dimers in the *ps* duplex F5·F6 is two times greater than that in the *aps* duplex F5·F7 and 1.5 times greater than in the *aps* duplex form of self-complementary oligonucleotide d(CCTTTA-AAGG) which was used as an additional double-

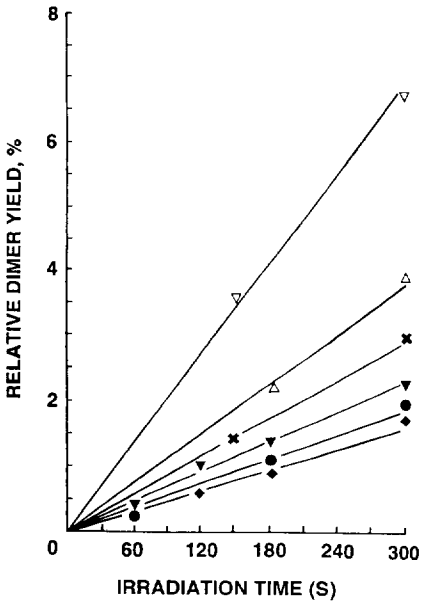

Fig. 3. Time courses of a single-position cyclobutane thymine dimer accumulation (calculated relative to the initial amounts of unmodified oligomers) in oligonucleotides in the different structural forms. Conditions: 20 m*M* sodium cacodylate, 0.5 *M* NaCl, pH 7.2, −7.5°C. ▽ = single-stranded d(GCGCG-CACGTGGGCCCT< >TGCAGCGCTCTAGACC); △ = AAAAATAAT< >TTATAT in *ps* F5·F6 duplex; × = CCT< >TTAAAGG in *aps* duplex; ● = TATATT< > TAATAAAAA in *aps* F5·F7 duplex; ▼ = AAAAATAAT < >TTATAT in F6 alone; ◆ = TATATT< >TAATAAAA in F7 alone.

stranded control. Under the same conditions the single strands F6 and F7, which we planned to use for the determination of the photosusceptibility of single-stranded nucleic acids, have lower degrees of modification than expected [9]. Examination of their sequences shows a possibility of their self-aggregation as they can form imperfect self duplexes with 10 base pairs (compare with 15 base pairs in perfect duplexes). The photomodification properties of these aggregated single strands were similar to those of the duplex conformations. In another single-stranded con trol, d(GCGCGCACGTGGGCCCTTGCAGC-GCTCTAGACC), the cyclobutane thymine dimers formed faster than in both *ps* and *aps* duplexes.

According to the early studies, the photosusceptibility of single-stranded DNA is greater than that of its double-stranded form [9,13]. The rate of thymine dimer formation in the *ps* F5·F6 duplex is intermediate between those for single-stranded form and the ordinary *aps* F5·F7 duplex. This could be explained by the conformational differences between the *ps* and *aps* duplexes and/or increased structural mobility of bases in the *ps* duplex relative to the *aps* duplex. Compared to the B-DNA *aps* duplex, the *ps* duplex has a very different surface shape, and the two grooves are of approximately equal width [14]. There was no direct experimental measurement of the base mobilities in the *ps* nucleic acids. Molecular dynamics calculations show that in comparison with the *aps* DNA the *ps* DNA is more fluctuating structure [15]. This gives it some single-stranded character, which is confirmed by its increased susceptibility to the single-strand specific reagents (e.g., Os-pyridine, or potassium permanganate) [16]. However, the photosusceptibility of adjacent thymines in both single- and double-stranded nucleic acids may significantly depend on the nearest neighbors [13]. Clearly, more possible sequence variants should be tested to reach a general conclusion about the differences in photosusceptibilities of *ps* and *aps* nucleic acids. Yet, the present results allow us to conclude that unlike other non-B DNA forms (A, B′, triplex) where the formation of dipyrimidine photoproducts is significantly inhibited, the *ps* DNA has the photosusceptibility comparable to that of the usual *aps* B-DNA.

The oligonucleotide models are useful because (i) the short lengths and relative simplicities allow a quantitative analysis of various modifications in them by a chromatographic technique, and (ii) they approximate short DNA sequences (a few dozen nucleotides long) where unusual structures (B′, Z forms, cruciform, triplex, etc.) different from a regular B form DNA have been found [17–19]. The present results show the usefulness of a new oligonucleotide model of nucleic acid structure which can be analyzed by the HPLC technique. In order to separately quantify the photoproducts formed in different strands of the double-stranded DNA fragment, we artificially increased the hydrophobicity of one strand leaving it in a partially protected form. This allowed the products of

photomodification to be separately analyzed for each of the complementary strands. We used the simplest way to obtain the hydrophobically modified oligonucleotide. Yet, other hydrophobicity modifications (e.g., oligonucleotide conjugation with cholesterol [20]) can be used for a significant increase in the oligonucleotide retention time.

We described a new oligonucleotide model which is suitable for the structure-dependent UV modification. Many chemical compounds known to react with DNA are used as the structure-dependent probes or to produce some mutagenic lesions [21,22]. Their sequence specificity and the structures of adducts are often studied using the oligonucleotide models. The new oligonucleotide model is suitable also for such multiple-site reacting chemicals. In many cases the formation of chemical adducts results in an increase of the on-column oligomer retention, which is significantly lower than expected for the difference in retention between partially protected and completely deprotected unmodified strands [23–25]. Thus, our model seems to be applicable to the chromatographic analysis of both chemical and photochemical modifications of oligonucleotide duplexes.

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