

Complementary addressed modification of double-stranded DNA within a ternary complex

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Double-stranded DNA containing a $d(pG)_{18} \cdot d(pC)_{18}$ sequence was shown to be selectively alkylated in the vicinity of this fragment using the 5'-*p*-(*N*-2-chloroethyl-*N*-methylamino)benzylamide of deoxyribooligocytidylate, $CIRCH_2NH(pdC)_n$ ($n = 9, 15$), in conditions favouring triple-stranded complex formation.

Complementary addressed alkylation; dsDNA

1. INTRODUCTION

Single-stranded nucleic acids may be modified at a definite site with reagents containing an oligonucleotide moiety complementary to the nucleotide sequence of the target nucleic acid properly chosen in the vicinity of the site. The oligonucleotide fragment of a reagent serves as an address which directs the reactive group to this site. This approach proposed in [1] is usually referred to as complementary addressed modification. A number of model oligonucleotides, RNA molecules and single-stranded DNA fragments were shown to be modified with high level of selectivity using this approach. Significant decrease in the level of expression of the immunoglobulin gene in MOPC myeloma cells as well as suppression of multiplication of influenza virus in the chicken fibroblasts cell cultures were achieved by treatment of the cells with the respective oligonucleotide derivatives [2]. However the approach in its

original version cannot be applied to modification of the selected regions of genome presented by double-stranded DNA. Here we demonstrate that double-stranded DNA (dsDNA) containing a $(pdG)_n \cdot (pdC)_n$ fragment can be selectively alkylated in the vicinity of this fragment using the 5'-*p*-(*N*-2-chloroethyl-*N*-methylamino)benzylamide of deoxyribooligocytidylate, $CIRCH_2NH(pdC)_n$, in conditions favouring triple-stranded complex formation.

2. MATERIALS AND METHODS

The ssDNA fragment used was prepared and 3'-labelled with [^{32}P]TTP as described in [3]. Conversion to partially dsDNA was carried out using Klenow fragment of DNA polymerase I and $d(pTGACCCCTCTTCCC)rA$ as a primer complementary to the 261-274 sequence of ssDNA. The 275-302 region remained in a single-stranded form (fig.1). The reaction mixture contained 0.05 mM of four dNTPs, 10^{-8} M dsDNA, 2.5×10^{-6} M primer in 100 μ l of 10 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 50 mM NaCl, 0.1 mM β -mercaptoethanol. Incubation was carried out in the presence of 0.5 a.u. of enzyme for 10 min at 37°C and stopped by addition of 10 μ l of 250 mM EDTA, pH 8.3. dsDNA was isolated by gel electrophoresis in non-denaturing conditions. To check the completion of the second DNA chain synthesis, the presence of restriction sites *Pst*I (12-17), *Msp*I (166-169) and *Bsp*RI (216-219) was proved. Reactive oligodeoxycytidylate derivatives $CIRCH_2NH(pC)_9$ and $CIRCH_2NH(pC)_{15}$ were synthesized as described in [4] and purified by reversed-phase chromatography on a Nucleosil 5-C-18 column in a methanol gradient.

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Abbreviations: CIR, *p*-(*N*-2-chloroethyl-*N*-methylamino)phenyl residue; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; other abbreviations are in accordance with IUPAC IUB recommendations

Addressed alkylation of DNA was performed in 0.1 M NaCl, 0.01 M CH_3COONa , 1 mM EDTA at 25°C and pH values as indicated in the text. The reaction was stopped by addition of 10 vols of LiClO_4 in acetone, the precipitate was washed by ethanol, redissolved in 1 M piperidine and heated for 10 min at 90°C. DNA fragments were repeatedly precipitated, washed, redissolved in 5 μl of 80% formamide and heated for 1 min at 100°C to complete denaturation. Gel electrophoresis was carried out in 8% polyacrylamide gel/7 M urea. Autoradiographs were taken using an X-ray film (RM-1) and scanned using an Ultrascan laser densitometer (LKB, Sweden).

3. RESULTS AND DISCUSSION

It is known that purine homopolynucleotides can form triple-stranded complexes with two complementary polypyrimidinic chains. In the present investigation we have chosen a double-stranded, 302 base pair long DNA fragment containing the $(\text{pdG})_{18} \cdot (\text{pdC})_{18}$ sequence in position 17–34 (fig.1). Alkylating reagents $\text{ClRCH}_2\text{NH}(\text{pdC})_n$, $n = 9, 15$, were taken as reactive oligonucleotide derivatives. According to the well known properties of oligoguanilyc sequences, triple-stranded structures $(\text{pdC})_{18}(\text{pdG})_{18}(\text{pdCH}^+)_n$ were expected to be formed in mild acidic medium [5].

Incubation of a dsDNA fragment labelled with

^{32}P at the 3'-end of the $(\text{pdG})_{18}$ -containing strand with the above mentioned oligodeoxycytidylate derivatives was carried out for 25 h at 25°C. This time corresponds to 5 half-times of the transformation of an aromatic 2-chloroethylamino group to an intermediate ethylene immonium cation [6] which is known to be the rate limiting step of alkylation by aromatic 2-chloroethylamines [7]. Similar experiments were performed with 3'-end labelled parent single-stranded fragments containing the $(\text{pdG})_{18}$ sequence. The experiments were carried out at several pH values ranging from 4.0 to 7.5. After piperidine treatment, to split modified fragments at the alkylation points, the samples were subjected to gel electrophoresis and autoradiographs were taken. Both fragments were shown to be selectively alkylated at definite areas. This means that specific alkylation inside the complexes of target DNA and reagent takes place. ssDNA at pH 7.0 is modified at the 3'-side of the $(\text{pdG})_{18}$ sequence in accordance with the antiparallel orientation of the oligocytidylate moiety of the reagent within the duplex (fig.2). At the same time, dsDNA at pH 4.5 is modified at the 5'-side of the $(\text{pdG})_{18}$ fragment, thus indicating

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1                                     50
GATCC GTCGA GCTGC AGGGG GGGGG GGGGG GGGGT TGCTC AGGGT GAGGC
CTAGG CAGCT CGACG TCCCC CCCCC CCCCC CCCCC ACGAG TCCCA CTCCG

                                     100
GGAAA AGAGT CGACC CAACC TTCCG CCGGC CGTCA CTGGC ACAGG CTGGA
CCTTT TCTCA GCTGG GTTGG AAGGC GGCCG GCAGT GAGCG TGTCC GACCT

                                     150
CAGCA AAAGG GCAGA TCACA GTGCT GGACA TGCAC CCAGG CTCTG GGAAG
GTGCT TTTCC CGTCT AGTGT CACGA CCTGT ACGTG GGTCC GAGAC CCTTC

                                     200
ACCCA CAGAG TCCTC CCGGA GCTCA TTGCG CAATG CATTG ACAGA CGCCT
TGGGT GTCTC AGGAG GGCCT CGAGT AAGCG GTTAC GTAAC TGTCT GCGGA

                                     250
AAGGA CATTG GTGTT GGCCC CAACC CGTGT GGTGC TTAAG GAAAT GGAGC
TTCCT GTAAC CACAA CCGGG GTTGG GCACA CCACG AATTC CTTA CCTCG

                                     300
GTGCC TTGAA TGGGA AGAGG GTCAG GTTCC ATTCT CCTGC AGGCT CGACGG*PA
CACGG AACTT ACCCT TCTCC CTGT

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Fig.1. Primary structure of the DNA fragment under investigation.

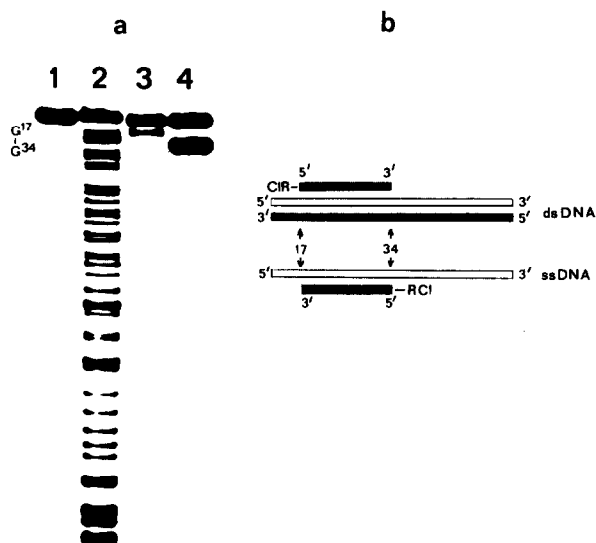


Fig.2. (a) An autoradiograph of the gel electrophoretogram of the piperidine-treated DNA fragments. Lanes: 1, control single-stranded fragment; 2, the same fragment split at guanine residues according to the Maxam-Gilbert method; 3, dsDNA fragment incubated with $\text{CIRCH}_2\text{NHd}(\text{pC})_9$ at pH 4.5; 4, ssDNA fragment incubated with $\text{CIRCH}_2\text{NHd}(\text{pC})_9$ at pH 7. (b) The orientation of reagent in the complex with dsDNA and ssDNA.

parallel orientation of the oligonucleotide address and the target sequence which is typical of the triple-stranded complex $(\text{pdG})_{18}(\text{pdC})_{18}(\text{pdCH}^+)_n$.

The results of the gel scanning obtained for dsDNA modified at different pH values are presented in fig.3. The first peak corresponds to the fragment split at the alkylation points, the second one to the unaffected fragment. It is seen that the greatest extent of modification is achieved at pH 4.5 which is optimal for triple-stranded complex formation.

It is noteworthy that at pH 4.0–5.0 modification of the ssDNA fragment downstream from the $(\text{pdG})_{18}$ sequence was observed (not shown) thus indicating that the triple-stranded complex, consisting of one DNA strand and two molecules of reagent, may be formed in these conditions.

Thus, addressed modification may be accomplished within triple-stranded complexes of a definite structure. It is reasonable to expect that oligoguanilyc sequences are not the only sequences which may undergo such modification. Similar structures are known to be formed by polypurinic

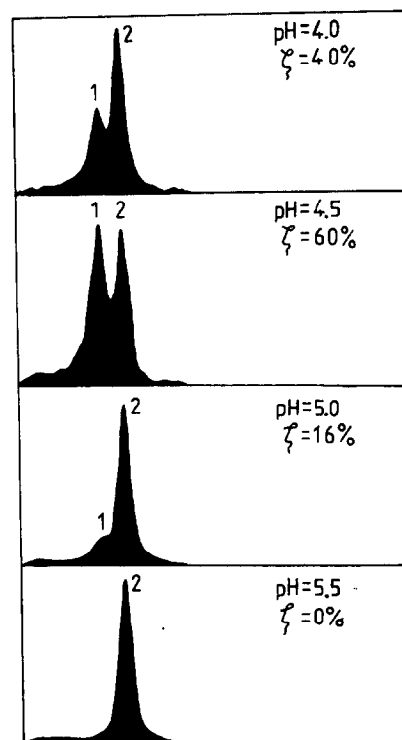


Fig.3. The scanned electrophoretogram of the piperidine-treated dsDNA fragment alkylated with $\text{CIRCH}_2\text{NHp}(\text{dC})_9$ at different pH values. ζ , extent of alkylation calculated as the ratio of the area under the first peak to the total shaded area.

sequences with A [8] or alternating G and A residues [5,9]. Triple-stranded helices may contain both polydeoxyribo- and polyribonucleotide sequences [10–12]. Also, it was reported that poly(pyrimidine)·poly(purine) DNAs containing 5-methylcytosine instead of cytosine form a triplex at pH values below 8 [13]. Therefore it may be expected that a significant number of dsDNA sites capable of addressed alkylation may be found in the majority of DNA molecules.

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