

Sequence-specific chemical modification of a 365-nucleotide-long DNA fragment with an alkylating oligonucleotide derivative

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An aromatic 2-chloroethylamino group was attached to the 5'-terminal phosphate of the oligodeoxyribonucleotide pCCCTCTTTCTT. The oligonucleotide derivative prepared was used for modification of the 365-nucleotide-long DNA fragment. It was found that modification of the fragment proceeds in a sequence-specific way at 3 guanosine residues within the sequence complementary to the oligonucleotide reagent.

Nucleic acid Affinity modification

1. INTRODUCTION

Complementary addressed modification of nucleic acids with oligonucleotide derivatives carrying reactive groups was proposed as an approach to sequence-specific derivatization of nucleic acids at arbitrary nucleotides [1–3]. The potential use of this approach in molecular biology and medicine was reviewed in [2–5]. Recently it was demonstrated that specific arrest of translation of preselected mRNAs can be achieved by means of the complementary addressed modification [6]. Modification with alkylating oligonucleotide derivatives was investigated with model systems, synthetic complementary oligonucleotides [7,8]. An attempt to accomplish complementary addressed modification of a 203-nucleotide-long DNA fragment was reported in [9].

Here we demonstrate the sequence-specific modification of a 365-nucleotide-long DNA fragment within the preselected region with the complementary alkylating oligonucleotide derivative.

2. MATERIALS AND METHODS

The undecanucleotide pCCCTCTTTCTT was synthesized by the triester method as described in

[10]. An alkylating derivative of the oligonucleotide carrying a 2-chloroethylamino group at its 5'-terminal phosphate was synthesized according to [11]. The oligonucleotide reagent was purified by chromatography on a Lichrosorb 5-C18 column with an acetonitrile gradient containing 0.2 M LiClO₄ and isolated by precipitation with a 10-fold excess of 2% LiClO₄ solution in acetone.

The 365-nucleotide-long DNA fragments were prepared by *Rsa*I digestion of the plasmid pEMC [12–14]. Plasmid DNA was isolated as described in [15]. Isolation of the DNA fragments and DNA strand separation by treatment with 5 mM oxy-methylmercury was performed by gel electrophoresis in 6% polyacrylamide gel. The fragment was labelled at the 3'-end with *E.coli* DNA polymerase and ³²P-labelled deoxyribonucleoside triphosphates. Purine-specific splitting of DNA fragments by treatment with 2% diphenylamine in 67% formic acid was described in [16].

DNA fragments were alkylated with the oligonucleotide reagent in 0.15 M NaCl, 20 mM Tris-HCl, pH 7.3, 1 mM EDTA. Concentrations of DNA fragments in reaction mixtures were ~0.1 A₂₆₀ units/ml and the oligonucleotide reagent was taken in 30-fold molar excess. The reaction was terminated by addition of 2 µg M13 mp9-21 DNA,

1:10 (v/v) 3 M sodium acetate, pH 4.8, and 3 vols ethanol. The mixture was chilled to -20°C and the DNA precipitate was collected by centrifugation. For splitting of modified DNA fragments treatment was performed with 10% piperidine [17].

3. RESULTS AND DISCUSSION

Here we have chosen a 365-nucleotide-long single-stranded DNA fragment as a target for complementary addressed modification. The sequence of the fragment represents a sequence in the 3'-terminal region of the mouse encephalomyocarditis RNA, positions 6688-7705 [13,14]. This fragment is a part of a longer 600-bp sequence cloned in plasmid pBR 322 at the *Pst* site [12]. A part of this sequence, fragment *Pst* 500, was also cloned into bacteriophage M13 mp9 at the *Pst*I site (fig.1). Hybrid phages containing the DNA fragment – analog of the viral RNA sequence and the DNA fragment complementary to this sequence were constructed (clones M13 mp9-21 and M13 mp9-22, respectively). To prepare the single-stranded DNA fragments replicative form DNA of the hybrid phages or plasmid pEMC DNA were digested with *Rsa*I restriction endonuclease and the 365-bp-long fragment was isolated by electrophoresis. After 3'-terminal labelling the DNA strands were separated by gel electrophoresis. Fig. 2 shows the sequence of the target DNA fragment. In experiments with ^{32}P -labelled oligonucleotide pCCCTCTTTCTT it was found that the oligonucleotide binds efficiently to the single-stranded DNA of the hybrid phage M13 mp9-21 but not to that of the phage M13 mp9-22. *E.coli* DNA polymerase 1 elongates the fragment bound oligonucleotide in the presence of an in-

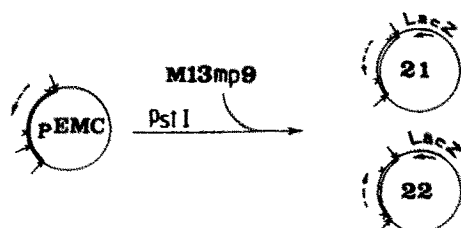


Fig.1. Cloning of the *Pst* 500 DNA fragment at the *Pst*I site of M13 mp9 bacteriophage DNA. (→ and ⇌) *Pst*I and *Rsa*I sites, respectively, in the cloned sequence. (---→) 5'-3' orientations of the DNA fragments representing the viral RNA sequence.

6650
5'...AAAAATTTTGAATTTGATGATGTGAAGGTGTTGCTG[✓]ACGGAGAT

GATCTCCTTGTGGCCACAAATTACCAATTGGATTTTGATAAGGTG

AGAGCAAGCCTCGCAAAGACAGGATATAAGATAACTCCCGCTAAC

A AAAACTTCTACCTTTCTCTTAATTCGACGCTGAAGACGTTGTC
TTCTTTCTCCpRC1
TTCTTAAAAAGAAAGTTTAAAGAAAGAGGGCCCTGTGATCGGCCT

GTGATGAACAGAGAGGCGTTGGAAGCAATGTTGCATACATCTCGT

CCAGGGACTCTATCTGAGAACTCACTTCGATCACTATGCTTGCC
7900
GTTCATTCTGGCAAGCAGGAATATGATCGGCTCTTTGCCCATTC

CGTGAGGTAGGGGTTGTCGTGCCATCATTCGAGAGTGTGGA[✓]GTAC...3'

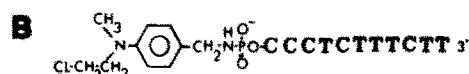


Fig.2. (a) 3'-terminal sequence of encephalomyocarditis RNA [14]. Arrows show the sequence corresponding to the *Rsa* 365 fragment which was used as a target for the complementary addressed modification. Guanosine residues attacked in the target DNA fragment by the oligonucleotide derivative are underlined. (b) Alkylating oligonucleotide derivative.

complete set of deoxyribonucleoside triphosphates with formation of the expected products of elongation thus demonstrating that the binding site in the DNA fragment is unique. The target DNA fragment representing the sequence of the viral RNA and the complementary DNA fragment were modified with the oligonucleotide reagent. The reaction was performed under conditions providing complementary complex formation. Alkylated DNA fragments were treated further with piperidine to split them at the positions of alkylated nucleotides and the splitting products were analysed by gel electrophoresis. It was found that splitting of the alkylated target DNA fragment occurs exclusively at positions of 3 guanosine residues located within the sequence complementary to the oligonucleotide reagent (fig.3). In the absence of salts and buffer, modification resulted in a low efficient random alkylation of guanosines in the DNA fragments.

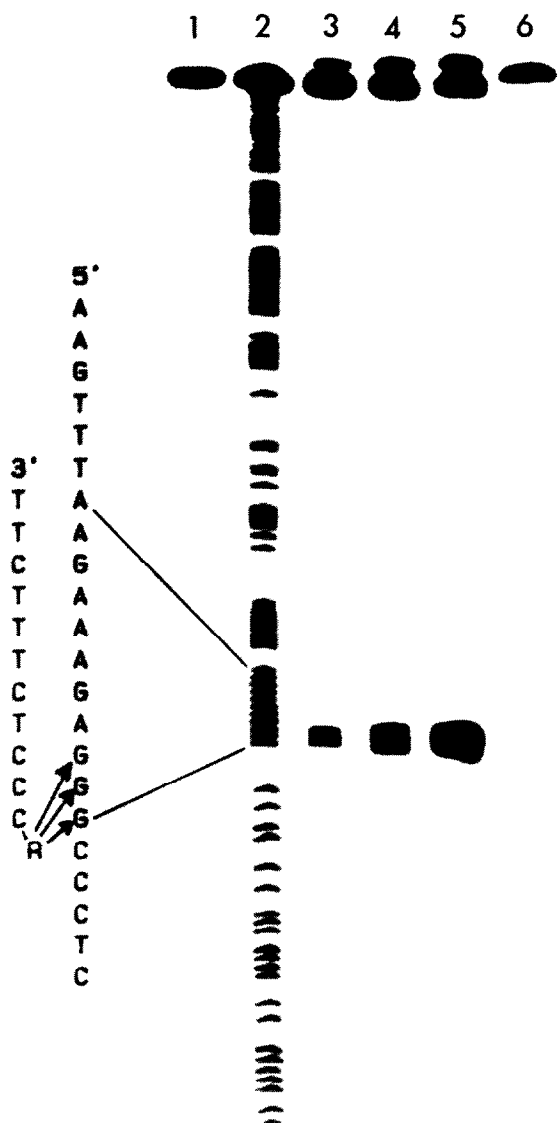


Fig.3. Analysis of the target *Rsa* 365 DNA fragment alkylated with the oligonucleotide derivative. (1) Control treatment of the unmodified fragment with piperidine, (2) A + G specific splitting of the fragment according to [16], (3,4,5 respectively) the fragment modification with the oligonucleotide reagent for 15, 60 and 150 min at 37°C and consequent treatment with piperidine (6) DNA fragment complementary to the target *Rsa* 365 DNA fragment, alkylation and treatments as in (4).

The results obtained clearly demonstrate sequence-specific complementary addressed modification of single-stranded DNA sequence with the alkylating oligonucleotide derivative. An unsuccessful attempt to accomplish sequence-specific

modification of a DNA fragment with an alkylating oligonucleotide derivative was reported in [9]. The alkylating group used in [9] was different from that used here. Modification of the fragment was found to proceed predominantly within a short DNA sequence which is, however, remote from the target sequence. The result was ascribed to the effect of the fragment tertiary structure in which the target sequence could be juxtaposed to other sequences. It was unclear whether the effect observed is unique or if the interference of the polynucleotide tertiary structure with the specificity of the complementary addressed modification is a general phenomenon. In the latter case the possibility of sequence-specific modification of polynucleotides could be doubtful. The present results demonstrate that sequence-specific complementary addressed modification with alkylating oligonucleotide derivatives can be achieved in the case of rather long nucleic acid fragments, thus providing one more tool for site-directed mutagenesis and a potential tool for specific damage of preselected nucleic acids.

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