Volume 231, number 2, 352–354 FEB 05796 April 1988

Sequence-specific chemical modification of a hybrid bacteriophage M13 single-stranded DNA by alkylating oligonucleotide derivatives

V.V. Vlassov, V.F. Zarytova, I.V. Kutyavin and S.V. Mamaev

Institute of Bioorganic Chemistry, Siberian Division of the USSR Academy of Sciences, Novosibirsk 630090, USSR

Received 16 February 1988

Alkylating oligonucleotide derivatives react with the complementary sequences in hybrid M13mp7 bacteriophage single-stranded DNA and destroy the infecting ability of the DNA. The reagents do not damage M13mp9 single-stranded DNA lacking the target nucleotide sequence.

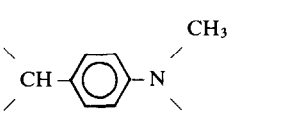
Nucleic acid; Affinity modification

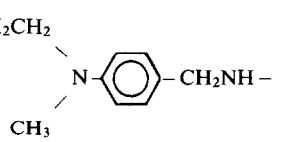
1. INTRODUCTION

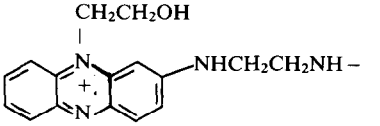
Recently we have demonstrated the possibility of accomplishing sequence-specific chemical modification of single-stranded DNA fragments by alkylating derivatives of oligonucleotides which can form complexes with the complementary nucleotide sequences in single-stranded nucleic acids [1–3]. In this communication sequence-specific chemical modification of a single-stranded bacteriophage DNA is described. We have compared damaging effects of reactive oligonucleotide derivatives bearing alkylating aromatic nitrogen mustard groups toward the single-stranded hybrid bacteriophage M13mp7 DNA which possesses the complementary target nucleotide sequence, and toward single-stranded DNA of bacteriophage M13mp9 lacking this sequence. The reagents were found to inactivate the target DNA much more efficiently as compared to the non-target one.

2. MATERIALS AND METHODS

Oligonucleotides were synthesized by the triester method [4]. Alkylating groups were coupled either to 3′-terminal ribose of oligonucleotides [5] or to 5′-terminal phosphate [6]. Oligonucleotide derivatives bearing a phenazine group were prepared essentially as described recently [7]. Three reagents were used for modification of DNA:

I pd(ACCCTCTTCCC)(rA)CHRCl CHRCl: 

II ClRCH$_2$NH-pd(ACCCTCTTCCC)(rA) ClRCH$_2$NH–: 

III Phn-pd(CCTCTTCCC)(rA)CHRCl Phn: 

Correspondence address: V.V. Vlassov, Institute of Bioorganic Chemistry, Siberian Division of the USSR Academy of Sciences, Novosibirsk 630090, USSR

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50 © 1988 Federation of European Biochemical Societies

Cloning, isolation and labelling of the 300-nucleotides long DNA fragment corresponding to a sequence present in the tick-borne encephalitis virus RNA were described earlier [2].

Bacteriophage DNAs and the DNA fragment were incubated with the oligonucleotide derivatives (2×10^{-4} M) for 12 h at 37°C in Tris-Cl^-, pH 7.6, 0.1 M NaCl, 1 mM EDTA. Concentration of the bacteriophage DNAs in the reaction mixtures was 2×10^{-7} M and that of the isolated DNA fragment was 4×10^{-8} M. After alkylation, the reaction mixture was added to a 10-fold excess of water, and 1–10 μl of this solution were used for infection of the competent JM103 *Escherichia coli* cells. The competent cells were prepared by the $CaCl_2$ treatment in ice [8]. In control experiments, it was found that components of the reaction mixture do not interfere with the infection of the bacterial cells. The cells were grown on plates containing XGal and IPTG-supplemented medium, and the plaques formed after overnight incubation at 37°C were counted.

3. RESULTS AND DISCUSSION

As a target for sequence-specific modification, the single-stranded DNA of hybrid bacteriophage M13mp7 containing a 300-nucleotides long insert sequence was used [3]. Oligonucleotide reagents I–III were targeted to the complementary sequence

rACCCTTCTCCCApNHCH$_2$RCl

–AGCGTGCCTTGAATGGGAAGAGGGTCAGGTTCCAT–

RClCHrACCCTTCTCCCAp

RClCHrACCCTTCTCCpPhn

in this insert. Recently we have demonstrated sequence-specific chemical modification of this isolated insert DNA fragment with reagents I and II [3]. As a control non-target DNA, bacteriophage M13mp9 DNA was used. This DNA does not possess the target sequence. The most closely related sequences present in this DNA are at least different from the target sequence by 4 nucleotides.

The single-stranded DNAs were reacted with all the oligonucleotide derivatives under conditions providing >90% efficient alkylation of the target sequence, judging from the data of parallel experiments in which the isolated insert fragment was modified in the presence of equivalent amount of the M13mp9 DNA (fig.1). The treated DNA samples were used for transfection of the competent JM103 *E. coli* cells. DNA infectivity was determined as the number of plaques formed under standard conditions. Table 1 represents the

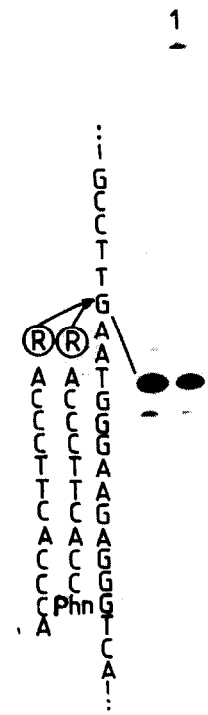


Fig.1. Autoradiogram of gel electrophoresis of the target 3′-end labelled single-stranded DNA fragment, cleaved by alkylation with oligonucleotide reagents I (lane 1) and III (lane 2) and piperidine treatment. The fragment (2×10^{-8} M) was incubated for 12 h at 37°C with 1×10^{-4} M reagents in 0.01 M Tris-HCl, pH 7.5, 0.1 M NaCl, 0.001 M EDTA, containing 1×10^{-7} M M13mp9 DNA. Lane 3, piperidine-treated DNA fragment.

Table 1

Inactivation of hybrid bacteriophage M13mp7 DNA and bacteriophage M13mp9 DNA by complementary oligonucleotide derivatives

Oligonucleotide derivative	Infectivity of DNA (%)	
	Hybrid M13mp7 DNA	M13mp9 DNA
No reagents added	100	100
Oligonucleotide d(ACCCTCTTCCC)	100	100
Phn-pd(CCTCTTCCC)	35	95
I	72	95
II	8	97
III	4	84

Experimental details are described in section 2

results of a typical experiment on the DNA inactivation. It is seen that non-reactive oligonucleotide derivatives and reagent I do not influence the infectivity of the DNAs while reagents II and III substantially inactivate the target DNA in contrast to the non-target one.

The target nucleotide sequence is not essential for the functioning of the bacteriophage DNA, therefore the damaging effect of the modification is most probably related to the interference with the DNA replication. *E. coli* has an efficient system for reparation of DNA damages caused by the alkylation of single bases, and oligonucleotides attached to DNA can be eliminated by the bacterial DNA polymerase which possesses nuclease activity [9]. Therefore it is clear why reagent III, which forms a more tight complex with the complementary DNA sequence and should be more resistant toward nuclease hydrolysis, is more efficient in the DNA inactivation. It is interesting to note that non-reactive oligonucleotide derivatives bearing a phenazine group at their 5′-termini show some effect on the target DNA infectivity probably due to the formation of a tight complex with the DNA.

The obtained results show that alkylating oligonucleotide derivatives targeted to specific nucleotide sequences can be used as reagents for the specific inactivation of predetermined single-stranded DNAs.

Acknowledgement: The authors thank Dr I.V. Morozov for the contextual analysis of DNAs.

REFERENCES

[1] Vlassov, V.V., Gaidamakov, S.A., Gorn, V.V. and Grachev, S.A. (1985) FEBS Lett. 182, 415–418.
[2] Brosalina, E.B., Vlassov, V.V., Kutyavin, I.V., Mamaev, S.V., Pletnev, A.G. and Podyminogin, M.A. (1986) Bioorg. Khim. 12, 240–247.
[3] Vlassov, V.V., Zarytova, V.F., Kutiavin, I.V., Mamaev, S.V. and Podyminogin, M.A. (1986) Nucleic Acids Res. 14, 4065–4076.
[4] Zarytova, V.F., Ivanova, E.M. and Kutyavin, I.V. (1982) Bioorg. Khim. 8, 224–230.
[5] Gorn, V.V., Karpova, G.G., Knorre, D.G., Kutyavin, I.V. and Pichko, N.P. (1982) Bioorg. Khim. 8, 1225–1232.
[6] Mishenina, G.F., Samukov, V.V. and Shubina, T.I. (1979) Bioorg. Khim. 5, 886–894.
[7] Zarytova, V.F., Kutyavin, I.V., Silnikov, V.N. and Shishkin, G.V. (1986) Bioorg. Khim. 12, 911–920.
[8] Maniatis, T., Fritsch, E.E. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[9] Wilman, P.E.V. and Connors, T.A. (1983) in: Molecular Aspects of Anticancer Drug Action, Weinheim, Peerfield Beach, FL.