Complementary addressed reagents carrying EDTA-Fe(II) groups for directed cleavage of single-stranded nucleic acids

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An octathymidylate derivative carrying an EDTA residue at its 5'-terminus was synthesized. In the presence of Fe^{2+}, O_2 and dithiotreitol, this derivative cleaves poly(dA) and poly(A) more efficiently than the non-complementary polynucleotide poly(dT).

Affinity modification *Nucleic acid* *Directed cleavage*

1. INTRODUCTION

The principle of complementary addressed modification with reactive oligonucleotide derivatives was proposed for directed sequence-specific chemical action on single-stranded nucleic acids [1–3]. Modification of polynucleotides with reactive oligonucleotide derivatives occurs in the neighbourhood of the nucleotide sequences complementary to those of oligonucleotides carrying reactive groups. In [1–5], oligonucleotides carrying alkylating groups were used as the complementary addressed reagents. Recently, complementary addressed reagents carrying platinum complexes as reactive groups have been prepared [6,7]. Alkylating and platinating oligonucleotide derivatives can be used for chemical modification of certain nucleotide residues in preselected regions of nucleic acids. The sequence-specific reagents which cleave nucleic acids under physiological conditions should be more useful for many purposes.

It was shown that a complex of Fe^{2+} with EDTA cleaves DNA in the presence of reducing agents [8]. More efficient cleaving reagents were prepared by attachment of EDTA groups to compounds which form complexes with DNA [8–11]. Recently, an EDTA–Fe(II) pentapeptide derivative has been shown to cleave pBR322 plasmid DNA in specific regions [11].

Here we have prepared an octathymidylate derivative carrying the EDTA–Fe(II) group. It was found that this reagent cleaves complementary polynucleotides more efficiently than non-complementary polynucleotides.

2. MATERIALS AND METHODS

Poly(A), poly(dA) and poly(dT) were purchased from Sigma; dithiothreitol, TPS and methylimidazole were from Merck (Darmstadt).

Polynucleotides (average length about 200 nucleotide residues) were isolated by column gel chromatography on Sephadex G-100 superfine (Pharmacia, Uppsala). The 5'-ends of isolated polynucleotides were labeled with ^{32}P by treatment with T4 polynucleotide kinase and [γ-^{32}P]ATP from the Institute of Nuclear Physics (Tashkent, USSR).

Concentrations of polynucleotides and oligonucleotide solutions were determined spectrophotometrically at pH 7 using the following extinction coefficients (per mol nucleotide residues):

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Abbreviations: TEA, triethanolamine; TPS, triisopropylbenzenesulphonyl chloride; DMF, dimethylformamide; $(pT)_8$-EDTA, heptathymidilyl (5'-3')thymidine 5'-*N*-[*N*,*N*,*N*'-ethylenediaminetriacetate)]-*N*'-acetylaminopropyl phosphate

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$\epsilon^{260\,nm}_{poly(A)} = 9.4 \times 10^3\ M^{-1} \cdot cm^{-1}$;

$\epsilon^{257\,nm}_{poly(dA)} = 8.6 \times 10^3\ M^{-1} \cdot cm^{-1}$;

$\epsilon^{264\,nm}_{poly(dT)} = 8.5 \times 10^3\ M^{-1} \cdot cm^{-1}$;

$\epsilon^{267\,nm}_{d(pT)_8} = 8.5 \times 10^3\ M^{-1} \cdot cm^{-1}$

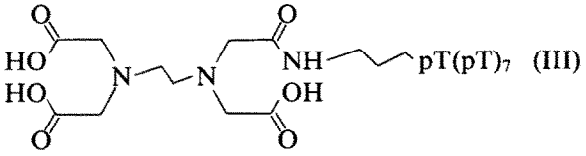
Octathymidylate derivative I containing protecting groups was synthesized as in [12]. The synthesis of oligonucleotide derivative III was carried out according to the following scheme:

(CNEt)(ClPh)pT[(ClPh)pT]$_6$p(PhCl)T(Lev) (I)

↓ (1) TEA, acetonitrile
(2) $F_3CCONH(CH_2)_3OH$, TPS, methylimidazole

$(F_3CONH(CH_2)_3$(ClPh)pT[(ClPh)pT]$_6$p(PhCl)T(Lev) (II)

↓ (1) NH_4OH
(2) EDTA dianhydride, DMF



At the first stage of synthesis an aliphatic amino group was introduced into the oligonucleotide terminus by condensation of aminopropanol, protected at the amino group by the trifluoracetyl residue, with the 5'-phosphate group of the oligonucleotide. This amino group was then actylated with EDTA dianhydride prepared as in [13].

The synthesis will be published in detail elsewhere. Purification and analysis of oligonucleotide derivatives were carried out by macroporous anion exchanger microcolumn chromatography [14].

Octathymidylate derivative III was allowed to equilibrate for 5 min at 20°C with equimolar quantities (per nucleotide residue) of 0.3 mM polynucleotides in 10 mM Tris–HCl (pH 7.6), 0.1 M NaCl, 1 mM $MgCl_2$ and 2 mM DTT. The polynucleotide cleaving reactions were initiated by adding freshly prepared $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ to the reaction mixture (Fe^{2+}/EDTA residue = 1:1) and allowed to proceed for 5 h at 20°C.

^{32}P-labelled polynucleotides and oligonucleotides were analysed by gel electrophoresis on a 2 mm thick 10% polyacrylamide (1:40 cross-linked) gel containing 7 M urea, 50 mM Tris–borate (pH 8.3) and 2.5 mM EDTA.

For estimation of the extent of polynucleotide cleavage, radioactive bands after gel autoradiography were excised from gels and determined by Cerenkov counting.

3. RESULTS AND DISCUSSION

Here, an oligothymidylate derivative carrying an EDTA–Fe(II) group and homopolynucleotides poly(A), poly(dA) and poly(dT) have been chosen as a model system to study the possible use of EDTA–Fe(II) as a reactive cleaving group for complementary addressed reagents. ^{32}P-labelled polynucleotides were incubated with the EDTA–Fe(II)–$(pT)_8$ derivative under the conditions of complementary complex formation. The extent of polynucleotide degradation was estimated by gel electrophoretic analysis. Results of the experiments are shown in fig.1,2 and in table 1. It is seen that the EDTA–Fe(II)–$(pT)_8$ complex cleaves complementary polynucleotide poly(dA) more efficiently than the non-complementary poly(dT). Under the same conditions the EDTA–Fe(II) complex cleaves poly(dA) considerably slower than the $(pT)_8$–EDTA–Fe(II) derivative and in this case the efficiency of cleavage does not depend on the nature of the polynucleotide. Addition of oligo(dT) to the reaction mixture inhibited degradation of poly(dA) by EDTA–Fe(II)–$(pT)_8$ but did not influence degradation of poly(dA) by EDTA–Fe complex. Therefore, it can be concluded that the oligothymidylate derivative carrying the EDTA–Fe(II) group cleaves poly(dA) in a complementary complex and that this reaction can be considered as a complementary addressed modification.

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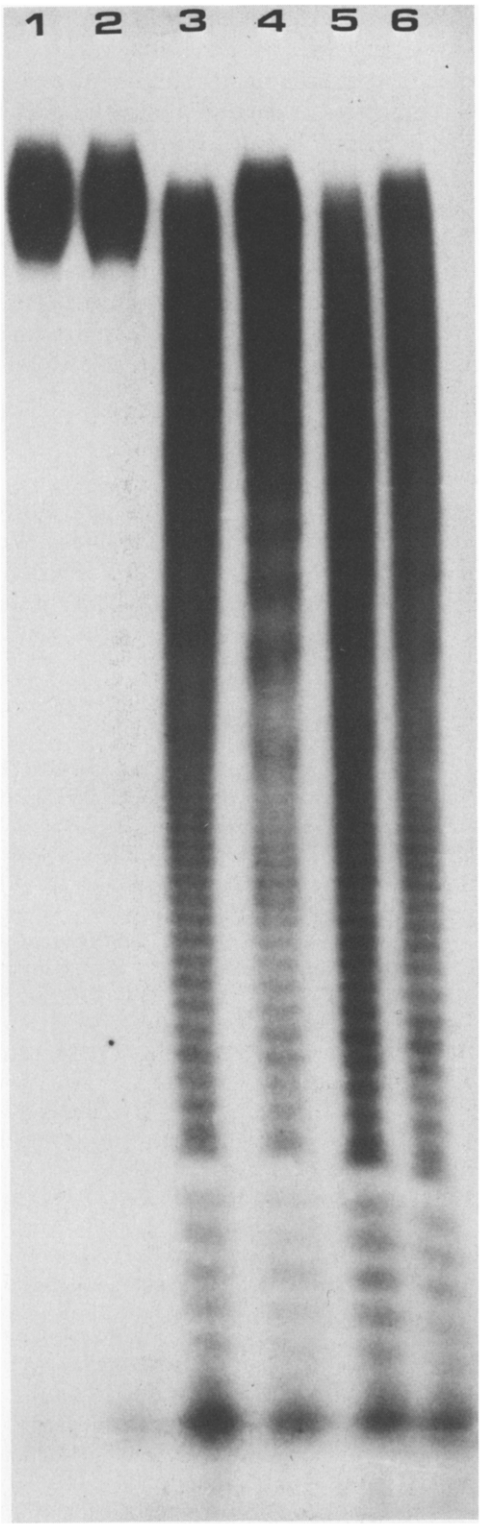
Fig.1. Autoradiograph of 10% polyacrylamide gel electrophoretic analysis of ^{32}P-labelled poly(dA) incubated for 5 h: (1) in water at 20°C; (2) in reaction buffer at 20°C; (3) with 50 μM Fe(II)–EDTA–$(pT)_8$ at 20°C; (4) with 0.25 mM Fe(II)–EDTA–$(pT)_8$ at 4°C; (5) with 0.25 mM Fe(II)–EDTA–$(pT)_8$ and 0.5 mM H_2O_2 at 20°C; (6) with 0.25 mM Fe(II)–EDTA–$(pT)_8$ at 20°C. Cleavage reaction buffer: 10 mM Tris–HCl (pH 7.6), 100 mM NaCl, 1 mM MgCl, 2 mM DTT. Concentration of poly(dA) (per nucleotide residue) was 30 mM.



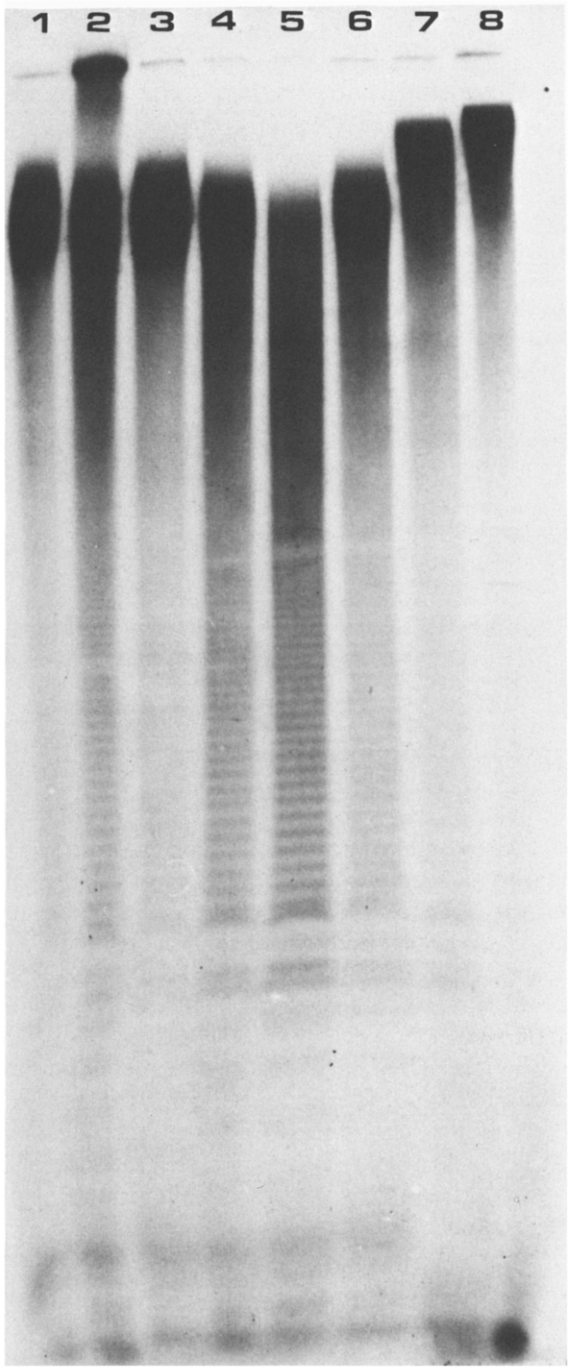


Fig.2. Autoradiograph of 10% polyacrylamide gel electrophoretic analysis of ^{32}P-labelled polynucleotides incubated under conditions described in the legend to table 1: (1) poly(dA); (2) poly(dA) with 50 μM Fe^{2+}; (3) poly(dA); (4) poly(dA) with 50 μM Fe(II)–EDTA; (5) poly(dA) with Fe(II)–EDTA–$(pT)_8$; (6) poly(dA) with Fe(II)–EDTA–$(pT)_8$ and 0.2 mM $T(pT)_9$; (7) poly(dT) with Fe(II)–EDTA–$(pT)_8$; (8) poly(dT).

Table 1
Cleavage of poly(dA) and poly(dT) by the octathymidylate derivative carrying the EDTA–Fe(II) group and by the EDTA–Fe(II) complex

Polynucleotide	Reagent	Cleavage of polynucleotide (%)
Poly(dA)	–	0
Poly(dA)	EDTA–Fe(II)	12
Poly(dA)	EDTA–Fe(II)-$(pT)_8$	50
Poly(dT)	–	0
Poly(dT)	EDTA–Fe(II)	17
Poly(dT)	EDTA–Fe(II)-$(pT)_8$	14

Polynucleotides were incubated with the reagents for 5 h at 20°C in 10 mM Tris–HCl (pH 7.6), 100 mM NaCl, 1 mM MgCl and 2 mM DTT. Concentrations of the polynucleotides per nucleotide residue were 30 mM. Concentrations of EDTA–Fe(II) and of $(pT)_8$–EDTA–Fe(II) were 50 μM

In the experiments with poly(A) it was demonstrated that the EDTA–Fe(II)–$(pT)_8$ complex is able to cleave polyribonucleotides; however, the eficiency of this reaction was rather lower than the reaction with poly(dA).

When the derivative EDTA–Fe(II)–$(pT)_8$ was incubated under the modification conditions in the absence of polynucleotides, degradation of the derivative was observed. According to ion-exchange chromatography data, the extent of $(pT)_8$–EDTA degradation was 50–60% after 5 h incubation. Obviously this is the result of self-cleaving of the derivatives.

The results obtained lead to the conclusion that oligonucleotide derivatives carrying the EDTA–Fe(II) group can be used for directed cleavage of nucleic acids. The reaction proceeds under mild conditions and does not require additional chemical treatments of the polynucleotides.

The EDTA–Fe(II) group can be considered a model of antibiotic bleomycin cleaving DNA in vivo [8]. Therefore, it is hoped that oligonucleotide derivatives carrying EDTA–Fe(II) groups can be used for directed degradation of certain preselected nucleic acids in living cells. In contrast to the reactive moieties of other complementary addressed reagents, the EDTA–Fe(II) group does not undergo chemical transformations and functions as a catalyst [11]. Therefore, the oligonucleotide derivatives carrying EDTA–Fe(II) groups should be more efficient compared to non-catalytic complementary addressed reagents. The reaction proceeds in the presence of reducing agents and dissolved oxygen only. Therefore, the derivatives can be stored and manipulated in an inactive state and can be easily activated when necessary.

It should be noted that under the conditions used the efficiency of polynucleotide degradation by the oligonucleotide derivative was not very high. However, one can expect that the efficiency of the reaction can be increased by improvement of the reagent structure and by appropriate choice of the reaction conditions.

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