Volume 280, number 2, 271–273 FEBS 09509 March 1991

ADONIS 0014579391002161

Direct cleavage of a DNA fragment by a bleomycin-oligonucleotide derivative

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Received 11 January 1991

Bleomycin A_5 oligonucleotide derivative was used for direct cleavage of a DNA target. In the presence of Fe^{2+} ions and 2-mercaptoethanol, Blm-R-pd(CCAAACA) (I) damaged the target, pd(TGTTTGGCGAAGGA), with the yield of 80%, without affecting its own oligonucleotide tail. The sites of cleavage were T^3-T^4 and G^6-G^7. Unbound bleomycin A_5 damaged the G^6-G^7-C^8 site. Reagent I formed more stable complementary complexes with the target than parent oligonucleotide ($\Delta T_m = 11°C$).

Reactive oligonucleotide derivative; Antitumour antibiotic; Bleomycin; Direct damage of nucleic acids

1. INTRODUCTION

A number of oligonucleotides bearing EDTA [1,2], phenantroline [3] and porphyrine [4] residues with chelated transition metal ions were prepared and used for site-specific modification and chain scission of DNA. These groups imitated the active part of an antitumour antibiotic, bleomycin. The latter was shown to produce a variety of DNA damage including loss of bases [5,6], production of apurinic or apyrimidinic sites [7] and single-stranded or double-stranded breaks [8–10]. GC and GT selective cleavage of DNA by bleomycin is now considered to be due to the following two chemical characteristics of the glycopeptide. A bithiazole terminal amine residue contributes to binding and sequence-specific recognition of DNA [9,10] while β-aminoalanine, pyrimidine and β-hydroxyhistidine moieties can form Fe^{2+}-oxygen center. The latter produces reactive oxygen forms mediating oxidative cleavage of the DNA target.

We have used a bleomycin A_5 oligonucleotide for direct cleavage of DNA. In the presence of Fe^{2+} ions BLM-R-pd(CCAAACA) cleaved the target DNA fragment, pd(TGTTTGGCGAAGGA) with an 80% yield.

2. MATERIALS AND METHODS

Ia, **II** and target were synthesized by a phosphotriester method [11]. BLM-RH was purchased from a Pilot plant of the Institute of Organic Synthesis, Latvian Academy of Sciences. Oligonucleotide **Ia** was activated with a mixture of Ph_3P and 2,2'-dipyridyldisulfide as in [12]. Cu^{2+}-bleomycin A_5 was attached to the activated 5'-phosphate of oligonucleotide **Ia** through a spermidine amino group. The details of this synthesis are to be published elsewhere. The concentrations of the **Ia**, **II**, and Cu^{2+}-bleomycin A_5 oligonucleotide, and the target were determined spectrophotometrically. Molar extinction coefficients ($\lambda = 260$ nm) for **Ia**, target and **II** were 66 000 $M^{-1} \cdot cm^{-1}$, 140 000 $M^{-1} \cdot cm^{-1}$ and 57 000 $M^{-1} \cdot cm^{-1}$, respectively [13,14]. Those for Cu^{2+}-bleomycin A_5 oligonucleotide were determined by radioisotope labelling method [13] and equaled 86 000 $M^{-1} \cdot cm^{-1}$ ($\lambda = 260$ nm) and 1×10^2 $M^{-1} \cdot cm^{-1}$ ($\lambda = 610$ nm). T_m of the

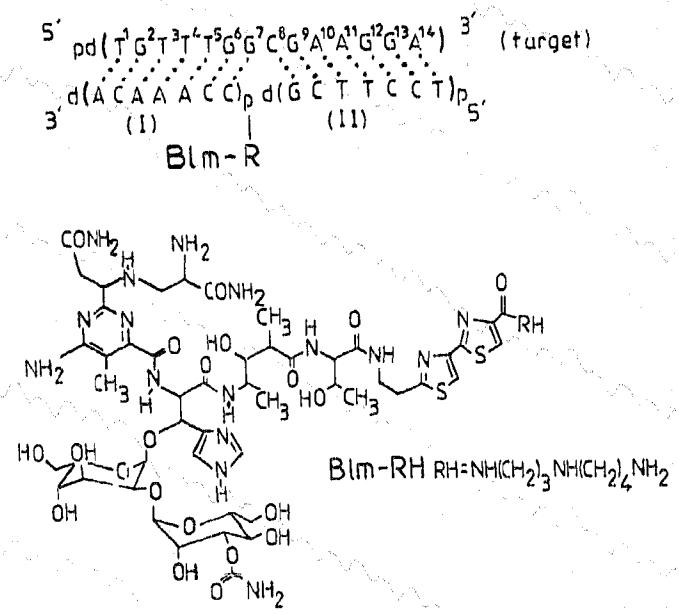

Fig. 1. A model system used for cleaving the DNA target by **I**.

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Abbreviations: BLM-RH, bleomycin A_5; **I**, BLM-R-pd(CCAAACA); **Ia**, pd(CCAAACA); **II**, pd(TCCTTCG)

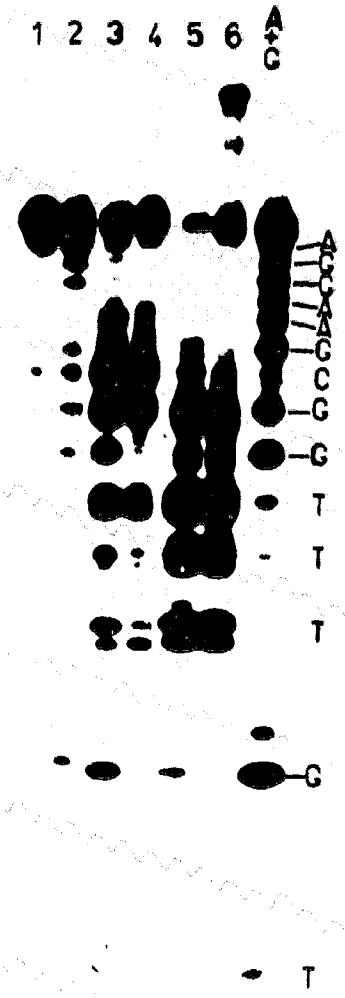

Fig. 2. Electrophoresis of the DNA target cleavage in the presence of **II**. (Lanes 1,2) The target under the reaction conditions without reagent; (lanes 3,4) the target with free bleomycin and **Ia**; (lanes 5,6) the target with reagent **I**. (Lanes 2,3,5) The reaction was followed by piperidine treatment (95°C, 45 min). Reaction mixture contained 1×10^{-4} M $Fe(NH_4)_2(SO_4)_2$, 0.05 M $HSCH_2CH_2OH$, 0.2 M LiCl, 0.01 M Tris-HCl (pH 7.5) (20°C, 9 h). **I**, **Ia**, **II**, the target and bleomycin were each present at a concentration of 1×10^{-5} M.

complementary complexes, target + **I** and target + **Ia**, were measured using equipment for thermal denaturing based on a Milichrom spectrophotometer [13]. The concentration of oligonucleotide components was 2.5×10^{-5} M in 0.16 M NaCl, 0.02 M Na_2HPO_4 and 0.1 mM EDTA (pH 7.4). Cu^{2+}-ion was removed from the bleomycin A_5 oligonucleotide derivative, adsorbed on a RP-18 Lichrosorb column, using 0.1 M EDTA (pH 6.3) washing for 50 min. The obtained **I** was used for degradation of the DNA target in the presence of Fe^{2+}-ions (Fig. 1). The conditions for degradation are described in the legend to Fig. 2. After the reaction, the oligonucleotide material was precipitated by addition of a 2% $LiClO_4$ solution in acetone. The reaction mixtures with or without piperidine treatment (45 min, 95°C) were electrophoresed in denaturing conditions: 20% PAAG, pH 8.2, 8 M urea, 0.089 M Tris-H_3BO_3, 40°C. The gels were further autoradiographed with RM-V film at −20°C, cut and radioactivity was counted according to Cherenkov in a Rackbeta scintillation counter (Wallac Oy, Finland). We performed control experiments with all components of the reaction mixture except **I** or free antibiotic. We calculated degradation extent of the DNA target subtracting the degradation value obtained in the controls.

3. RESULTS AND DISCUSSION

It is known that the efficiency of site-specific modification of DNA by oligonucleotide reagents depends very much on stability of complementary complexes formed. We investigated the effect of a bulky antibiotic, covalently attached to **Ia**, on complex formation. It was found that T_m of the duplex **I**/target (33°C) was higher than that of the duplex with the parent oligonucleotide **Ia** (22°C). It means that, thanks to a stabilizing effect of the BLM-RH residue, bleomycin oligonucleotide derivatives should destroy functions of nucleic acids more effectively than parent oligonucleotides without bleomycin.

Fe^{2+}-complex of bleomycin is known to damage DNA effectively in the presence of oxygen and SH-compounds [15]. It was important to check this ability of the antibiotic covalently attached to an oligonucleotide. A model system shown in Fig. 1 was used. Oligonucleotide **II** was in the system to imitate double-stranded DNA. The results of destruction of the target are shown in Fig. 2. Slight 'doubling' of spots in cases of both **I** and free bleomycin is likely to be due to accumulation of target scraps with similar structure. It is known that DNA cleavage by free bleomycin can yield 3'-phosphates and 3'-phosphoglycolates of the target [10]. The DNA target was effectively cleaved by both free (lines 3,4) and bound (lanes 5,6) bleomycin. Along with breaks a few cross-links were observed (lanes 4,6) that disappeared after piperidine treatment (lanes 3,5). It is noteworthy that most of the breaks caused by both agents were direct, piperidine treatment causing very slight changes in modification products (cf. lanes 3,4 and 5,6). The modification by free bleomycin (lanes 3,4) and I (lanes 5,6) yielded rather different products. In the case of free BLM-RH maximal cleavage was observed at G^7-C^8 and G^6-G^7 of the target, whereas

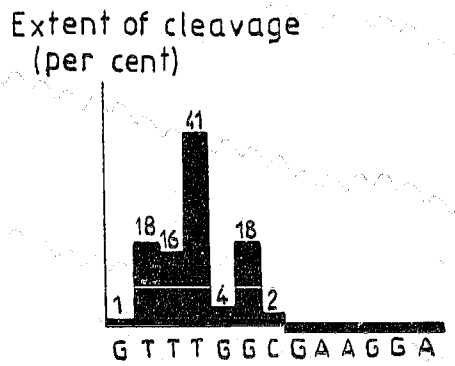

Fig. 3. Distribution of products upon cleavage of the DNA target by **I**. Total extent of the target cleavage was 80%.

reagent I mostly damaged the sites -T^4-T^4-T^5 and G^6-G^7 (Fig. 3). It did not affect G^7-C^8. Presumably this difference is due to the oligonucleotide moiety of I which directs the antibiotic group to the site of modification.

We have proven that the oligonucleotide part of I did not degrade during modification of the target (Fig. 1) in the following way. Reagent I was ^{32}P-labelled and incubated with the unlabelled target under conditions of the reaction. Then it was treated by piperidine and stored for 16 h at pH 3.5, to remove the antibiotic. Electrophoresis did not reveal any noticeable destruction of the heptanucleotide Ia.

To conclude, bleomycin A_5 covalently bound to an oligonucleotide can induce direct breaks of a DNA target without affecting its own oligonucleotide tail.

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