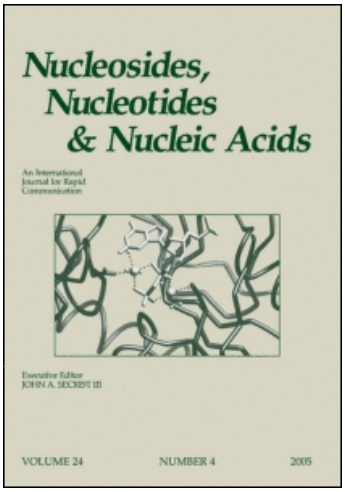

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 10 July 2004

To cite this Article Vorobjev, Pavel , Tchaika, Olessia and Zarytova, Valentina(2004) 'Efficient Cleavage of DS DNA by Bleomycin Conjugated via Hexaethylene Glycol Linker to Triplex-Forming Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 23: 6, 1047 — 1051
To link to this Article: DOI: 10.1081/NCN-200026066
URL: http://dx.doi.org/10.1081/NCN-200026066

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 6 & 7, pp. 1047–1051, 2004

Efficient Cleavage of DS DNA by Bleomycin Conjugated via Hexaethylene Glycol Linker to Triplex-Forming Oligonucleotides

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ABSTRACT

New conjugates of bleomycin A_5 with oligonucleotides are synthesized. The bleomycin residue was attached to the 3′- or 5′- terminus of hexadecathymidilate via a hexaethylene glycol phosphate linker. Newly designed conjugates were shown to cleave site-specifically both strands of a dsDNA fragment within a triplex. The maximum extent of cleavage for individual strand amounts to 61%.

Key Words: DNA cleavage; Triplex; Bleomycin-oligonucleotide conjugate; Hexaethylene glycol linker.

INTRODUCTION

Bleomycin (Fig. 1) is a glycopeptide antitumor antibiotic, which induces selective oxidative cleavage of nucleic acids (NA)[1] in a multiple turnover manner[2] in the presence of certain cofactors—molecular oxygen, Fe (II) ions and reducing agent. Bleomycin produces base-labile lesions on DNA as well as its direct breaks,[3] the latter

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DOI: 10.1081/NCN-200026066
1525-7770 (Print); 1532-2335 (Online)

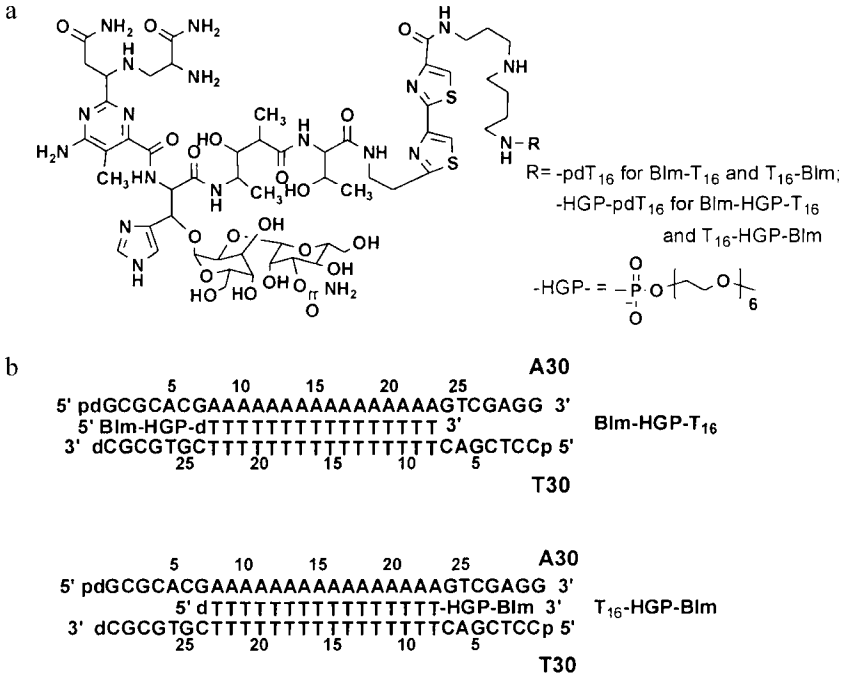

Figure 1. Structure of the bleomycin-hexadecathymidilate conjugates (a) and their triplexes with the target **M30** (b).

being especially important. These properties make bleomycin very prospective as a warhead for reactive oligonucleotide conjugates targeting pathogenic NA.

Previously, bleomycin was conjugated directly to the terminal phosphate group of triplex-forming oligonucleotides.[4] Bleomycin conjugates of hexadecathymidilate were shown to cleave both strands of a dsDNA fragment with moderate efficiency. Depending on the cleaved strand and the position of bleomycin attachment (5′ or 3′), the extent of cleavage varied from 25 to 48%. The minor groove of DNA duplex is believed to be a favorable region for interaction of bleomycin with DNA. On the other hand, the third strand interacts with the duplex in the major groove. The native bleomycin's spermine linker is apparently not long enough to allow bleomycin to be bound to DNA in an appropriate conformation.

In the current work we present an attempt to increase the efficiency of dsDNA cleavage. We suggest introducing a long flexible linker between the oligonucleotide and bleomycin moieties believing that it would facilitate the binding of the bleomycin residue within the minor groove, thus increasing the cleavage efficiency of the target duplex.

RESULTS AND DISCUSSION

As an example of such a linker flexible hexaethylene glycol phosphate (HGP) residue containing 21 bonds has been chosen (Fig. 1). It can be introduced into

oligonucleotides during the process of the automated synthesis. Bleomycin was conjugated to hexadecathymidilates (T_{16}) containing the HGP residue at the 3′- (T_{16}-HGP-Blm) or 5′-end (Blm-HGP-T_{16}).[5] Bleomycin-T_{16} conjugates (T_{16}-Blm and Blm-T_{16}) containing no linkers were synthesized analogously. Experiments on cleavage of dsDNA by triplex-forming bleomycin-oligonucleotide conjugates involved specially designed double-stranded 30-mer **M30** (Fig. 1) that met some essential criteria.[4] First, the target must contain a region of homopurine sequence necessary to form a triplex. Second, a triplex must be at a neutral pH, since bleomycin cleaves DNA efficiently in the pH range of 7–9. Third, the sequence involved in the triplex formation with the conjugate should be flanked with favorable targets for the bleomycin residue, i.e. GT, GC, AT, or GA motifs. Either purine-rich (**A30**) or pyrimidine-reach (**T30**) strands were ^{32}P-labeled.

Under conditions of the triplex formation and in the presence of Fe^{++} and β-mercaptoethanol, conjugates induce the cleavage of the target. All conjugates cleave DNA at both strands in the vicinity of the antibiotic residue. The cleavage of **A30** strand is shown in the Fig. 2; an analogous picture (not shown) is observed for **T30**

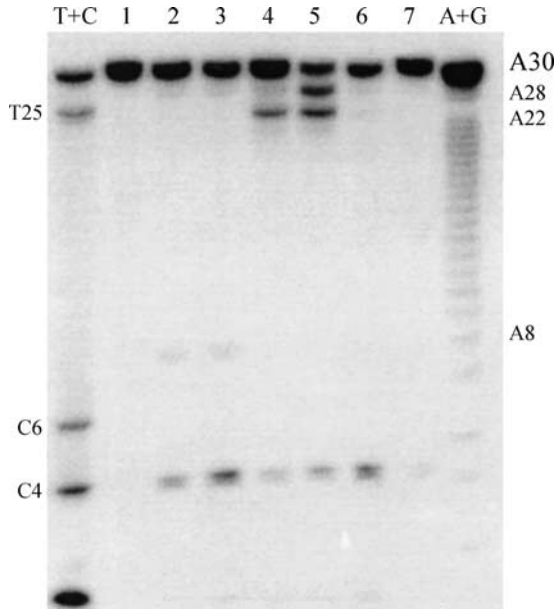

Figure 2. Authoradiogram of the electrophoretic analysis of the **A30** strand cleavage products in 20% PAGE. All reactions mixtures contained 1 M LiCl, 0.1 M Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and $3 \cdot 10^{-6}$ M **M30** target. Lane 1—no conjugate added, lane 2—Blm-T_{16}, lane 3—Blm-HGP-T_{16}, lane 4—T_{16}-Blm, lane 5—T_{16}-HGP-Blm, lane 6—non-conjugated bleomycin, lane 7—scrambled conjugate Blm-TTTCATTGTTTTCCA. Concentration of the conjugates or free bleomycin was $1.5 \cdot 10^{-5}$ M. Reaction mixtures were pre-incubated during 18 h at 20°C, then the reaction was started by addition of 10^{-4} M $Fe(NH_4)_2(SO_4)_2$, 0.05 M β-mercaptoethanol, and run for 5 h at 20°C. Prior to electrophoresis all reaction mixtures were treated with 0.2 M 1-buthylamine at 95°C for 8 min.

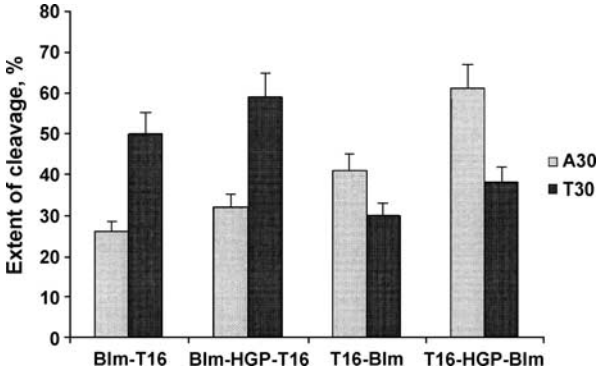

Figure 3. The extent of cleavage of each strand (A30 and T30) by conjugates Blm-HGP-T_{16} and T_{16}-HGP-Blm in comparison with the conjugates Blm-T_{16} and T_{16}-Blm.

strand. The third strand binds in parallel to purine and antiparallel to pyrimidine strands. This is consistent with the published data on the cleavage of double-stranded DNA by Fe(III)-EDTA-containing oligonucleotide conjugates which form a triplex with the target DNA.[6]

The cleavage of **M30** by HGP containing conjugates occurs virtually at the same sites, which are damaged by conjugates T_{16}-Blm and Blm-T_{16} (Fig. 2). A weak non-specific cleavage can be observed at both strands. These ''non-specific'' sites are cleaved in reactions with non-conjugated bleomycin (with the cleavage extent up to 25%, Fig. 2, lane 6) and control conjugate which is incapable of forming the triplex (up to 5%, Fig. 2, lane 7). Conjugates T_{16}-HGP-Blm and Blm-HGP-T_{16} induce enhanced cleavage of the target DNA at both strands as compared to conjugates T_{16}-Blm and Blm-T_{16} (Fig. 3). For instance, 61% of **A30** strand is cleaved by conjugate T16-HGP-Blm, whereas for conjugate T16-Blm the extent of cleavage achieves only 41%. Thus, the cleavage of the target DNA fragment by newly synthesized HGP containing conjugates is by 20–30% more efficient than the cleavage induced by the conjugates containing no linkers.

ACKNOWLEDGMENTS

Hexadecathymidilates containing HGP linkers were kindly provided by Inna Pyshnaya, Institute of Chemical Biology and Fundamental Medicine.

The work was supported by RFBR (grant 02-04-49597-a) and BRHE (grant REC-008).

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Received December 2, 2003
Accepted April 23, 2004