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Nick-containing oligonucleotides as human topoisomerase I inhibitors

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ABSTRACT

A series of oligonucleotides with various lengths that contain nick and topoisomerase I-binding sites were designed. The interactions between these oligonucleotides and human topoisomerase I were investigated and the most efficient one among them has displayed IC_{50} value of 6.3 nM. Our studies have also demonstrated that the position of the nick as well as the length of the oligonucleotides were crucial factors for the inhibition of this nuclear enzyme.

DNA topoisomerase I (topo I) is an enzyme that plays vital roles in releasing the topological stress of DNA generated by cellular metabolic processes such as replication and transcription. During the course of its function, this nuclear enzyme introduces a nick into one strand of supercoiled DNA, allows free rotation about sigma bonds in the strand opposite the nick and then reseals the original break,[1–4] thus leading to relaxation of the supercoiled DNA (Fig. 1a). This nuclear enzyme has been found to be abundant in the fast proliferating tumor cells and is thus known to be the molecular target of several anticancer agents such as camptothecins, indolocarbazoles and indenoisoquinolines.[5,6] In their actions, these potent anticancer agents can bind to the nuclear enzyme to form a transient topoisomerase I–DNA covalent complex which consequently inhibits the resealing of a single-strand nick that the enzyme creates to relieve superhelical tension in duplex DNA.[7] Besides these quinoline alkaloids that serve as topo I inhibitors, efforts have been made in the past few years to examine the mechanism of topo I interaction with several linear duplex oligonucleotides (ONs).[8,9] It was shown in these studies that certain designed ONs containing either mismatched base pairs or internal C3-spacers exhibited high potency on the activity of this DNA relaxing enzyme as well as resistance to degradation by DNA repair enzyme.[10,11]

With the aim of further examining whether other structural variation of ONs could act as irreversible inhibitors of this nuclear enzyme, we have designed some ONs that contain nicks located at different positions in the sequences of their scissile strands.

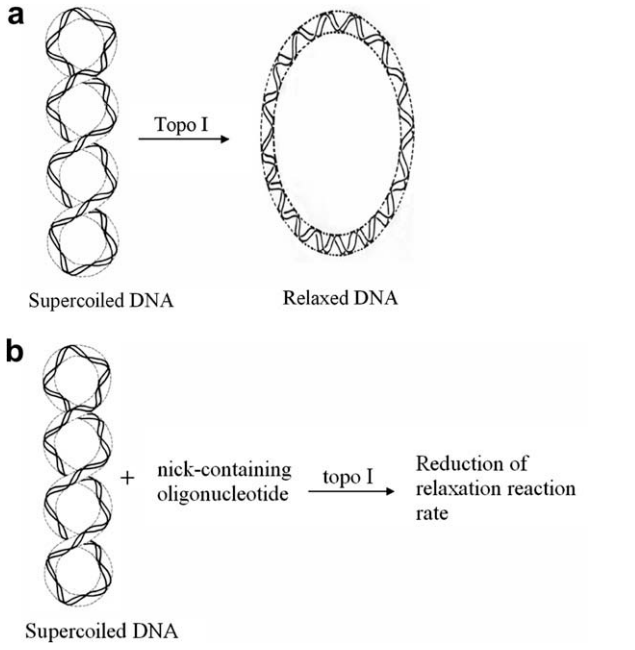

Figure 1. (a) Illustration of relaxation reaction of supercoiled DNA catalyzed by topo I. (b) Anticipated roles of nick-containing ON in the current studies.

Abbreviations: Topo I, topoisomerase I; Oligonucleotide, ON; IC_{50}, the concentration of a drug that is required for 50% inhibition of the activity.

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Figure 2. Sequences of oligonucleotides used in the current studies. The underlined tracts in Duplex 1 denote the topoisomerase I binding sequences. The cutting site by topoisomerase I is indicated by ↓.

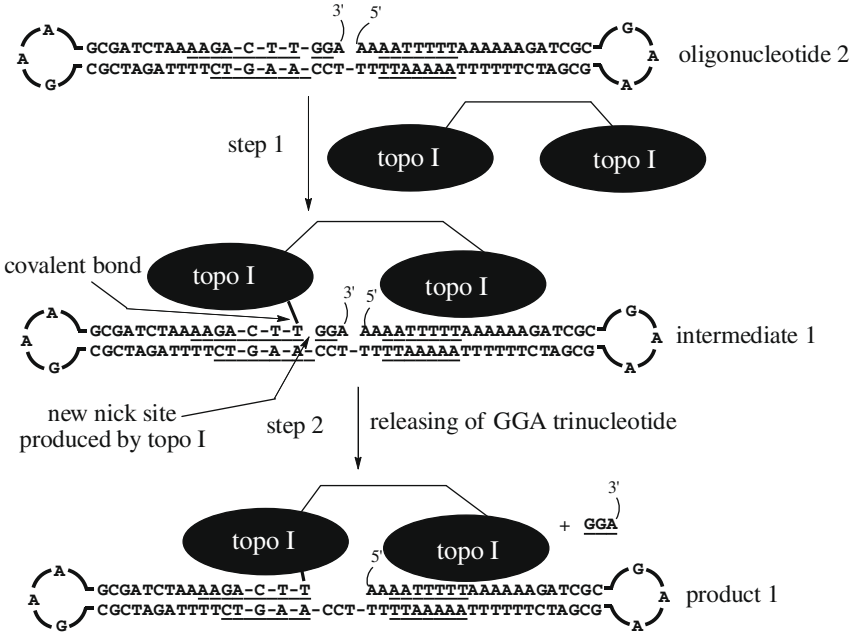


Figure 3. Schematic illustration of anticipated inhibitory effect of Oligonucleotide 2 on the activity of human topo I in our studies.

Further examination revealed that some of these designed ONs have demonstrated high inhibitory effect on the activity of human topo I in its relaxation reaction of negatively supercoiled pBR322. Our detailed studies on these ONs as well as the correlation between inhibitory effects and the locations of the nicks are discussed in this report.

It is known that topo I could bind and cleave some duplex forms of ONs besides its innate substrate of supercoiled DNA. The minimal requirements for the duplex structure at its binding are $A_{-7}A_{-6}G_{-5}A_{-4}C_{-3}T_{-2}T_{-1}G_{+1}G_{+2}$ and $A_{+6}A_{+7}T_{+8}T_{+9}T_{+10}T_{+11}T_{+12}$ on the scissile strand and $C_{-5}T_{-4}G_{-3}A_{-2}A_{-1}$ and $T_{+6}T_{+7}A_{+8}A_{+9}A_{+10}A_{+11}A_{+12}$ on the non-scissile strand[12] (underlined segments of Duplex 1 in Fig. 2) while the cutting site by topo I occurs between T_{-1} and G_{+1}.[8,12] Oligonucleotide 2 (Fig. 2) was accordingly designed during our investigations in order to study the inhibitory effect of a nick ON on topo I. Two 'extremely stable hairpin' were introduced onto both ends of the duplex structure of Oligonucleotide 2 as these hairpin structures could in theory lead to an increase of the thermal stability of the ON and the resistance of hydrolysis by exonuclease.[13,14] Since Oligonucleotide 2 contains a topo I-recognizing site, it is anticipated that this topoisomerase could in theory bind to this oligonucleotide and subsequently cause a strand cleavage between T_{-1} and G_{+1} (Fig. 3).[15,16] A trinucleotide stretch ($G_{+2}G_{+3}A_{+4}$) would consequently be generated and dissociate away from its complementary strand once a cutting site is created within the sequence of Oligonucleotide 2 by topo I (Intermediate 1). It is anticipated that the rejoining reaction between these cut fragments would not be able to take place properly due to the dissociation of $G_{+2}G_{+3}A_{+4}$ (Step 2), resulting in chemically irreversible damages to the nuclear enzyme (Product 1 in Fig. 3).[17,18]

Through determining the efficiency of relaxation reaction of pBR322 catalyzed by topo I in the presence of our designed ON, the inhibitory effect of Oligonucleotide 2 on topo I was investigated (Fig. 4). Oligonucleotide 2 was accordingly incubated with topo I for 3 min followed by the addition pBR322 to the corresponding reaction mixture. As shown in Figure 4, relaxation of the negatively supercoiled form of pBR322 by topo I proceeded to near completion in the absence of Oligonucleotide 2 (lane 8). With the increase of concentrations of Oligonucleotide 2 from 0.1 to 1000 nM, however, the relaxation reactions of

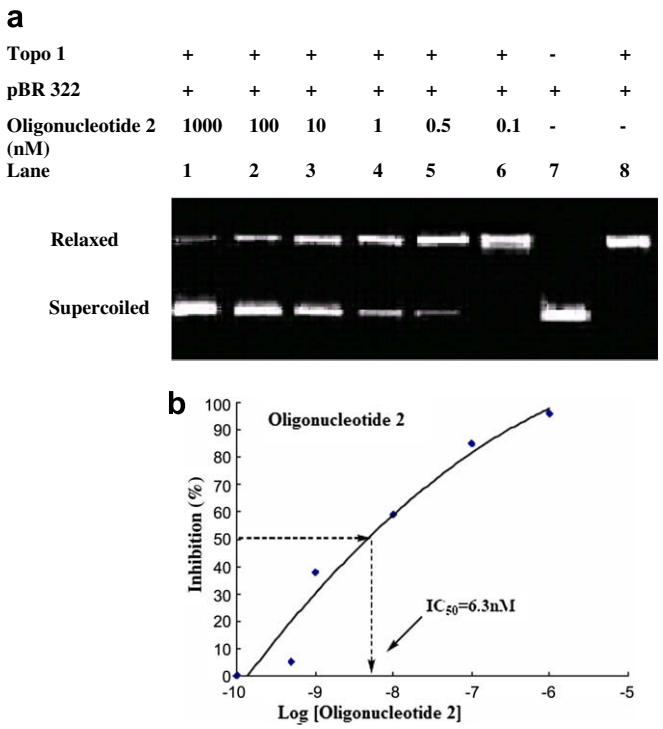


Figure 4. (a) Agarose gel electrophoretic analysis of inhibitory effects of Oligonucleotide 2 on human topoisomerase I. (b) Plotting of percentage of inhibition vs concentration of ONs. Lane 7, substrate pBR 322 alone; lane 8, pBR 322 and 1 U topo I in the absence of Oligonucleotide 2; lanes 1–6, pBR 322, topo I and concentrations of Oligonucleotide 2 loaded (1000 nM, 100 nM, 100 nM, 1 nM, 0.5 nM and 0.1 nM, respectively).

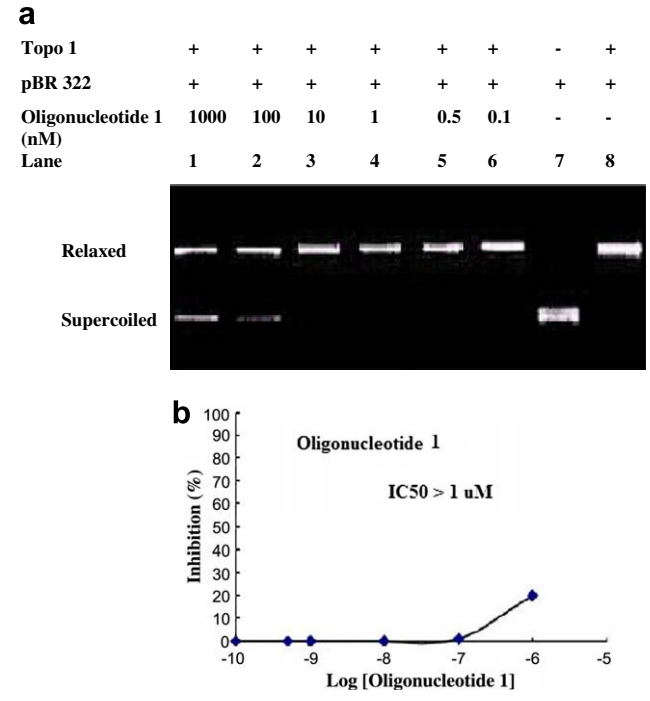


Figure 5. (a) Agarose gel electrophoretic analysis of inhibitory effects of Oligonucleotide 1 on human topoisomerase I. (b) Its IC_{50} curve. Lane 7, substrate pBR 322 alone; lane 8, pBR 322 and 1 U topo I in the absence of Oligonucleotide 1; lanes 1–6, pBR 322, topo I and concentrations of Oligonucleotide 1 loaded (1000 nM, 100 nM, 100 nM, 1 nM, 0.5 nM and 0.1 nM, respectively). The same procedure was carried out as Figure 4 except for replacing Oligonucleotide 2 with Oligonucleotide 1.

pBR322 decrease drastically (lanes 1– 6). The IC_{50} value of Oligonucleotide 2 obtained from our studies is 6.3 nM, which is smaller than those of many organic inhibitors of topo I used in clinical studies.[19,20] The above observations indicate that Oligonucleotide 2 could indeed serve as a highly effective inhibitor of topo I in the relaxation reaction of negatively supercoiled DNA.

For comparison purpose, Oligonucleotide 1 was synthesized next in our studies through ligation reactions of a linear 86-mer ON catalyzed by DNA T_4 ligase. The inhibitory effect of Oligonucleotide 1 on the activity of topo I was further examined in the same way as those designed for Oligonucleotide 2. As shown in Figure 5, the IC_{50} value Oligonucleotide 1 is greater 1.0 μM, which indicates that the inhibitory efficiency of Oligonucleotide 1 on topo I is very low. This low inhibitory efficiency of Oligonucleotide 1 could be caused by the fact that Oligonucleotide 1 contains no nick site and hence is not capable of forming an irreversible linkage with topo I[17,18] as Oligonucleotide 2 does.

In order to establish the correlation between the positions of the nick sites in the dumbbell-shaped ONs and their inhibitory effects on human topo I, a series of new ONs (Table 1) were subsequently designed and investigated. The observed inhibitory efficiencies of these designed ONs (Fig. 6) are as follows:

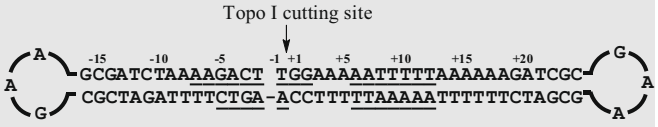
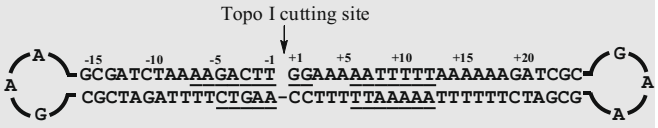
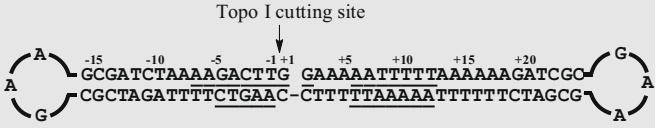
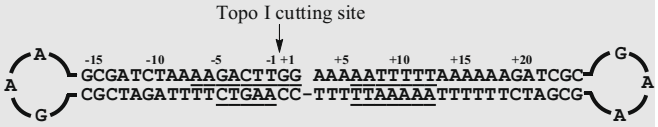
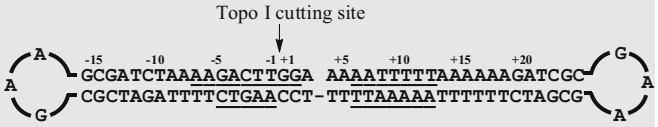
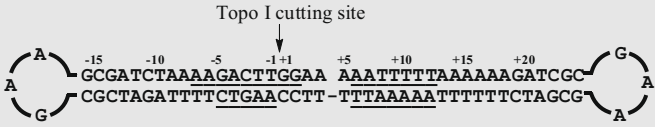
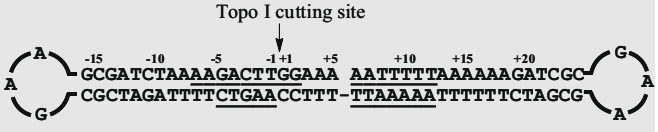
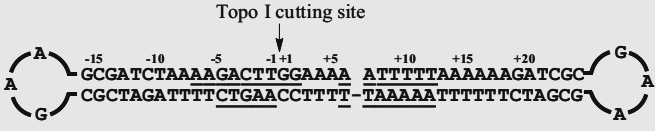
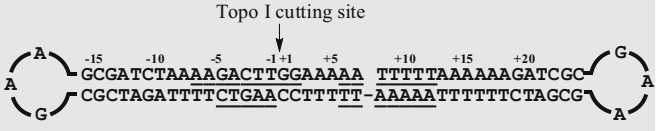
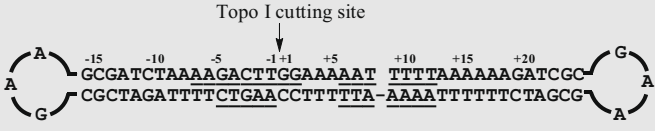
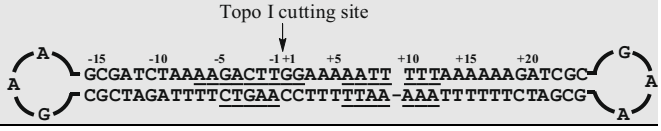
Oligonucleotide 2 (81% inhibition) > Oligonucleotide 8 > Oligonucleotide 7 > Oligonucleotide 6 > Oligonucleotide 9 > Oligonucleotide 5 > Oligonucleotide 10 > Oligonucleotide 4 > Oligonucleotide 3 ≈ Oligonucleotide 11 ≈ Oligonucleotide 12.

As seen in Table 1, the nick sites of Oligonucleotide 3, Oligonucleotide 4, Oligonucleotide 5, Oligonucleotide 9, Oligonucleotide 10, Oligonucleotide 11 and Oligonucleotide 12 occur within the binding domains of topo I, which could consequently interfere the binding of topo I to these ONs. Unlike the above-mentioned seven ONs, on the other hand, the nick sites of Oligonucleotide 2, Oligonucleotide 7 and Oligonucleotide 8 appears outside the topo I-binding domains, which may have consequently little effect on the binding of topo I to the oligonucleotide sequences. This could in turn explain the relatively high inhibitory efficiencies of these three oligonucleotides on the activity of the human topoisomerase. In addition, once Oligonucleotide 2, Oligonucleotide 7 and Oligonucleotide 8 are cleaved by topo I, trimer (GGA), tetramer (GGAA) and pentamer (GGAAA) will be generated. The easiness of dissociation of trimer from its complementary strand than the corresponding tetramer and pentamer could be the cause of relatively high efficiency of inhibition of Oligonucleotide 2 than those of Oligonucleotide 7 and Oligonucleotide 8.

In order to examine how the lengths of ONs will affect their inhibitory effects on topo I, Oligonucleotide 13 and Oligonucleotide 14 (Table 2) were designed and investigated during our studies, which were 3 bp shorter than that of Oligonucleotide 2. As shown in Figure 7, the inhibitory effects of these oligonucleotides on the activity of topo I decreased significantly as compared to Oligonucleotide 2. In addition, the oligonucleotide that was 6 (Oligonucleotide 15) and 9 bp (Oligonucleotide 16 and Oligonucleotide 17) shorter than that of Oligonucleotide 2 was examined. It turned out that these three oligonucleotides exhibited little or no observable inhibitory effects on topo I (Fig. 7).

In conclusion, a series of ONs containing nick and topo I-binding site were designed and their inhibitory effects on the activity of human topo I were examined in our studies. The IC_{50} value of one of the ONs is as low as 6.3 nM, which is smaller than those previously reported.[10,11] In addition, our studies demonstrated that both the locations of nick sites and the lengths of ONs affect the inhibitory action significantly. It is our hope that the information provided in these studies could benefit future design of new ON-based therapeutical agents[21–23] as inhibitors of human topo I.

Table 1
Sequences of oligonucleotides used in our studies that contain nick sites at different positions

Name	Structure
Oligonucleotide 3	
Oligonucleotide 4	
Oligonucleotide 5	
Oligonucleotide 6	
Oligonucleotide 2	
Oligonucleotide 7	
Oligonucleotide 8	
Oligonucleotide 9	
Oligonucleotide 10	
Oligonucleotide 11	
Oligonucleotide 12	

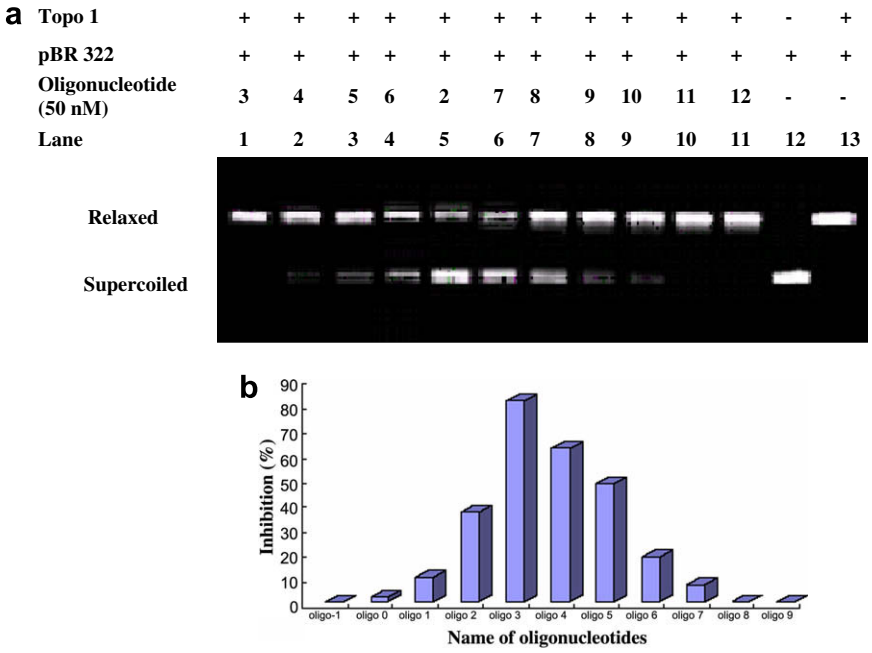


Figure 6. (a) Agarose gel electrophoretic analysis of inhibitory effects of Oligonucleotide 2 to Oligonucleotide 12 on human topoisomerase I and (b) percentage inhibition of relaxation reaction by these oligonucleotides. Lane 12, substrate pBR 322 alone; lane 13, pBR 322 and 1 U topo I in the absence of oligonucleotides; lane 1–11, pBR 322, topo I and oligonucleotides with different nick positions. The same procedure was carried out as Figure 4 except for replacing Oligonucleotide 2 with different nick positions ONs.

Table 2
Sequences of oligonucleotides used in our studies that possess different lengths

Name	Structure
Oligonucleotide 2	Topo I cutting site -15 -10 -5 -1 +1 +5 +10 +15 +20 GCGATCTAAAAGACTTGGA AAAATTTTTAAAAAAGATCGC CGCTAGATTTTCTGAACCT-TTTTAAAAATTTTTTCTAGCG
Oligonucleotide 13	Topo I cutting site -12 -10 -5 -1 +1 +5 +10 +15 +20 GCGTAAAAGACTTGGA AAAATTTTTAAAAAAGATCGC CGCATTTTCTGAACCT-TTTTAAAAATTTTTTCTAGCG
Oligonucleotide 14	Topo I cutting site -15 -10 -5 -1 +1 +5 +10 +15 +20 GCGATCTAAAAGACTTGGA AAAATTTTTAAAAAACGC CGCTAGATTTTCTGAACCT-TTTTAAAAATTTTTTGCG
Oligonucleotide 15	Topo I cutting site -12 -10 -5 -1 +1 +5 +10 +15 +20 GCGTAAAAGACTTGGA AAAATTTTTAAAAAACGC CGCATTTTCTGAACCT-TTTTAAAAATTTTTTGCG
Oligonucleotide 16	Topo I cutting site -10 -5 -1 +1 +5 +10 +15 +20 GCGAAGACTTGGA AAAATTTTTAAAAAACGC CGCTTCTGAACCT-TTTTAAAAATTTTTTGCG
Oligonucleotide 17	Topo I cutting site -12 -10 -5 -1 +1 +5 +10 +15 GCGTAAAAGACTTGGA AAAATTTTTAAACGC CGCATTTTCTGAACCT-TTTTAAAAATTTGCG

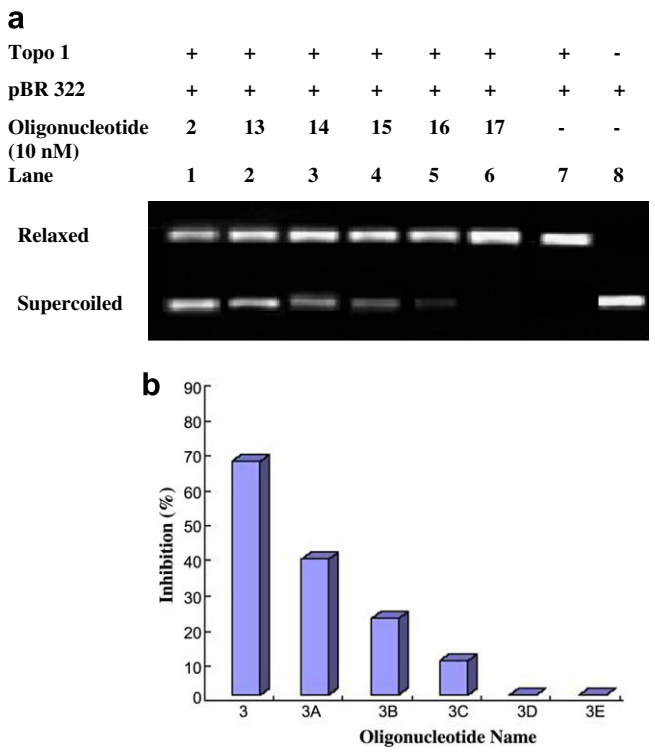


Figure 7. (a) Agarose gel electrophoretic analysis of inhibitory effects of different length of oligonucleotides on human topoisomerase I and (b) percentage inhibition of relaxation reaction by these oligonucleotides. Lane 8, substrate pBR 322 alone; lane 7, pBR 322 and 1 U topo I in the absence of oligonucleotides; lanes 1–6, pBR 322, topo I and oligonucleotides with different lengths. The same procedure was carried out as Figure 4 except for replacing Oligonucleotide 2 with different length ONs.

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