High affinity interaction of mammalian DNA topoisomerase I with short single- and double-stranded oligonucleotides

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Abstract The interaction of DNA topoisomerase I (topo I) with a set of single- and double-stranded oligonucleotides containing 5–27 mononucleotides was investigated. All single- and double-stranded oligonucleotides were found to inhibit competitively the supercoiled DNA relaxation reaction catalyzed by topo I. The enzyme affinity for specific sequence pentanucleotides of the scissile (GACTT, $K_i = 2$ μM) and non-cleaved chain (AAGTC, $K_i = 110$ μM) is about 2–4 orders of magnitude higher than that for non-specific oligonucleotides. This specific sequence affinity increases in several cases: lengthening of single-stranded oligonucleotides, formation of stable duplexes between complementary oligonucleotides and preincubation of the enzyme with ligands before addition of supercoiled DNA. We assume that oligonucleotides having a high affinity to the enzyme can offer a unique opportunity for rational design of topoisomerase-targeting drugs.

Key words: DNA topoisomerase I; Oligodeoxynucleotide; Competitive inhibition

1. Introduction

DNA topoisomerases are enzymes that change the topological state of DNA by transient breakage and rejoining of the sugar–phosphate backbone [1–5]. Topos have been shown to be involved in various biological processes such as DNA replication, transcription, recombination and chromosome dynamics [1–9], and are the pharmacological targets of a number of antitumor drugs [10–12]. This holds for topo I [13–17] and thus a better understanding of the molecular mechanisms of functioning of this enzyme is important from fundamental and applied points of view.

Topo I is a sequence-dependent enzyme. It protects both strands of the binding sequence over a 20-bp region in which the cleavage site is centrally located [18]. Minimal DNA duplex requirements for DNA cleavage in the recognition sequence have been delimited to nine nucleotides on the scissile strand and five nucleotides on the non-cleaved strand as illustrated below [19]:

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                point of cleavage
5'-TAA AAGACTT↓AG AAAAATT-3'
3'-ATT TTCTGAA TC·TTT TT AA-5'
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Abbreviations: IC_{50}, the concentration giving 50% inhibition of the activity; topo, DNA topoisomerase; ODN, oligodeoxynucleotide; ss and ds, single- and double-stranded, respectively; sc DNA, supercoiled DNA.

Since cleavage in DNA occurs only at sites situated in regions with the potential for intrastrand base-pairing due to distal complementary sequences [20], topo I has been considered to be a ds-specific enzyme. In fact in the literature there are no data as to whether topo I is capable of binding to short ss ODNs. Furthermore, it is a general belief that ds DNA-dependent enzymes cannot interact with short ss ODNs (for review see [21]). We have shown previously that this is not true using various kinds of DNA polymerases and repair enzymes [21]. Thus it is relevant and of great interest to examine whether topo I binds with any affinity to short ss ODNs with specific sequences.

We therefore investigated the possibility of complex formation of topo I with ss and ds short ODNs of specific and non-specific sequences. The results demonstrated that both specific and non-specific ODNs have a high affinity to the enzyme and they are effective inhibitors of topo I-dependent relaxation of supercoiled DNA.

2. Materials and methods

Sc colE1 DNA was purchased from Wakojunyaku Co. Ltd. (Japan). All ODNs were synthesized as in [22]. Concentrations of ODNs were determined according to [23]. Topo I was obtained from mouse Ehrlich ascites tumor cells as before [24].

Topo I activity was measured by assessing relaxation of sc colE1 DNA at 30°C. The reaction mixture (20 μl) contained: 50 mM Tris-HCl (pH 8.0), 70 μg/ml BSA, 0.5 mM DTT, 0.5 mM EDTA, 15% glycerol, 130 mM NaCl, 10 μg/ml colE1 sc DNA and 1 unit of topo I. ODNs were used at different concentrations.

Samples were incubated for 15 min and the results for ODN inhibition of topo I-dependent relaxation of sc DNA were estimated with two methods: (a) agarose gel electrophoresis as in [24] and the pH 12 ethidium fluorescence assay according to [25]. Preincubation of topo I with ODNs was done using standard conditions without DNA for 15 min at 23°C. The relaxation reaction was started by addition of colE1 DNA and the mixture was further incubated for 15 min at 30°C. Analysis of the reaction mixtures was performed using two methods as described above.

^{32}P-labeled at the 5'-end (using T4 polynucleotide kinase and γ-[^{32}P]ATP) ss ODNs and their duplexes were analyzed for covalent suicidal binding with the enzyme analogously to [19].

The K_i values for ODNs were estimated from fluorescence assay using dependencies $1/V$ versus $1/S$ according to [26].

3. Results and discussion

We examined whether topo I, like some other ds DNA-dependent enzymes, is capable of binding different short ss and ds ODNs. The ODNs containing cleaved strand sequences (corresponding to duplex region required for topo I-mediated cleavage, see section 1) were marked as C-ODNs and those

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Table 1
Concentrations of oligonucleotides inhibiting the DNA relaxation activity of DNA topoisomerase I by 50% (IC_{50})

Oligos and their mixtures	IC_{50} (1) – preincubation (mM)	IC_{50} (2) + preincubation (mM)	Ratio IC_{50} (1)/ IC_{50} (2)
d(pT)5	15,600	5,200	3
d(pA)5	12,600	4,200	3
d(pT)5 + d(pA)5	2,100	700	3
NC5	110	10	11
C5	2.0	0.4	5
NC9	2.5	0.2	12.5
C9	2.0	0.4	5
C5 + NC5	9.0	1.2	7.5
C9 + NC5	0.8	0.2	4.0
C5 + NC9	35.0	2.0	17.5
C9 + N9	0.8	0.1	8.0
NC27 + C27	0.07	0.01	7.0

All ODNs and their mixtures were heated for 5 min at 95°C and were slowly cooled to 20°C, and then were assayed for their inhibition of topo I relaxation activity. Sequences of oligonucleotides used (5'-3'): C5, GACTT; C9, AAGACTTAG; C27, AAAAAGACTTAGAAAA-ATTTTTAAAG; NC5, AAGTC; NC9, CTAAGTCTT; NC27, CTT-TAAAAATTTTTCTAAGTCTTTTTT. Standard errors did not exceed 20–40%.

containing non-cleaved strand sequences as NC-ODNs (see Table 1). Results for ODN inhibition of topo I-dependent relaxation of sc DNA were obtained with two methods; standard agarose gel electrophoresis and the pH 12 ethidium fluorescence assay coincided very well, as illustrated in Fig. 1 using NC5 as an example. Only the ethidium fluorescence assay allows an accurate quantitation of the enzyme inhibition. Interference by ODNs with the ethidium fluorescence measurement was not observed. Therefore the latter method was applied for estimation of the relative amounts of relaxed and sc colE1 DNA in the reaction mixtures in the absence and in the presence of ODNs.

All ss- and ds-specific and non-specific ODNs were found to inhibit topo I. Fig. 2 demonstrates that C9 is a competitive inhibitor (K_i = 1.5 ± 0.6 μM) towards sc DNA. The same result was obtained for C9 × NC9 and $d(pT)_5$ (K_i = 0.5 ± 0.2 μM and 10 ± 4 mM, respectively). The above K_i values are comparable with IC_{50} values estimated for ODNs (Table 1). For all other ODNs IC_{50} values were analyzed and are summarized in Table 1.

Preincubation of topo I at 23°C without ligands for 1 h did not change its activity. As shown in Fig. 3, addition of 30 or 100 μM NC5 to the reaction mixture resulted in 30% and 50% inhibition, respectively, (no preincubation), when preincubation of topo I with the NC5 for 10 min (effect completed within 10 min) caused a pronounced increase in inhibition up to 60% and 90%, respectively. Similar results were revealed for all ss and ds ODNs (Table 1).

Incubation of topo I with sc DNA leads usually to the 'suicide covalent complex' formation [27,28]. We preincubated topo I with 5'-[^{32}P]-labeled ss ODNs C27, C9, $d(pT)_5$ and ds ODNs C27 × NC27, C9 × NC9, $d(pT)_5$ $xd(pA)_5$ for 15 min at 23°C. No detectable covalent binding of the label with the protein was observed by polyacrylamide gel electrophoresis. Thus increase of the inhibition level after topo I preincubation with ODNs is not due to covalent attachment of ODNs to the enzyme but rather to reversible interaction and probably transition of ODNs to optimal conformation.

As one can see from Table 1, the enzyme affinity for specific ODNs (GACTT and AAGTC) is about 2–4 orders of magnitude higher than that for non-specific $d(pT)_5$ or $d(pA)_5$. This specific sequence affinity increases in several cases: lengthening of ss ODNs, formation of stable duplexes between complementary ODNs and preincubation of the enzyme with ligands before addition of sc DNA.

As the melting point of the C27 × NC27 complex should be significantly higher than the reaction temperature (30°C) [29,30], it is not surprising that the C27 × NC27 duplex demonstrated the highest affinity to the enzyme. The melting point of ODNs with complementary penta- and nonanucleotides are lower than 7–8°C and 25°C, respectively [27,28]. In spite of this, mixtures of C9 + NC5 or C9 + NC9 gave IC_{50} values which are at least 4–20 times lower than those of corresponding ss NC5, C9 or NC9 (Table 1). Thus after initial recognition of ss ODNs, which cannot form duplexes by themselves at 30°C, the enzyme combines and stabilizes them as duplexes.

The present findings (Table 1) speak in favor of a different contribution to the affinity of two chains of complementary duplexes. The contribution to the affinity of two chains of complementary oligonucleotides is far from being simply additive (Table 1). It seems most likely that one chain of the duplex forms a strong specific contact with the enzyme and the other chain a significantly weaker one.

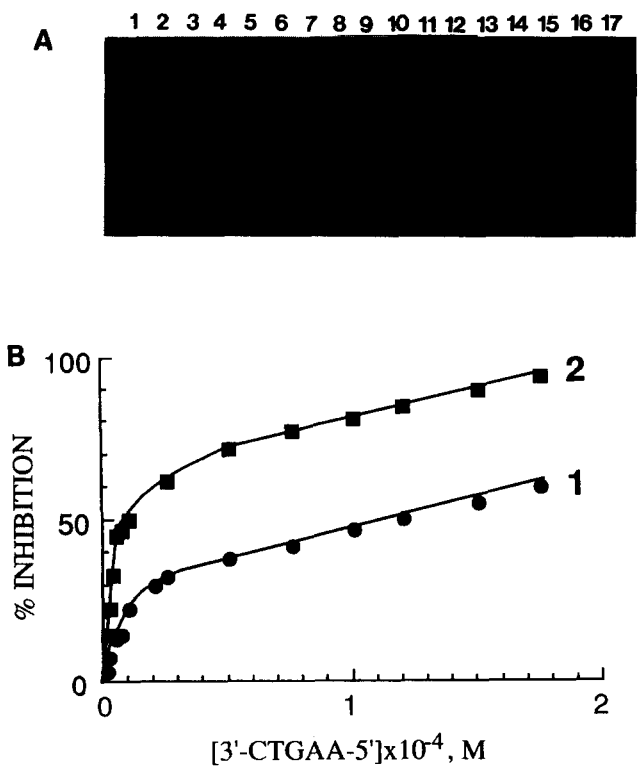

Fig. 1. Comparison of NC5 inhibition of topo I relaxation activity by agarose gel electrophoresis (A) and by ethidium fluorescence assay (B) before (A, lanes 3–9; B, 1) and after preincubation of the enzyme with NC5 (A, lanes 10–16; B, 2). The concentrations of NC5 used: 1 (lanes 3,10), 2 (lanes 4,11) 5 (lanes 5,12), 10 (lanes 6,13), 20 (lanes 7,14), 100 (lanes 8,15) and 200 μM (lanes 9,16). Standard reaction mixtures incubated without topo I (lanes 1,17) or without NC5 (lane 2) were used as controls.

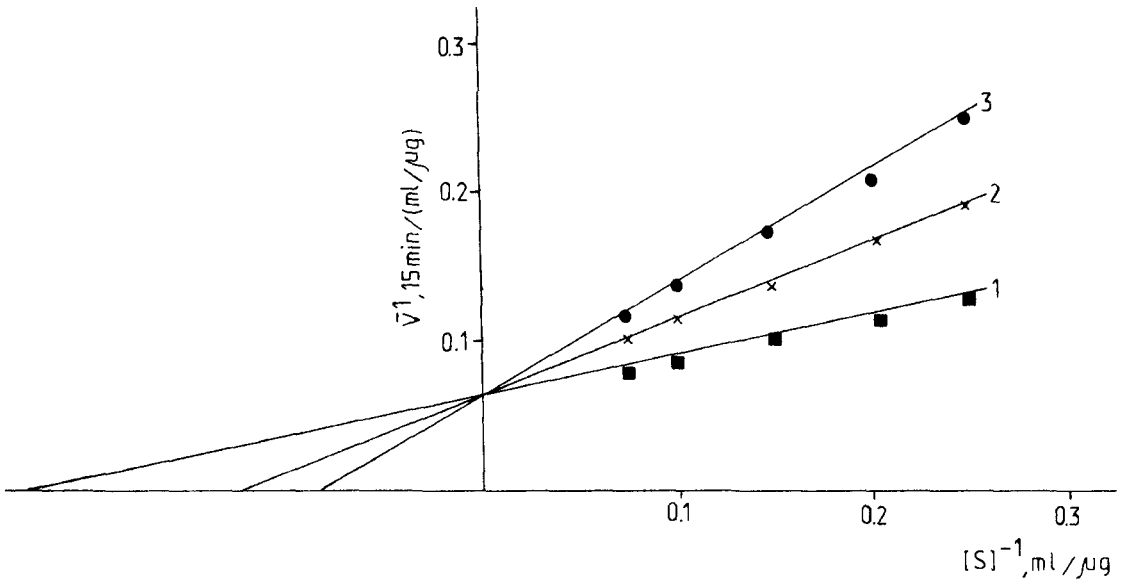

Fig. 2. Inhibition of the relaxation catalyzed by topo I was measured at various concentrations of supercoiled DNA substrate (S) in the presence of C9 at different concentrations: 1.0 (1), 2.0 (2) and 3.0 μM (3), respectively.

We have shown here, for the first time, that topo I can interact with high affinity even for short ODNs of specific sequence. The natural substrate of topo I is sc DNA having torsional stress, and the lack of this stress prevents, or at least markedly decreases, the efficiency of substrate topoisomerization [30,31]. Therefore the highest affinity observed for the C27 × NC27 complex was still about 2–3 orders of magnitude lower than that for sc DNA (K_m = 0.1 nM). At the same time, affinity of some short specific ODNs is higher than that of most known specific inhibitors of topo I, e.g. the IC_{50} value for camptothecin (IC_{50} = 30 μM) [14,16]. Thus specific oligonucleotides offer a unique opportunity for rational design of topoisomerase-targeting drugs.

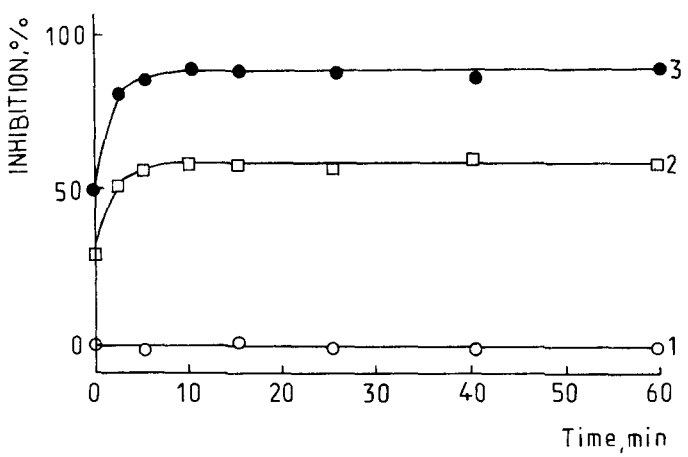

Fig. 3. Effect of preincubation of topo I with oligonucleotides. Topo I was incubated at 23°C in the absence (1) or in the presence of NC5: 30 (2) or 100 μM (3) in the standard reaction mixture without sc DNA. Aliquots of the mixture (16 μl) were drawn at intervals of 1–20 min and the relaxation reaction was started by addition of 4 μml solution of sc DNA, followed by additional incubation for 15 min at 30°C. The difference between topo I activity in the absence and in the presence of the NC5 at zero time incubation was taken as the enzyme inhibition by NC5 without preincubation.

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