FEBS Letters 407 (1997) 18–20 FEBS 18236

High affinity interaction of mouse DNA topoisomerase I with di- and trinucleotides corresponding to specific sequences of supercoiled DNA cleaved chain

Dmitry V. Bugreev[a], Elena L. Vasyutina[a], Valentina N. Buneva[a], Yoshihiro Yasui[b], Miwako Nishizawa[b], Toshiwo Andoh[b], Georgy A. Nevinsky[a,*]

[a]*Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of the Russian Academy of Sciences, Novosibirsk 630090, Russia*
[b]*Aichi Cancer Center Research Institute, Laboratory of Biochemistry, 1-1 Kanokoden Chikusa-ku, Nagoya 464, Japan*

Received 14 December 1996

Abstract Recently mouse DNA topoisomerase I (topo) was shown to possess high affinity for a single-stranded AAGACTTAG nonanucleotide (K_i = 2.0 μM) corresponding to the scissile strand of the minimal DNA duplex, which is necessary for cleavage of supercoiled DNA. In order to determine the most important part of the above sequence for the DNA recognition by topo, the interactions of the enzyme with a set of extremely short (2–5 nucleotides in length) oligonucleotides corresponding to different parts of the nonanucleotide have been investigated. The affinities of different oligonucleotides corresponding to the CTTAG part of the sequence (K_i = 0.13–0.92 mM) were shown to be significantly lower than that for the AAGA tetranucleotide (K_i = 9.0 μM). Topo effectively recognized even short oligonucleotides containing only two or three bases (AGA and pAG, K_i = 20 and 50 μM). We suppose that oligonucleotides having a high affinity to the enzyme can offer a unique opportunity for the 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 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]:

point of cleavage

5' - TAA **AAGACTT**↓**AG** AAAAATT - 3'

3' - ATT TT **CTGAA** TC TTT TT AA - 5'

It is a general belief that ds DNA dependent enzymes cannot interact with short ss ODNs (for review see [20]). Since cleavage in DNA occurs only at sites situated in regions with the potential for intrastrand base-pairing due to distal complementary sequences [21], topo has been considered to be a ds specific enzyme. Nevertheless, we have shown recently [22] that mouse topo can recognize and effectively bind specific and non-specific sequence ODNs containing 9–27 nucleotide units. All ss and ds specific and non-specific ODNs have been shown to inhibit competitively the sc DNA relaxation reaction, catalyzed by mammalian topo. The enzyme affinity for specific sequence ODNs of the scissile chain was higher than that for oligonucleotides of the non-cleaved chain; both types of specific ODNs had affinities about 2–4 orders of magnitude higher than that for non-specific ODNs. 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.

To elucidate the factors governing the sequence specificity of eukaryotic topo, the enzyme binding to various defined DNA (depurinated, depyrimidinated and methylated DNA) and substrate properties of such DNA were analyzed [18]: ACTT and AAGACT minimal consensus sequences were found to be the most essential for recognition and cleavage of sc DNA by the enzyme.

Taking into account the high affinity of the ss nonanucleotide for topo and the correct interaction of ss oligonucleotides with the enzyme [22], we examined whether topo can bind very short ss ODNs, containing 2–5 mononucleotide units, in order to determine the minimal consensus sequence that contributes significantly to the overall sc DNA affinity. The results obtained favor the possibility of the location of the high affinity region (2–4 bases) at a distance of 3 bases from the cleavage point.

2. Materials and methods

Sc colE1 DNA was purchased from Wakojunyaku Co., Ltd. (Japan). All ODNs were synthesized as in [23]. Concentrations of ODNs

*Corresponding author. Fax: (7) (3832) 351665.
E-mail: Nev@modul.bioch.nsk.su

Abbreviations: IC_{50}, the concentration giving 50% inhibition of the activity; topo, DNA topoisomerase I; ODN, oligodeoxynucleotide; ss and ds, single- and double-stranded, respectively; sc DNA, supercoiled DNA

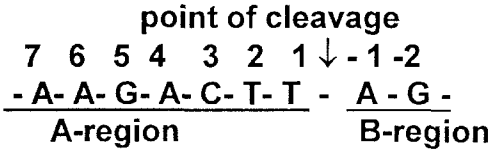
PII S0014-5793(97)00091-4

were determined according to [24]. Topo was obtained from mouse Ehrlich ascites tumor cells as before [25].

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. ODNs were used at different concentrations. Samples were incubated for 15 min and the results for ODN inhibition of topo dependent relaxation of sc DNA were estimated with two methods: agarose gel electrophoresis as in [25] and the pH 12 ethidium fluorescence assay according to [26]. The IC_{50} and K_i values for ODNs were estimated from fluorescence assay (K_i values using dependencies 1/V versus 1/S) according to [27].

3. Results and discussion

According to Christiansen et al. [28] topo mediated cleavage requires two separate regions of topo for interaction with a duplex DNA, one encompassing the cleavage site, region A, and the other holding the 5′-OH end generated by cleavage, region B. Corresponding domains on the enzyme have been hypothesized [28] for the bipartite interaction with DNA. As we showed previously [22], specific sequence ODNs possess significantly higher affinity to the A-region of topo than for the B-region: the specific nonanucleotide interacts with A- and B-regions of the enzyme according to the scheme:



In order to determine the most important parts of the specific sequence of the scissile DNA chain for its recognition by topo, we have analyzed interaction of the enzyme with extremely short ODNs. Results for ODN inhibition of topo dependent relaxation of sc DNA may be estimated with two methods: standard agarose gel electrophoresis and pH 12 ethidium fluorescence. As was shown earlier [22] and is apparent from Fig. 1, the results from both approaches coincide very well. However, only the ethidium fluorescence assay allowed an accurate quantitation of the enzyme inhibition. All ODNs were shown to be competitive inhibitors towards sc DNA in the relaxation reaction, catalyzed by topo, and their affinity may be characterized by K_i values. But here we have used IC_{50} values (Table 1) since in our conditions K_i values were only 1.5–2.0 times lower than IC_{50} [22].

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

oligonucleotide (5'----------3')	CI_{50} (μM)*
TAG	360
CTTAG	130
AAGACTTAG (C9)	2.0
GACTT (C5)	2.0
ACTT	90
pACTT	1.2
CTT	920
GACT	170
pGACT	160
ACT	720
AGAC	25
pAGAC	20
GAC	80
AAGA	9
AGA	20
pGA	80
AAG	130
pAG	50
pGG	90
PO_4^{3-}	150000

[a]Errors in IC_{50} were within 20–30%.

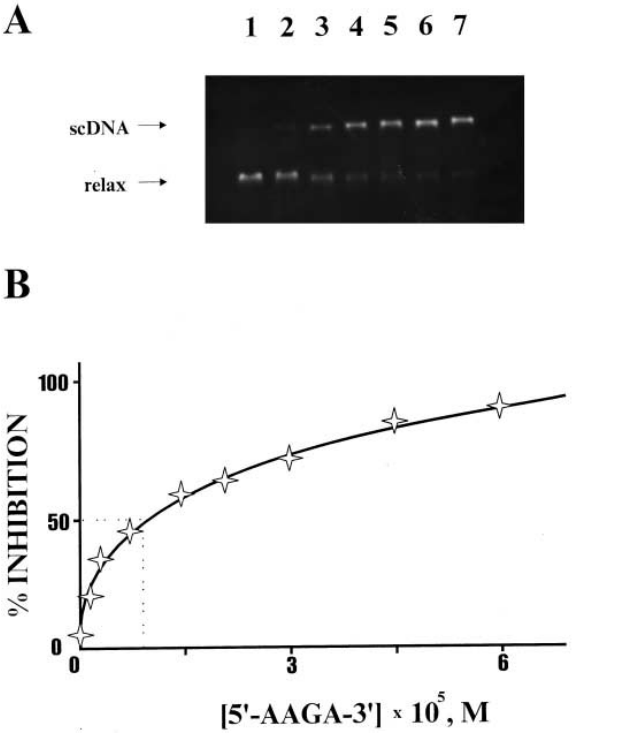


Fig. 1. Topoisomerase relaxation activity inhibition by oligonucleotides determined by agarose gel electrophoresis (A) and by the ethidium fluorescence assay (B). The concentrations of oligonucleotide used were: 0.15 (lane 2), 1.5 (93), 3.0 (4), 4.5 (5), and 6.0×10^{-5} M (6). Standard reaction mixtures incubated without inhibitor (line 1) or without topo I (line 7) were used as controls.

The ODNs containing 5 (5′-GACTT-3′) and 9 (5′-AAGACTTAG-3′) nucleotide units of the cleaved chain were marked as C5 and C9 respectively.

As one can see from Table 1, transition from C9 to ODNs, corresponding to the specific sequence directly before the site of the cleavage (bases from +3 to +1) and two bases after the site of cleavage leads to a decrease of the affinity by a factor of 65–180. In contrast, the affinity of C5, which does not have an AG dinucleotide interacting with the B-region, is comparable with that of C9. These data show that the specific sequence before the point of cleavage is more important for DNA recognition.

The affinity of the ODNs CTT, ACT, ACTT and GACT, without nucleotide units with numbers from +5 to +7, is also lower by a factor of 45–460 than that for C5 or C9. The affinity enhancement of short specific ODNs takes place when they contain an AGA trinucleotide or at least a GA and/or AG dinucleotide sequence corresponding to bases +4–+6 of the specific sequence. Among very short ODNs, the trinucleotide AGA (IC_{50} = 20 μM) has an affinity only 10 times lower than that for C9.

It is well known that enzyme recognition of small ligands may be the result of strong contact formation like electrostatic

or hydrogen bonds [29,30]. The recognition of pyrophosphate [31,32], nucleotides [29,30,32], amino acids and various other small substrates [33] takes place usually due to the formation of strong contacts. The particular role of the phosphate group in recognition and conversion of mono-, oligo- and polynucleotides has been recorded in many works [29,30,34–37]. The contribution of the electrostatic contact of mono-triphosphate groups to the total affinity of small ligands (NMP-NTP) correlates with the K_d range ($0.1–1 \times 10^{-4}$ M) estimated in various model experiments [38–40]. According to the data of studies of replication and repair enzymes, K_d values, characterizing the formation of one strong electrostatic contact between the enzymes and the internucleoside phosphate group of DNA, are usually within the range of 0.08–0.16 M [20].

We have shown that the orthophosphate inhibits the topo dependent relaxation of sc DNA by 50% at concentrations of 0.14–0.15 M. Thus, it is reasonable to suppose that topo (like other enzymes investigated [20]) forms one electrostatic contact with orthophosphate as well as with one internucleoside group of any ss ODNs. To determine the position of the internucleoside phosphate group involved in ODN-topo interaction, different ODNs with and without 5′-phosphate have been tested for the inhibition of the reaction. Table 1 demonstrates that the affinity of pGACT and pAGAC ODNs is practically the same as that for corresponding ODNs without the 5′-end phosphate group. At the same time the affinity of pACTT turns out to be about two orders of magnitude higher than that for ACTT. These findings speak in favor of direct involvement of an internucleoside phosphate group located between bases +4 and +5 in the interaction with the enzyme recognition center.

Thus, it is evident that different subsites of the topo active center, which can interact with different nucleotide units of the specific sequence of sc DNA, contribute differently to the total efficiency of sc DNA recognition. An AAGA sequence located upstream of the cleavage site forms strong specific contacts with topo, while the contacts with nucleotide units around the cleavage site are significantly lower. This high affinity of the tetranucleotide is most probably provided partly by strong specific contact with the negatively charged internucleoside phosphate group situated between bases +4 and +5 of the specific sequence.

It has been shown recently that topo can induce significant structural changes of ODNs [22] as a result of the adaptation of the ligand structure to the optimal one. We suppose that the relatively weak interaction of topo with the cleavage site region of sc DNA may be crucial for substrate catalytic cleavage, since rigid fixation of the CTTAG part of sc DNA on the enzyme should lead to a decrease of the maximal reaction rate. In contrast, a low affinity of this specific sequence may provide enhancement of flexibility of the cleaved sequence, which may be very important for the adaptation of the structure of sc DNA to the optimal for the catalysis.

Acknowledgements: This work was supported by a grant from the Russian Fond of Fundamental Investigation (95-04-13111), grants to G.A.N. from the Foundation for Promotion of Cancer Research, grants-in-aid to T.A. from the Ministry of Education, Science and Culture of Japan, and a grant from the Siberian Division of the Russian Academy of Sciences.

References

[1] Gellert, M. (1981) Annu. Rev. Biochem. 50, 879–910.
[2] Vosberg, H.-P. (1985) Curr. Top. Microbiol. Immunol. 114, 19–102.
[3] Wang, J.C. (1985) Annu. Rev. Biochem. 54, 665–697.
[4] Maxwell, A. and Gellert, M. (1986) Adv. Protein Chem. 18, 69–107.
[5] Osheroff, N. (1989) Pharmacol. Ther. 41, 223–241.
[6] Uemura, T., Ohkura, H., Adachi, Y., Morino, K., Shiozaki, K. and Yanagida, M. (1987) Cell 50, 917–925.
[7] Holm, C., Stearns, T. and Botstein, D. (1989) Mol. Cell. Biol. 9, 159–168.
[8] Wang, J.C., Caron, P.R. and Kim, R.A. (1990) Cell 62, 403–406.
[9] Wang, J.C. (1991) J. Biol. Chem. 266, 6659–6662.
[10] Lui, L.F. (1989) Annu. Rev. Biochem. 58, 351–375.
[11] Potmesil, M. and Kohn, K.W. (Eds.) (1991) DNA Topoisomerases in Cancer, Oxford University Press, New York.
[12] Andoh, T., Ikeda, H. and Aguro, M. (Eds.) (1993) Molecular Biology of DNA Topoisomerase and Its Application to Chemotherapy, CRC Press, Boca Raton, FL.
[13] Andoh, T., Ishii, K., Suzuki, Y., Ikegami, Y., Kusunoki, Y., Takemoto, H. and Okada, K. (1987) Proc. Natl. Acad. Sci. USA 84, 5565–5569.
[14] Hsiang, Y.-H., Liu, L.F., Wall, M.E., Wani, M.C., Nicholas, A.W., Manikumar, G., Kirschenbaum, S., Silber, R. and Pomesil, M. (1989) Cancer Res. 49, 4385–4389.
[15] Jaxel, C., Kohn, K.W., Wani, M.C., Wall, M.E. and Pommier, Y. (1989) Cancer Res. 49, 1465–1469.
[16] Yamashita, Y., Fujii, N., Murakata, C., Ashizawa, T., Okabe, M. and Nakano, H. (1992) Biochemistry 31, 12069–12075.
[17] Yoshinari, T., Yamada, A., Uemura, D., Nomura, K., Arakawa, H., Kojiri, K., Yoshida, E., Suda, H. and Okura, A. (1993) Cancer Res. 53, 490–494.
[18] Stevsner, T., Mortensen, U.H., Westergaard, O. and Bonven, B.J. (1989) J. Biol. Chem. 264, 10110–10113.
[19] Svejstrup, J.Q., Christiansen, K., Andersen, A.H., Lund, K. and Westergaard, O. (1990) J. Biol. Chem. 265, 12529–12535.
[20] Nevinsky, G.A. (1995) Mol. Biol. 29, 6–19.
[21] Been, M.D. and Champoux, J.J. (1984) J. Mol. Biol. 180, 515–531.
[22] Nevinsky, G.A., Bugreev, D.V., Buneva, V.N., Yasui, Y., Nishizava, M. and Andoh, T. (1995) FEBS Lett. 368, 97–100.
[23] Nevinsky, G.A., Levina, A.S., Podust, V.N., Khalabuda, O.V. and Lavrik, O.I. (1986) Bioorg. Khim. 12, 357–368.
[24] Fasman, G.D. (Ed.) (1975) Handbook of Biochemistry and Molecular Biology: Nucleic Acids Vol. 1, p. 589.
[25] Ishii, K., Hasegawa, T., Fujisawa, K. and Andoh, T. (1983) J. Biol. Chem. 258, 12728–12732.
[26] Andrea, J.E., Adachi, K. and Morgan, A.R. (1991) Mol. Pharmacol. 40, 459–501.
[27] Cornish-Bowden, E. (1978), in: Principles of Enzyme Kinetics, Mir, Moscow, pp. 260–266.
[28] Christiansen, K. and Westergaard, O. (1994) J. Biol. Chem. 269, 1–9.
[29] Nevinsky, G.A., Lavrik, O.I., Gasaryanz, M.G., Mkrchan, Z.S. and Akopyan, Zh.I. (1987) Bioorg. Khim. 13, 506–518.
[30] Doronin, S.V., Lavrik, O.I., Nevinsky, G.A. and Podust, V.N. (1987) FEBS Lett. 216, 221–224.
[31] Kuranova, I.I., Terzyan, S.S., Voronova, A.A., Smirnova, E.A., Vaiynshteiyn, B.K., Churu, V. and Hansen, G. (1983) Bioorg. Khim. 9, 1611–1624.
[32] Potapova, I.A., Nevinsky, G.A., Veniaminova, A.G., Khomov, V.V. and Lavrik, O.I. (1990) FEBS Lett. 277, 194–196.
[33] Fersht, A. (1977) in: Enzyme Structure and Mechanism, W.H. Freeman, New York.
[34] Lavrik, O.I., Nevinsky, G.A. and Akopyan, Zh.I. (1989) Sov. Rev. Physicochem. Biol. 9, 49–85.
[35] Kolocheva, T.I., Nevinsky, G.A., Levina, A.S., Khomov, V.V. and Lavrik, O.I. (1991) J. Biomol. Struct. Dynamics 9, 169–186.
[36] Beloglazova, N.G., Lokhova, I.A., Maksakova, G.A., Tsvetkov, I.V. and Nevinsky, G.A. (1996) Mol. Biol. 30, 136–142.
[37] Vinogradova, N.L., Yamkovoy, V.I., Tsvetkov, I.V. and Nevinsky, G.A. (1996) Mol. Biol. 30, 130–135.
[38] Mildvan, A.S. (1981) Phil. Trans. R. Soc. Lond. 239, 65–74.
[39] Siegel, H., Martin, R.B., Tribilet, R., Haring, U.K. and Malini-Balakrishman, R. (1985) Eur. J. Biochem. 152, 187–193.
[40] Siegel, H. (1987) Eur. J. Biochem. 165, 65–72.