Bioorganic & Medicinal Chemistry Letters 27 (2017) 4817–4822

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and examination of potent pseudosubstrate-based oligonucleotide inhibitors against bacterial topoisomerase IV

Tyler Li [a], Juanjuan Guo [b], Hao Zhang [b,*]

[a] Lexington High School, 251 Waltham Street, Lexington, MA 02421, USA
[b] Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371, Singapore

ARTICLE INFO

Article history:
Received 21 June 2017
Revised 16 August 2017
Accepted 27 September 2017
Available online 28 September 2017

Keywords:
Topoisomerase IV
Enzyme inhibitors
Oligonucleotides
Pseudosubstrates
Antibiotics

ABSTRACT

Topoisomerase IV is an enzyme that is mainly responsible for unwinding interlocked DNA strands at the final stage of prokaryotic DNA replication. Due to its exclusivity in prokaryotes, topoisomerase IV has been identified as a validated target for quinolone-based antibiotics in the past years for treating bacterial infection. In consideration that bacterial resistance to such antibiotics has occurred constantly, several newly designed pseudosubstrate oligonucleotides as DNA topoisomerase IV inhibitors have been examined during our recent investigations. Among them, the nick-, gap- and mismatched base pair-containing oligonucleotides displayed significantly high inhibitory effects toward topoisomerase IV. It is our anticipation that the outcomes of our current studies could be beneficial for the future development of pseudosubstrate-based enzyme inhibitors as well as new types of antibiotics.

Topoisomerase IV (topo IV) is a prokaryotic type II topoisomerase that was discovered originally by Kato et al. in 1990.[1] This enzyme, as a homolog of DNA gyrase, acts on the topology of DNA mainly by the following three types of action during bacterial replication and transcription processes[2–5]: (i) decatenation of interlocked daughter DNA molecules generated at the final stage of prokaryotic DNA replication, which is considered to be the major role of topo IV, (ii) relaxation of negatively supercoiled DNA molecules to control and maintain bacterial DNA supercoiling, and (iii) removal of the positive supercoils generated during transcription ahead of polymerase complexes. Similar to DNA gyrase, topo IV is able to cleave both DNA strands, form a covalent complex between enzyme and DNA, facilitate the passage of another double-stranded DNA through the transient DNA break, and reseal the broken DNA strand by catalyzing the formation of new phosphodiester bonds.[6] More specifically, high-resolution crystal structure of the topo IV-DNA complex was determined by recent X-ray crystallography studies, which revealed that during topo IV catalytic cycle, the hydroxyl groups on the active Tyr 118 residues of ParC (one of the subunits of topo IV) undergo nucleophilic attack on the phosphorus atoms in the DNA backbone, and therefore covalently link to the 5′ phosphate ends of the double-strand DNA break (formation of the topo IV-DNA complex illustrated in Fig. 1).[7]

Based on its catalytic mechanism and exclusivity in prokaryotic cells, topoisomerase IV has been taken as the target of several antibacterial agents specifically treating diseases caused by bacteria[8–10] (*e.g.* pneumonia, tuberculosis and malaria). One typical example of such antibiotics is quinolone, which acts by reversible trapping of topoisomerase-DNA complexes formed during the catalytic process.[9–14] At higher concentrations of quinolones, cell death can result due to the double-strand DNA breaks released from trapped topo IV complexes.[15–17] However, similar to many other antibiotics, quinolones and other widely-used topoisomerase-targeting molecules have constantly encountered resistance development by bacteria that could evolve rapidly.[18–20] Mutations at some of the crucial sites in the topoisomerase IV protein molecules could, for example, decrease their binding affinity to quinolones.[13,18,19] In addition, intracellular quinolone concentrations could be reduced by efflux pumps in some strains of bacteria.[18,20] Both above-mentioned actions in bacteria could consequently lead to the reduction of antibiotic drugs' effectiveness.

In consideration of the fact that bacterial resistance to small-molecule antibiotics has occurred continuously,[21–23] a series of pseudosubstrate oligonucleotides has been designed for the first time as new types of topoisomerase IV inhibitors, and was subsequently examined in our recent investigations. Different from any of the previously reported topo IV inhibitors, our newly designed topo IV-interacting molecules are composed of oligonucleotides for mimicking topo IV substrates that can be recognized and bound by topo IV, and contain certain modifications positioned

* Corresponding author.
E-mail address: h.zhang@ntu.edu.sg (H. Zhang).

https://doi.org/10.1016/j.bmcl.2017.09.053
0960-894X/

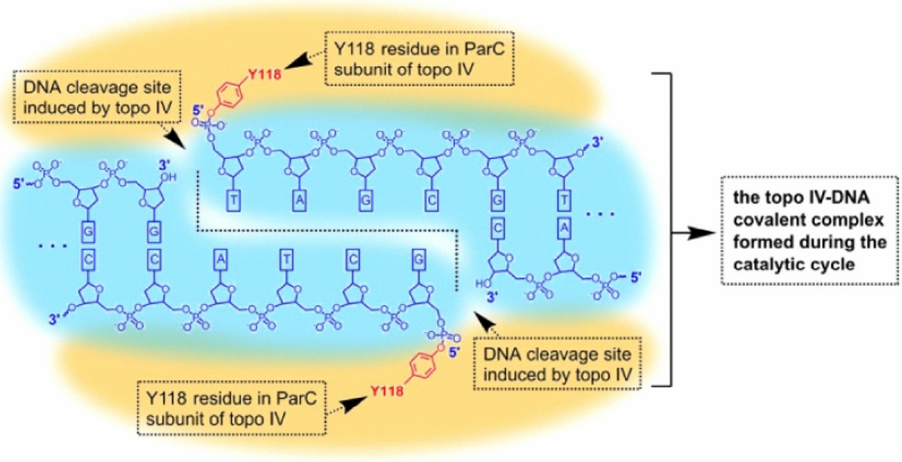

Fig. 1. Schematic illustration of the topoisomerase IV-DNA covalent complex formed during the catalytic cycle of topoisomerase IV.

exactly at or near the points of DNA scission generated by the action of topo IV. Our present study aims to find whether these particularly designed oligonucleotides could serve as potent inhibitors for topoisomerase IV; and to determine the inhibitory efficiencies (IC50 values) of these pseudosubstrate topo IV inhibitors.

DNase I-based footprinting studies indicated that topoisomerase IV is capable of recognizing and binding to particular segments of duplex DNA.[24–26] As described by Peng et al., *Escherichia coli* topo IV interacts closely with 34 nucleobases on one strand (strand A highlighted in red in Fig. 2) and 35 nucleobases on the opposite strand (strand B highlighted in blue in Fig. 2) when it binds to the target duplex DNA fragment.[24] The detailed nucleotide sequence of this topo IV preferential binding region has been determined previously as shown in Fig. 2.[24] Upon binding to this particular duplex sequence, *Escherichia coli* topoisomerase IV was demonstrated to cleave and religate each DNA strand at specific positions as indicated by the arrows shown in Fig. 2 (hydrolysis of phosphodiester bonds occurred between [+1 Thymine] and [−1 Guanine] on Strand A and between [+1 Guanine] and [−1 Cytosine] on Strand B) in the presence of ATP.[24]

On the basis of the reported topo IV preferential binding sequence, a self-complementary hairpin-like DNA structure was designed and constructed in our first study (structure shown in Fig. 3). This hairpin structure contains (i) a 46-base-pair self-complementary DNA duplex region, nucleotide sequence of which is exactly identical to that of topo IV binding region reported by Peng et al.[24], (ii) a single-stranded DNA break (both 3′ and 5′ ends terminating with a free hydroxyl group) positioned exactly at one of the topo IV-generated phosphodiester bond breaking sites,[24] and (iii) an extraordinarily stable mini-hairpin loop d(CGCGAAGCG)[27,28] for maintaining its structural integrity under physiological conditions.

In our opinion, the action of topoisomerase IV relies on the proper conformation of enzyme-DNA cleavage complexes which are known to be short-lived and readily reversible.[29,30] In such case, once the cleavage-religation equilibrium is shifted toward strand cleavage, topoisomerase IV will therefore be trapped on the DNA substrate, which will theoretically result in a significant inhibitory effect on topo IV enzymatic activity. Our newly-designed Inhibitor 1, on one hand, is supposed to closely fit the binding site of topo IV because of its identical sequence to that of topo IV preferential binding sequence. On the other hand, however, the topo IV-catalyzed re-ligation process on this nick-containing pseudosubstrate would be interrupted due to the lack of 5′ phosphate group at one of DNA cleavage sites. We therefore envisioned that this modified topo IV-binding oligonucleotide would be able to act as an endogenous poison of topoisomerase IV.

In order to quantify the inhibitory efficiencies of our newly designed oligonucleotide inhibitors, kinetoplast DNA (kDNA) was used in our studies as an ideal topoisomerase IV substrate. Unlike genomic or plasmid DNA, kDNA which is only found in protozoa contains thousands of DNA minicircles topologically interlocked in a massive network.[31–33] It has been demonstrated that topo IV is capable of completely decatenating kDNA under standard conditions.[34,35] We would therefore believe that the inhibitory effectiveness of the pseudosubstrate inhibitors could be quantitatively determined according to the kDNA decatenation rate. The actions of pseudosubstrate inhibitors is illustrated in Fig. 4.

As shown in Fig. 5a, in the absence of topo IV, massive interlocked kDNA remains in the wells of agarose gel during our agarose-gel electrophoretic study (experimental details described in

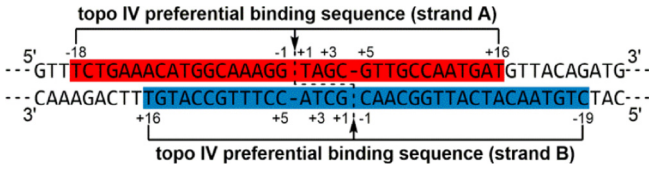

Fig. 2. Topoisomerase IV preferential binding region and topo IV-induced DNA cleavage sites reported by Peng et al.[24] The arrows denote the cleavage sites induced by topoisomerase IV.

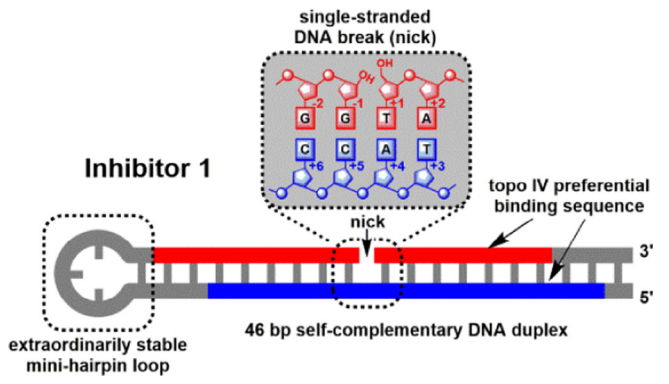

Fig. 3. Structural illustration of our newly designed oligonucleotide-based pseudosubstrate inhibitor (Inhibitor 1). Detailed nucleotide sequences are provided in Table S1 in Supplementary Information.