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An Overview of DNA Topoisomerase: The Cellular Magicians

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ABSTRACT

During any cellular process that involves DNA, a phenomenon might occur in the DNA double helix, which is known as supercoiling. The most common example of supercoiling is during helicase activity in DNA replication and transcription of DNA, which causes both positive and negative supercoiling on the DNA, and changes it's topology. Any sort of topological change such as supercoiling, imparts stress in the DNA double helix, and can affect the overall activity of DNA. Hence, the topology must be well regulated inside the cell. DNA Topoisomerase is the main enzyme, which regulates the DNA topology in the cell. In this paper, we focus on the types and mechanism of action of DNA Topoisomerase enzymes found in different genera of living organisms, from prokaryotes to higher eukaryotes. Also we have discussed the pharmacological significances of topoisomerase enzymes as potential drug targets.

Keywords- DNA Replication, DNA Topoisomerase, DNA Topology, Drug Targets, Gyrase, Quinolones, Supercoiling, Transcription

I. INTRODUCTION

DNA, the blueprint of life, is essential for almost every living organism in this world. It is a double-stranded polynucleotide helix consisting of two antiparallel strands. The classical structure of DNA (also known as B-DNA) was discovered by Watson and Crick in 1953. Frequently, the helix axis is curved and several 'unusual DNA structures' are formed under specific sequence and environmental conditions. Since the B-DNA has minimum energy, any bending or twisting of the DNA molecule will change its free energy [1]. In addition to different types of secondary structures, the DNA helix coils to form further complex helices, which leads to Supercoiling.

Supercoiling is an important phenomenon in various cellular processes. Without supercoiling, it would be impossible to pack large amounts of DNA inside the cell, or in the nucleus (for eukaryotes). Supercoiling significantly reduces the space occupied by the DNA and hence contributes to proper packaging of DNA. Also, supercoiling plays an important role for various biological pathways where DNA is involved. Thus, it is extremely important to regulate the topology and super coiling of DNA. One of the key regulators of DNA topology is the DNA Topoisomerase enzyme.

II. PARAMETERS OF DNA SUPERCOILING

Supercoiled DNA was first discovered and reported by Vinograd, Lebowitz, Radloff, Watson and Laipis. [2]. There are various quantitative parameters that can tell, whether a DNA double helix is supercoiled or not. Several mathematical studies have provided valuable insights related to supercoiling [3, 4]. The basic factors governing DNA topology and Supercoiling are described below. They are described with reference to covalently closed-circular DNA (cccDNA) molecules, but are also applicable for any stretch of DNA in the cell.

Linking Number- Linking Number is an invariant Topological Property of cccDNA. It is defined as the number of times one strand of a DNA would have to be passed through the other strand, such that the two strands are fully separated from each other [5]. It is always an integer.

Linking Number depends on two other factors, which are called *Twist* and *Writhe*.

Twist- Twist is the number of helical turns of one strand about the other. Which means, it is equal to the number of times one strand completely wraps around the other strand in a duplex DNA [5].

Writhe– Writhe is generated because of various torsional stresses, happening to the DNA strands. Writhe is classified into two types: *Interwound writhe*, where the long axis is twisted around itself. *Toroid or spiral writhe*, in which the long axis is wound in a cylindrical manner [5]. These kind of writhe is noticed when DNA is bound around proteins.

The writhing number (Wr) in a cccDNA is determined by the total number of interwound and/or spiral writhes present [5].

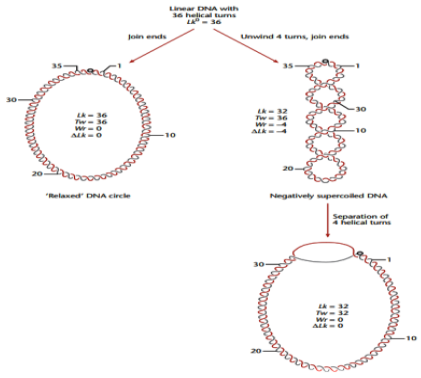

Figure 1: Relationship of linking number, twist and writhe in a cccDNA [1].

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[5] The sum of the twist number (Tw) and the writhing number (Wr) must remain equal to the linking number (Lk). Which means,

Lk = Tw + Wr.

All of these three factors contribute to the DNA topology, and hence can declare whether the DNA is supercoiled or not.

III. DNA TOPOISOMERASE

Also known as the cellular magicians, DNA Topoisomerase enzymes can solve various kinds of topological problems of DNA that might arise during replication, transcription and other cellular processes. Topoisomerases are present in almost all living organisms, including Archae, Prokaryotes and Eukaryotes and in some viruses, and they play an essential role in regulating the DNA topology and genome health.

DNA topoisomerases are able to change the linking number of a DNA, by interrupting the sugar–phosphate backbone, through transient single-strand or double-strand breaks into the DNA [5]. By this way, supercoiling of DNA can be regulated by this enzyme.

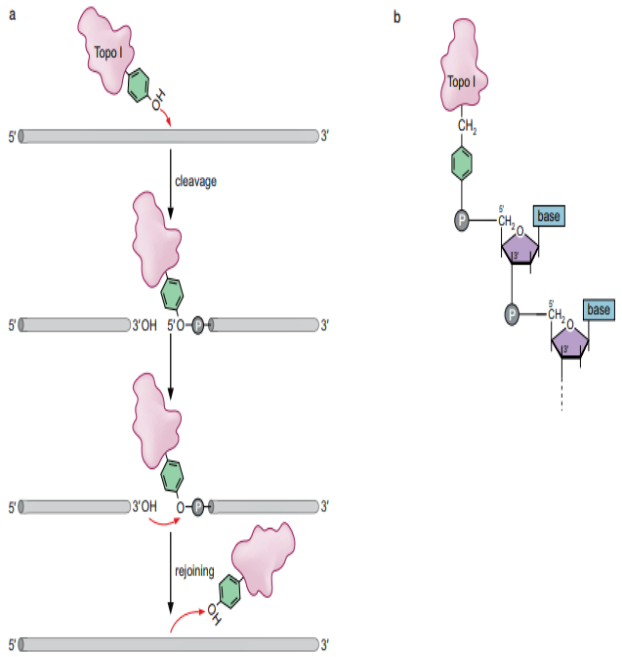

Figure 2: Overview of DNA topoisomerases cleaving DNA using a tyrosine residue present at its active site [5].

DNA Topoisomerase was first discovered by J.C. Wang in 1971. While he was studying negative supercoiling of DNA, he found out that one of his cell preparation lacked supercoils. He identified and isolated the enzyme that was responsible for this negative supercoiling. He named this protein ω [6]. After that, in 1976, DNA gyrase was isolated from *Escherichia coli*. Apart from circular DNAs in prokaryotes, long linear chromosomes of eukaryotes also experience topological problems. During a cycle of DNA replication, the double stranded daughter DNA molecules often become entangled, which blocks the separation of the daughter chromosomes during mitosis. This problem is eliminated by topoisomerase, and it ensures that DNA cleavage, strand passage and DNA rejoining all occur in a highly coordinated manner [1].

Dr. Wang kept on working on Topoisomerases. He explained in his 1991 *Journal of Biological Chemistry* (JBC) Mini review on topoisomerases "*As enzymes, the DNA topoisomerases are magicians among magicians; they open and close gates in DNA without leaving a trace, and they enable two DNA strands or duplexes to pass each other as if the physical laws of spatial exclusion do not exist*."[7]. After the discovery of protein ω, various types of Topoisomerase enzymes have been discovered, both in Prokaryotes as well as in Eukaryotes.

IV. TYPES OF DNA TOPOISOMERASE ENZYME

There are multiple families and subfamilies of DNA topoisomerase enzyme, which are isolated from different organisms. They are broadly classified into Type I Topoisomerase and Type II Topoisomerase.

Type I Topoisomerase

DNA type I topoisomerases can be classified into Topo IA, Topo IB, Topo IC (Topo V) [8]. Type IA and type IB topoisomerases exist in both prokaryotes and eukaryotes, with their own unique functions. A third type of topoisomerase I was identified, known as Type IC (or Topo V), which is considered to have viral origins [9]. Type I topoisomerases catalyze DNA by single strand breaks, through a forward cleavage reaction, and a backward ligation reaction. A tyrosine residue present in the DNA binding site of Topoisomerase forms a covalent DNA-tyrosine intermediate with the cleaved DNA. After cleavage, topoisomerase undergoes a conformational change that forms a protein bridge around the gap. The uncleaved DNA strand passes through the gap and binds to a DNA binding site of the protein. After the strand is passed, a second conformational change occurs in the enzyme. This change causes rejoining of the cleaved DNA ends by attacking the OH end on the phosphor-tyrosine bond. The enzyme opens up for the last time to release the DNA which is identical to the starting DNA except the change in its linking number by 1.

The overall reaction driven by Type I topoisomerases is isoenergetic in nature, and no external source of energy is required to drive the reaction.

Type II Topoisomerase

Type II Topoisomerases cleave both strands of the DNA helix at the same time; and so, they change the linking number of DNA by 2. They need ATP hydrolysis

for their function. In most of the type II topoisomerases; because of its dimeric structure, two topoisomerase subunits with their active-site tyrosine residues are required to cleave the two DNA strands and make the double stranded DNA break.

Type II topoisomerases is classified into type IIA and IIB. Type IIA is further classified into DNA gyrase, Topoisomerase II (topo II), and Topoisomerase IV (topo IV) [10]. DNA gyrase is a special type II topoisomerase, which catalyzes negative supercoiling in the DNA, which is a vital step for many reactions including initiation of both transcription and replication of prokaryotic DNA [5].

Whereas, Type IIB topoisomerases comprise only of topoisomerase VI (topo VI). Both type IIA and IIB use a duplex strand passage mechanism and have the same ATPase and cleavage domains but differ in overall tertiary structure [11]

Topo IIA and Topo IIB (Topo VI) are not homologous, indicating that they originated independently [8].

V. FUNCTIONS OF TOPOISOMERASE

a- Topoisomerases are required to relieve the torsional strain introduced into the system by changing the linking number through transient single-strand or double-strand breaks into the DNA.

b- Prokaryotes contain "DNA Gyrase", a special ATP Dependant [12] type II topoisomerase that results in the formation of negative supercoils in the helical DNA. This type of supercoiling facilitates the unwinding of the DNA duplex, and hence it stimulates initiation of both transcription and DNA replication [5].

c- Topoisomerases can both catenate and decatenate circular DNA molecules [5]. If atleast one of the two catenated DNA molecules carry a nick or a gap, then type I can also unlink the two molecules.

d- Topoisomerases can also use covalent –intermediate mechanism to promote both DNA cleavage and rejoining; thus they do not require the assistance of other proteins or high energy cofactors like ATP.

e- To initiate a relaxation cycle, topoisomerase binds to a segment of duplex DNA in which the two strands are melted [5]. A "protein bridge" present in the Topoisomerase enzyme facilitates DNA strand passage through the break.

f- Topoisomerases are essential for cell function and regulation of the genome inside the cell. Life would cease to exist if these enzymes are removed.

VI. QUINOLONE AND FLUOROQUINOLONE MEDIATED TARGETING OF TOPOISOMERASE

Quinolones are a group of broad-spectrum antimicrobials. The basic structure of Quinolone contains a bicyclic ring. Fluorine atom and various substitutions on the basic bicyclic structure produce different types of Fluoroquinolones [13]. Studies reveal that Fluoroqinolones are more potent, and they show bactericidal effect against numerous pathogens including Gram-positives, Gram-negatives, aerobes and anaerobes [14, 15]. Quinolones and Fluoroquinolones both target the DNA gyrase or topoisomerase IV to form reversible drug-enzyme-DNA cleavage complexes, after which the microbe undergoes cell cycle arrest and thus fails to carry out proper DNA replication and RNA synthesis. Studies show that Quinolones that contain a piperazin in position C7 are more effective on Gram-negative bacterias [16]. In this article, we have tried to make a comparative study of three Quinolone molecules, namely finafloxacin, norfloxacin and hydroxychloroquine, by docking them with topoisomerase IV-DNA cleavage complex of S. pneumonia, and infer that how they can be used to inhibit the proliferation of several pathogenic microbes.

We used Autodock Vina [17] for the docking, and the results are given below.

Table 1: Docking Results

Si No.	Name of the Drug	**Highest affinity with the protein (Kcal/mol)** {after 10 consecutive runs}
1	Finafloxacin	-8.1
2	Hydroxychloroquine	-6.3
3	Norfloxacin	-7.4

The following figures categorized under Figure 3 show the output files viewed in Pymol, where the quinolone molecules are docked with the protein.

(*Note*- The enzyme is shown in Blue, the intervening DNA is marked in Orange, and the drug ligand is marked in Red)

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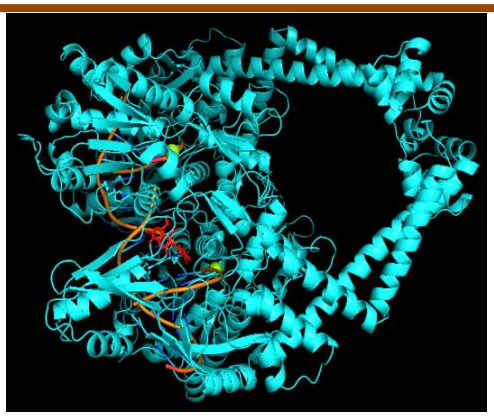

Figure 3a: Docking result of Finafloxacin with Topoisomerase IV- DNA cleavage complex

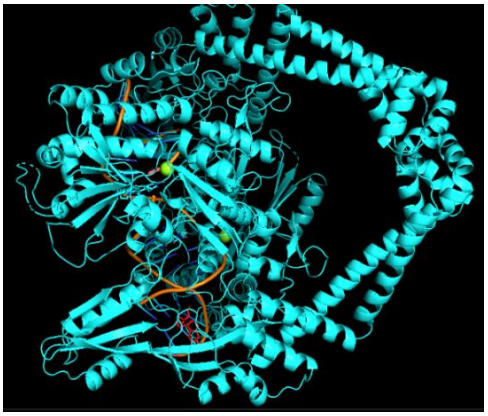

Figure 3b: Docking result of Hydroxychloroquine with Topoisomerase IV-DNA cleavage complex

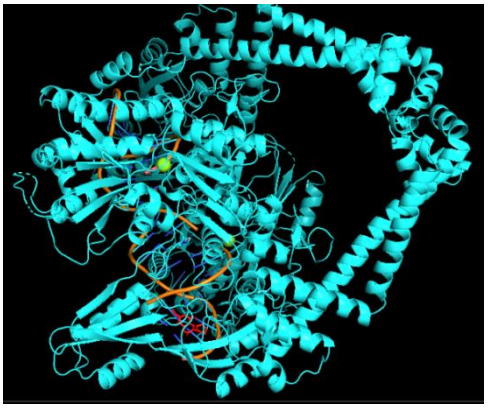

Figure 3c: Docking result of Norfloxacin withTopoisomerase IV-DNA Cleavage complex

VII. RESULT

The docking analysis shows a crude yet promising aspect of how these Quinolone molecules bind with a high affinity to regions near the DNA binding domains of the S. pneumoniae bacterial Topoisomerase IV-DNA cleavage complex. In this comparative study, it is clear that the resultant complexes thus formed are quite stable thermodynamically. The binding of Finafloxacin is most stable (-8.1 Kcal/mol), compared to Norflaxin (-7.4 Kcal/mol) and Hydroxychloroquine (-6.3 Kcal/mol). These features may be proved as useful for future drug designing and targeting of DNA Topoisomerase enzyme of several pathogenic organisms.

VIII. TARGETING TOPOISOMERASES FOR CANCER CHEMOTHERAPY

There are various compounds that target the Topoisomerase of Tumor or malignant cells in cancer. The mechanism of such "Topoisomerase poisons" are similar to those of Quinolones, which involve the formation of a stable ternary complex of cleaved DNA, topoisomerase, and the drug inside the target cells. These ternary complexes are accumulated, and ultimately cause a cytotoxic effect in the cell, resulting in cell death [18]. Anthracycline, anthracenedione and epipodophyllotoxin are few such anticancer agents, which act upon type IIA topoisomerases. Most of them act via an interfacial mechanism by intercalation between the -1and +1 DNA base pairs in the cleavage complex [18]. These agents contribute to various hydrophobic and electrostatic interactions with both the DNA and protein, which helps to stabilize the binding of the compound and prevent DNA re-ligation by the enzyme. Topoisomerase poison mechanism can also occur by redox-dependent, covalent formation of a drug-enzyme complex. These compounds bind to a site distal to the DNA active site and work to stabilize the enzyme –DNA cleavage complex, which accumulates, and cause cell death [18]. By this way, Topoisomerase poisons can be used for cancer chemotherapy.

IX. CONCLUSION

The DNA topoisomerases are indeed the "cellular magicians" of the cell, and they are of enormous importance for executing various functions inside the cell. As previously discussed, Topoisomerase acts through transient breakage of DNA strands. This property has been exploited by researchers for therapeutic purposes [19]. The study and design of several Quinolones and anticancer agents can be carried out using these enzymes as the target. Since the discovery of protein ω by Dr. Wang, different studies are still being conducted on Topoisomerases, and various new findings about these cellular magicians continue to emerge with respect to their cellular function, regulation and utility as several clinical purposes.

REFERENCES

[1] Bowater, R. P. (2001). Supercoiled DNA: Structure. In John Wiley & Sons, Ltd (Ed.), *Encyclopedia of Life Sciences* (p. a0001040). John Wiley & Sons, Ltd. https://doi.org/10.1038/npg.els.0001040

[2] Vinograd, J., Lebowitz, J., Radloff, R., Watson, R., & Laipis, P. (1965). The twisted circular form of polyoma viral DNA. *Proceedings of the National Academy of Sciences*, *53*(5), 1104–1111. https://doi.org/10.1073/pnas.53.5.1104

[3] Bauer, W. R., Crick, F. H., & White, J. H. (1980). Supercoiled DNA. *Scientific American*, *243*(1), 100–113. https://pubmed.ncbi.nlm.nih.gov/6256851/

[4] Banfalvi, G. (1994). DNA topology. *Biochemical Education*, *22*(2), 104. https://doi.org/10.1016/0307-4412(94)90102-3

[5] Watson, J. D., Baker, T. A., Bell, S. P., Gann, A., Levine, M., &Losick, R. M. (2004). *Molecular biology of the gene.*(7thed.). Pearson Inc.

[6] Depew, R. E., Liu, L. F., & Wang, J. C. (1978). Interaction between DNA and Escherichia coli protein omega. Formation of a complex between single-stranded DNA and omega protein. *The Journal of biological chemistry*, *253*(2), 511–518. https://pubmed.ncbi.nlm.nih.gov/338610/

[7] Wang J. C. (1991). DNA topoisomerases: why so many?. *The Journal of biological chemistry*, *266*(11), 6659–6662. https://pubmed.ncbi.nlm.nih.gov/1849888/

[8] Forterre, P., Gribaldo, S., Gadelle, D., & Serre, M.-C. (2007). Origin and evolution of DNA topoisomerases. *Biochimie*, *89*(4), 427–446. https://doi.org/10.1016/j.biochi.2006.12.009

[9] Forterre, P. (2006). DNA topoisomerase V: A new fold of mysterious origin. *Trends in Biotechnology*, *24*(6), 245–247. https://doi.org/10.1016/j.tibtech.2006.04.006

[10] Reece, R. J., & Maxwell, A. (1991). DNA Gyrase: Structure and Function. *Critical Reviews in Biochemistry and Molecular Biology*, *26*(3–4), 335–375. https://doi.org/10.3109/10409239109114072

[11] Kumar, P., Mina, U, Verma, P. (2013). *Biotechnology, A Problem Approach* (5thed). Pathfinder Publication.

[12] Piecing Together the Details of Bacterial DNA Gyrase Specialization♦: Mechanisms for Defining Supercoiling Set Point of DNA Gyrase Orthologs. I. A Nonconserved Acidic C-terminal Tail Modulates *Escherichia coli* Gyrase ActivityMechanisms for Defining Supercoiling Set Point of DNA Gyrase Orthologs. II. The Shape of the GyrA Subunit C-terminal Domain (CTD) Is Not a Sole Determinant for Controlling Supercoiling Efficiency. (2012). *Journal of Biological Chemistry*, *287*(22), 18655–18655. https://doi.org/10.1074/jbc.P112.345678

[13] Kocsis, B., Domokos, J., & Szabo, D. (2016). Chemical structure and pharmacokinetics of novel quinolone agents represented by avarofloxacin, delafloxacin, finafloxacin, zabofloxacin and nemonoxacin. *Annals of Clinical Microbiology and Antimicrobials*, *15*(1), 34. https://doi.org/10.1186/s12941-016-0150-4

[14] Wright, D. H. (2000). Application of fluoroquinolone pharmacodynamics. *Journal of Antimicrobial Chemotherapy*, *46*(5), 669–683. https://doi.org/10.1093/jac/46.5.669

[15] Van Bambeke, F., & Tulkens, P. M. (2009). Safety Profile of the Respiratory Fluoroquinolone Moxifloxacin: Comparison with Other Fluoroquinolones and Other Antibacterial Classes. *Drug Safety*, *32*(5), 359–378. https://doi.org/10.2165/00002018-200932050-00001

[16] Walsh, C. T., & Wencewicz, T. A. (2014). Prospects for new antibiotics: A molecule-centered perspective. *The Journal of Antibiotics*, *67*(1), 7–22. https://doi.org/10.1038/ja.2013.49

[17] Trott, O., & Olson, A. J. (2009). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, NA-NA. https://doi.org/10.1002/jcc.21334

[18] Hevener, KirkE., Verstak, T. A., Lutat, K. E., Riggsbee, D. L., & Mooney, J. W. (2018). Recent developments in topoisomerase-targeted cancer chemotherapy. *Acta Pharmaceutica Sinica B*, *8*(6), 844–861. https://doi.org/10.1016/j.apsb.2018.07.008

[19] Vos, S. M., Tretter, E. M., Schmidt, B. H., & Berger, J. M. (2011). All tangled up: How cells direct, manage and exploit topoisomerase function. *Nature Reviews Molecular Cell Biology*, *12*(12), 827–841. https://doi.org/10.1038/nrm3228