MS05 Nucleic acids and their interaction

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High resolution cryo-EM structure of a type II topoisomerase cleavage core-complex with bound 18mer dsDNA

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Abstract

Type II topoisomerases perform essential roles in DNA replication, chromosome segregation and recombination [1]. Bacteria have two type II enzymes: DNA gyrase, whose function is to supercoil chromosomal DNA and remove positive supercoils ahead of replication forks, and topoisomerase (topo) IV which unlinks catenated daughter chromosomes at cell division [2]. They use ATP to cross one DNA segment through a transient double-stranded DNA break in another DNA duplex involving a 'DNA cleavage complex'. This covalent enzyme-DNA intermediate is formed by reversible attack of the ParC or GyrA active-site tyrosines of the tetrameric topo IV (ParC2ParE2) or gyrase (GyrA2GyrB2) complex. Many important antibacterial and anticancer agents exert their cytotoxic effects by stabilising the cleavage complex. Thus, both topo IV and gyrase are targets for clinically important quinolones such as ciprofloxacin and moxifloxacin and quinazolinedione inhibitors, and human topo Iα and Iβ are targets for the anticancer drugs etoposide and doxorubicin [3]. Structures of several quinolone-topo IV/gyrase (and drug-topo II) core cleavage complexes have been determined by X-ray crystallography [4-6] but the approach has limitations. For example, not all topoisomerases and quinolones yield suitable crystals. Moreover, X-ray crystal structures of full-size holoenzyme complexes [7] are not easily obtained and the few cryoEM structures available are at relatively low resolution. Here we describe the use of cryoEM to solve the structure to 2.7 Å of a DNA-core complex of a topoisomerase, that of topo IV from Streptococcus pneumoniae, a major cause of pneumonia. To our this is the first reported sub-3 Å cryoEM structure of a topoisomerase and provides high resolution insights on novel enzyme-DNA-interactions.

References

[1] Schoeffler, A.J. and Berger, J.M. (2008) Q. Rev. Biophys. 41, 41-101.
[2] Hirsch J. and Klostermeier D. (2021) Nucleic Acids Res. 49, 6027-6042.
[3] Jaswal S., Nehra B., Kumar S. and Monga V. (2020) Bioorg. Chem. 104, 104266
[4] Laponogov, I., Sohi, M.K., Veselkov, D.A., Pan, X.-S., Sawhney, R., Thompson, A.W., McAuley, K., Fisher, L.M. and Sanderson M.R. (2009) Nat. Struct. Mol. Biol. 16, 667-9.
[5] Veselkov, D.A., Laponogov, I., Pan, X.-S., Selvarajah, J., Skamrova, G.B., Branstrom, A., Narasimhan, J., Vara Prasad, J.V.N., Fisher, L.M. and Sanderson, M.R. (2016) Acta Cryst. D 72, 488-496.
[6] Laponogov, I., Pan, X.-S., Veselkov, D.A., Cirz, R.T., Wagman, A., Moser, H.E., Fisher, L.M. and Sanderson, M.R. (2016) Open Biol. 6, 160157.
[7] Laponogov, I., Veselkov, D.A., Crevel, I.M.-T., Pan, X.-S., Fisher, L.M. and Sanderson, M.R. (2013) Nucleic Acids Res. 41, 9911-9923.