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Topoisomerase IV belongs to the type II class of DNA topoisomerases, which are responsible for changing and stabilizing DNA supercoiling and are also involved in chromosome segregation in prokaryotes. Topo IIs are essential enzymes for bacterial replication and are targeted by antibacterial drugs such as quinolones or diones. They change DNA topology by forming a transient covalent cleavage complex with a gate-DNA (G-segment) duplex and transporting the second duplex (T-segment) though a double-stranded break in the formed protein-DNA gate. Although the biological importance of these enzymes is well known, cleavage complex formation and reversal is not fully understood for any type II topoisomerase. In order to further our understanding of the topo II action, we have solved the crystal structures representing sequential states in the formation and reversal of a DNA cleavage complex by topoisomerase IV from
S. pneumoniae. A 3.1 Å resolution structure of the complex captured by a novel antibacterial dione represents a drug-arrested form of the cleavage intermediate and reveals two drug molecules intercalated at a symmetrically cleaved B-form DNA gate and stabilized by drug-specific protein contacts. Similar protein/DNA/drug complex formation was observed for the 2.9 Å resolution structure of topo IV/DNA/levofloxacin solved by us and representing the first high-resolution quinolone-stabilized cleavage complex. Subsequent dione release allowed us to obtain drug-free cleaved and resealed DNA complexes in which the DNA gate, in contrast to the previous state, adopts an unusual A/B-form helical conformation. It also revealed an important reposition of a Mg^{2+} ion towards scissile phosphodiester group allowing its coordination and promoting reversible cleavage by active-site tyrosines. These are the first structures solved for putative reaction intermediates of a type II topoisomerase. They indicate how a type II enzyme reseals DNA during its normal reaction cycle as well as how the complex is stabilized by different antibacterial drugs, which is important for the development of new topoisomerase-targeting therapeutics.

Keywords: Topoisomerases, breakage-reunion, Protein-DNA complexes

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Crystal structure of a homodimeric 4-thiouridine synthetase - RNA complex. Piotr Neumann[a], Kristina Lakomek[a], Peter-Thomas Naumann[b], Achim Dickmanns[a], Charles T. Lauhon[b], Ralf Ficner[a], [a]*Abteilung für Molekulare Strukturbiologie, Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany.* [b]*School of Pharmacy, Univeristy of Wisconsin, Madison, Wisconsin 53705, USA.*
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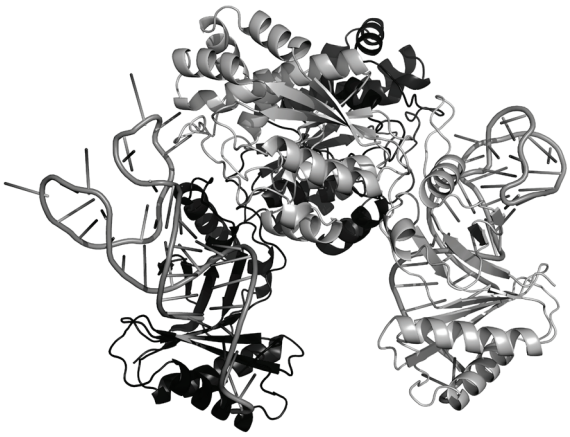
The maturation of all types of RNA in all domains of life includes the posttranscriptional modification of nucleosides. A wide variety of rare nucleosides has been identified, among them there are 16 different thio-nucleotides including the 4-thiouridine, the 2-thiouridine and several alkylated derivatives of 2-thiouridine. Modified nucleotides have been reported to influence vastly different cellular processes. Thiouridines are prerequisites to a correct and efficient translation process and contribute to the structural stability of the tRNA molecule. The modified nucleoside 4-thiouridine (s^4U) is ubiquitously located at position eight (U8) of eubacterial and archaeal tRNAs in the loop region between the acceptor and the D stem. s^4U does not only stabilize the fold of the tRNA, but also plays a central role in bacterial UV protection acting as a sensor for near-UV radiation. U8 is post-transcriptionally modified by a set of enzymes including the 4-thiouridine synthetase ThiI.
Here we report the crystal structure of ThiI from *T. maritima* in complex with a truncated substrate tRNA. The structure demonstrates that ThiI functions only as homo-dimer, since the tRNA acceptor stem including the 3'-recognition element ACCA is bound by the N-terminal ferredoxin-like and THUMP domains of one monomer thereby correctly positioning U8 close to the active site in the pyrophosphatase domain of the other monomer. The structure also indicates that full-length substrate tRNA has to adopt a non-canonical conformation upon binding to ThiI.



Keywords: protein-RNA complexes, RNA-binding proteins, RNA structure

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Combined biophysical techniques used to derive a model for alpha crustacyanin John R Helliwell[a], Ming-chuan Wang[b], Natasha Rhys[b], Clair Baldock[b] and J Günter Grossmann[c,*], [a] *School of Chemistry, University of Manchester M13 9PL, UK,* [b]*Faculty of Life Sciences, University of Manchester M13 9PL, UK,* [c] *STFC Daresbury Laboratory, Warrington WA4 4AD, UK*
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