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

FA1-MS06-P14

Novel Agonists and Antagonists Question Molecular Recognition and Information Transmission in TetR.

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The tetracycline repressor (TetR) protein is considered to represent a paradigm of an effector-regulated DNA-binding protein and is biochemically, genetically and structurally well characterized. TetR forms a homo-dimer and in the tetracycline-free (Tc-free or "repressed") state binds with two helix-turn-helix (HTH) DNA-binding motifs to its operator DNA (*tetO*). The "induced" complex (Tc-bound) is formed upon binding of Tc to TetR and causes the dissociation of TetR from *tetO*, so that the genes *tetR* (encoding TetR) and *tetA* (encoding antiporter TetA) responsible for Tc-resistance can be transcribed. The conformational switch between the two states has been characterized as a shift from a Tc-bound conformation with the DNA-binding domains locked in an orientation that is unfavourable for *tetO* binding to more flexible behaviour of the DNA-binding domains in the Tc-free state [1]. However, recently, several TetR mutants [2] and Tc peptide mimetics [3] have been identified that appear not to behave according to this "classical mechanism".
One such example is the peptide inducer Tip (also called transcription inducing peptide) that triggers a conformational change that differs from Tc [3]. The solved crystal structure of TetR in complex with Tip [4] reveals that Tip uses alternative interactions to transmit the information between the effector-binding and the DNA-binding domain, however, leading to the same conformational end points during induction. Further to the discovery of Tip, several novel TetR interacting peptides with very little sequence conservation among them have been identified using an *in vivo* screening system in *Saccharomyces cerevisiae* (Goeke, Dissertation). Most of the peptides acted as inducers of TetR-regulated transcription, while a few of them showed anti-induction activity. We solved crystal structures of several complexes of TetR with both agonist and antagonist peptides. The structures reveal an unexpected diversity of peptide sequences that can be accommodated in the effector-binding pocket of TetR and which elicit an allosteric response. A detailed analysis of 4 different complexes questions whether allosteric regulation in TetR occurs via a defined information transmission pathway or whether, instead, a conformational selection mechanism applies.

[1] Saenger, W., Orth, P., Kisker, C., Hillen, W., and Hinrichs, W. (2000). Angew Chem Int Ed Engl. 39, 2042-2052. [2] Henssler, E. M., Bertram, R., Wisshak, S., and Hillen, W. (2005). Febs J. 272, 4487-4496. [3] Klotzsche, M., Berens, C., and Hillen, W. (2005). J Biol Chem. 280, 24591-24599. [4] Luckner, S. R., Klotzsche, M., Berens, C., Hillen, W. & Muller, Y. A. (2007). J. Mol. Biol. 368, 780-790.

Keywords: TetR, Induction, Anti-induction