

Protein phosphorylation transduces a large set of intracellular signals. One mechanism by which phosphorylation mediates signal transduction is by prompting conformational changes in the target protein or interacting proteins. Previous work described an allosteric site mediating phosphorylation-dependent activation of AGC protein kinases. The AGC kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) is activated by the docking of a phosphorylated motif from substrates.

PDK1 is a central component of the growth factor and insulin signaling pathways. PDK1 is responsible for the stimulus-dependent phosphorylation and activation of many AGC kinases like Akt/PKB, S6K, RSK and SGK. Thus, our studies on PDK1 are relevant both for the growth factor/cancer field (PDK1 is a validated drug target for cancer treatment) and for insulin/diabetes research. PDK1 is also required for the constitutive phosphorylation of the activation loop of other protein kinases, such as all 12 protein kinase C (PKC) isoforms and the protein kinase C-related protein kinases (PRKs). In all, at least 23 protein kinases are known to be phosphorylated by PDK1.

We present the crystal structure^[1] of PDK1 bound to a rationally developed low-molecular-weight activator^[2] and describe the conformational changes induced by small compounds in the crystal and in solution using a fluorescence-based assay and deuterium exchange experiments. Our results indicate that the binding of the compound produces local changes at the target site, the PIF binding pocket, and also allosteric changes at the ATP binding site and the activation loop. Altogether, we present molecular details of the allosteric changes induced by small compounds that trigger the activation of PDK1 through mimicry of phosphorylation-dependent conformational changes.

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Structural investigation of antidepressant drug and dipalmitoyl phosphatidylcholine interactions by Small Angle X-Ray Scattering method. Dilek Yonar^a, Elif Hilal Soylu^b, M. Maral Sunnetcioglu^a, Semra Ide^a.

^aHacettepe University, Faculty of Engineering, Dept. of Physics Engineering, 06800, Beytepe, Ankara, Turkey.

^bKaradeniz Technical University, Faculty of Science and Literature, Dept. of Physics, 61080, Trabzon, Turkey.

E-mail: ddogan@hacettepe.edu.tr

In this work, interaction of antidepressant drugs clomipramine hydrochloride and trazadone hydrochloride with dipalmitoyl phosphatidylcholine (DPPC) liposomes with/without cholesterol have been investigated. On these samples EPR spin labeling studies were performed recently [1, 2]. To receive further structural information on membrane thickness and the change of it by drug incorporation Small Angle X-Ray Scattering (SAXS) profiles have been recorded. The components were mixed in a wide capillary and left for 1 h at room temperature. The resulting complex was centrifuged into a narrow X-ray capillary. SAXS experiment was carried out

with a Hecus SWAXS camera (Hecus X-Ray Systems Graz-Austria). X-ray generator operating at 50 kV and 50 mA with Cu anode ($\lambda = 1.54 \text{ \AA}$) in q range of 0.008 – 1.22 (\AA^{-1}). Measurements were made by a step –scanning procedure and in the fixed time mode, with a sampling time 300 s for each step.

SAXS scans were also carried out from 15 to 55 °C by using an external temperature control unit causing temperature increasing in 2 °C to investigate thermal effects on the structure of drug incorporated and pure liposomes.

In the result of these studies, the changing in the lamellar repeat distances of DPPC have been determined after the incorporation of three drug compounds. The observed increase in the bilayer thickness which may allow to PBS and drug compound locations was in the range of 12-15 Å. As known as, these interactions occur by four effective types of forces defined as van der Waals, electrostatic, hydration, and undulation forces between DPPC bilayers, and during the incorporation of drug molecule into the DPPC structure [3]. The possible 3-D molecular conformations have been also investigated by using molecular mechanic calculations to explain the interaction of the drug molecules with DPPC. The results have been compared and discussed by using the related literature knowledge [4, 5].

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Drug-stabilized and drug-free complexes of topo IV from *S. pneumoniae* shed light on the mechanisms of reversible DNA scission and selective drug

resistance. Ivan Laponogov^{a,b}, Xiao-Su Pan^b, Dennis A. Veselkov^a, Katherine McAuley^c, L. Mark Fisher^b, Mark R. Sanderson^a. ^aRandall Division of Cell and Molecular Biophysics, King's College London, 3rd Floor New Hunt's House, Guy's Campus, University of London, London, UK. ^bMolecular Genetics Group, Molecular and Metabolic Signalling Centre, Division of Basic Medical Sciences, St. George's, University of London, London, UK. ^cDiamond Light Source, Didcot, Oxford, UK.

E-mail: mark.sanderson@kcl.ac.uk, lfisher@sgul.ac.uk, ivan.laponogov@kcl.ac.uk

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) through 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 Mg²⁺ 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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Novel Agonists and Antagonists Question Molecular Recognition and Information Transmission in TetR.

Madhumati Sevvana^a, Dagmar Goeke^b, Christoph Götz^a, Fabian Gruß^a, Wolfgang Hillen^b, Yves Muller^a.
^aInstitute for biotechnology, ^bInstitute for microbiology, FAU Erlangen-Nuernberg, Germany.

E-mail: msevvana@biologie.uni-erlangen.de

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.

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