

s1.m6.p10 **Binding of KAR-2 to a new binding domain of calmodulin and beyond: structure and function.** István Horváth,<sup>a</sup> Veronika Harmat,<sup>b</sup> Villő Pálfi<sup>c</sup>, András Perczel<sup>c</sup>, László Nyitrai<sup>d</sup>, Gábor Náray-Szabó<sup>e</sup> and Judit Ovádi<sup>a</sup>, <sup>a</sup>Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary, <sup>b</sup>Protein Modelling Group, Hungarian Academy of Sciences - Loránd Eötvös University, Budapest, Hungary, <sup>c</sup>Department of Organic Chemistry, <sup>d</sup>Department of Biochemistry and <sup>e</sup>Department of Theoretical Chemistry, Loránd Eötvös University, Budapest, Hungary. E-mail: horvathi@enzim.hu

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Intracellular Ca<sup>2+</sup> serves as a second messenger in response to external stimuli. One of the major Ca<sup>2+</sup> receptor proteins is calmodulin (CaM), an ubiquitous, multifarious protein which regulates at least 30 different proteins and enzymes. Complex structures of CaM with several target peptides and nonpeptidic antagonists show that the hydrophobic pocket in the C-terminal part of the protein is the primary binding domain of the ligand. CaM adapts its shape to its ligand among the complexes.

Our objective was the localization of the binding domain of KAR-2, [3'-(β-chloroethyl)-2',4'-dioxo-3,5'-spiro-oxazolidino-4-deacetoxy-vinblastine] a new potential antimitotic agent on CaM and the characterization of the structure of the CaM-KAR-2 complex at atomic level in order to interpret the unique effect of KAR-2 on the CaM-modulated processes. KAR-2 impedes the microtubule assemblies, consequently arrest mitosis in cancer cells with modest toxic side effect. KAR-2 binds to CaM with comparable affinity with that of the classical antagonist TFP, however, it can neither disrupt the CaM-enzyme complexes nor suspend the modulating effect of CaM in contrast to TFP, as demonstrated by surface plasmon resonance measurements and by enzymatic assay.

In order to localize the binding domain, crystallographic and NMR studies were carried out. The results from HSQC spectra suggests global conformation change upon KAR-2 binding. Crystals of the CaM-KAR-2 complex were obtained by co-crystallization. 2.12 Å resolution data were collected from one crystal (sg P6<sub>2</sub>22 unit cell constants a=b=37.57Å, c=356.66Å) at DESY beamline X11. The crystal structure shows that KAR-2 does not bind to the well-characterized hydrophobic pocket of the C-terminal domain as TFP, rather to a new binding site. By overlaying of the crystal structures of CaM complexed with TFP or KAR-2 it is clear that different residues are responsible for the binding of the two drugs, consequently simultaneous binding of KAR-2 and target enzyme on CaM may occur which is in agreement with its partial inhibitory effects detected by SPR and enzymatic assay. The fact, that KAR-2 does not suspend the modulating function of CaM might be the reason for its low toxicity in complex biological systems. The overall conformation of the CaM-KAR-2 complex is similar to that of CaM-TFP. However, there are few common residues involved in the binding of both the KAR-2 and TFP which might play crucial role in the global conformational changes caused by the ligands due to their binding to CaM.

s1.m6.p11 **Structural and biochemical studies of human PCNA complexes provide the basis for association with CDK/cyclin and a rationale for inhibitor design.** George Kontopidis, Su-Ying Wu, Daniella Zheleva, Paul Taylor, Campbell McInnes, David Lane, Peter Fischer, Malcolm Walkinshaw, Cyclacel Ltd., Drug Discovery, Structure Based Design Group, James Lindsay Place, Dundee, UK. E-mail: gkontopidis@cyclacel.com

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The interactions between the tumour suppressor protein p21 WAF1 and cyclin-dependent kinase (CDK) complexes, as well as with proliferating cell nuclear antigen (PCNA), regulate and coordinate the processes of cell-cycle progression and DNA replication. We present an X-ray crystal structure of PCNA complexed with a 16mer peptide related to p21, which binds with a Kd of 100 nM. Two additional crystal structures of native PCNA provide the first structures of free human PCNA and show that the only significant changes on ligand binding involve rigidification of a number of flexible regions on the surface of PCNA. In the competitive binding experiments described here we show that a 20mer sequence from p21 can associate simultaneously with PCNA and CDK/cyclin complexes. A structural model for such quaternary complexes is presented, in which the C-terminal sequence of p21 acts as a double-sided tape in that it docks to both the PCNA and cyclin molecules. The quaternary complex shows little direct interaction between PCNA and cyclin, assigning to p21 the role of an adaptor. Taken together, the biochemical and structural results delineate a compact inhibitor site on the surface of PCNA that may be exploited in the design of peptidomimetics, which will act independently of cyclin-groove inhibitors. Blocking this site with drug-like small molecules may be both chemically feasible and therapeutically relevant in proliferative diseases.