Binding of KAR-2 to a new binding domain of calmodulin and beyond: structure and function

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Intracellular Ca^{2+} serves as a second messenger in response to external stimuli. One of the major Ca^{2+} 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 nonpetidic 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-oxazol-idino-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 $P6_122$ 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