Probing the Binding of Indolactam-V to Protein Kinase C through Site-Directed Mutagenesis and Computational Docking Simulations

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Protein kinase C (PKC) comprises a family of ubiquitous enzymes transducing signals by the lipophilic second messenger sn-1,2-diacylglycerol (DAG). Teleocidin and its structurally simpler congener indolactam-V (ILV) bind to PKC with high affinity. In this paper, we report our computational docking studies on ILV binding to PKC using an automatic docking computer program, MCDOCK. In addition, we used site-directed mutagenesis to assess the quantitative contribution of crucial residues around the binding site of PKC to the binding affinity of ILV to PKC. On the basis of the docking studies, ILV binds to PKC in its cis-twist conformation and forms a number of optimal hydrogen bond interactions. In addition, the hydrophobic groups in ILV form “specific” hydrophobic interactions with side chains of a number of conserved hydrophobic residues in PKC. The predicted binding mode for ILV is entirely consistent with known structure–activity relationships and with our mutational analysis. Our mutational analysis establishes the quantitative contributions of a number of conserved residues to the binding of PKC to ILV. Taken together, our computational docking simulations and analysis by site-directed mutagenesis provide a clear understanding of the interaction between ILV and PKC and the structural basis for design of novel, high-affinity, and isozyme-selective PKC ligands.