Acta Cryst. (2002). A**58** (Supplement), C231

STRUCTURAL BASIS FOR THE SIGNAL TRANSDUCTION BY THE TRAF PROTEINS

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The TNF receptor associated factors (TRAF1-6) are major signal transducers for the TNF receptor (TNFR) superfamily and the IL-1 receptor (IL-1R)/Toll-like receptor (TLR) superfamily. As a first step towards understanding this signal transduction, we determined the crystal structures of the TRAF domain of human TRAF2, both alone and in complex with several receptor peptides, revealing a conserved trimeric self-association of TRAF2 and a symmetrical interaction with the receptor. We also determined the crystal structure of the complex between the TRAF domain of TRAF2 and TRADD. In conjunction with biochemical and cellular studies, we revealed a novel mechanism of TRAF signaling. Recently, we determined the crystal structures of TRAF6, alone and in complex with receptor peptides from CD40 and TRANCE-R. These structures revealed a distinct molecular mechanism for TRAF6 to initiate signal transduction of both the TNFRs and the IL-1R/TLRs.

Keywords: TRAF, SIGNAL TRANSDUCTION, CRYSTAL STRUCTURES

Acta Cryst. (2002). A**58** (Supplement), C231

INHIBITION OF p38 MAP KINASE BY UTILIZING A NOVEL ALLOSTERIC BINDING SITE

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The p38 MAP kinase plays a crucial role in regulating the production of proinflammatory cytokines such as tumor necrosis factor (tnfa) and interleukin-1 (IL-1b). Blocking this kinase may offer an effective therapy for treating many inflammatory diseases. Here we report a novel, allosteric binding site for a diaryl urea class of highly potent and selective inhibitors against human p38 MAP kinase. The formation of this binding site requires a large conformational change for the highly conserved Asp-Phe-Gly motif in the active site of the kinase, previously not observed for any of the protein Ser/Thr kinases. Solution studies show that this class of compounds exhibits slow binding kinetics, consistent with the requirement for conformational change. A 12,000-fold enhancement in the affinity of the inhibitors was obtained by improving interactions in this allosteric pocket as well as establishing binding interactions in the ATP pocket. One of the best compounds in this series, BIRB 796, has picomolar affinity for the kinase and low nanomolar inhibitory activity in cell culture.

Keywords: P38 MAP KINASE, DRUG DESIGN, CONFORMATIONAL CHANGES

Acta Cryst. (2002). A**58** (Supplement), C231

STRUCTURE BASED DESIGN OF SELECTIVE PTP1B INHIBITORS

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Several Protein-Tyrosine Phosphatases have been proposed to act as negative regulators of insulin signaling. Recent studies have shown increased insulin sensitivity and resistance to obesity in PTP1B knockout mice, thus pointing to this enzyme as a potential drug target in diabetes. Structure based design guided by PTP mutants and X-ray protein crystallography was used to optimize a relatively weak, non-phosphorus, non-peptide general PTP inhibitor into a highly selective PTP1B inhibitor. Selectivity has been achieved by the usage of two different principles; attraction vs. repulsion and steric fit vs. hindrance. We have used residue 48 as a selectivity determining residue. By introducing a basic nitrogen in the core structure of the inhibitor, a salt bridge was formed to Asp 48 in PTP1B. In contrast, the basic nitrogen causes repulsion in other PTPs containing an asparagine in the equivalent position resulting in a remarkable selectivity for PTP1B. Recently we have demonstrated that Gly259 in PTP1B forms the bottom of a gateway that allows easy access to the active site for a broad range of substrates, while bulky residues in the same position in other PTPs cause steric hindrance and reduced substrate recognition capacity. Utilizing these differences in accessibility to the active site among various PTPs we show that a general, low molecular weight PTP inhibitor can be developed into a highly selective inhibitor for PTP1B by introducing a substituent, which is designed to address the region around residues 258 and 259.

Keywords: PTPASE INHIBITOR DRUG DESIGN

Acta Cryst. (2002). A**58** (Supplement), C231

DISCOVERY OF SMALL MOLECULAR Bcl-2 INHIBITORS AS POTENTIAL ANTICANCER DRUGS

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Bcl-2 family proteins are key regulators of programmed cell death implicated in many human diseases. Bcl-2 protein is a potent suppressor of apoptosis, and its overexpression contributes to tumorigenesis in many types of human cancers. To define the possibility of modulating Bcl-2 function as an anticancer strategy, here we report the discovery of a small molecular specific targeted to Bcl-2 protein surface pocket, by using a virtual screening strategy based on the three dimension structure of Bcl-2 protein. The Bcl-2 binding assay demonstrated the design small molecular may target Bcl-2 protein, and effectively induced apoptosis of Hela-Bcl2 and siha-Bcl2 cells that Bcl-2 gene was transinfected into Hela and siha tumor cells which overexpress Bcl-2 protein. Furthermore, the design small molecule had notability *in vivo* activity in inhibiting tumor growth in several naked mice model. These results demonstrate a novel approach for therapeutic intervention of tumor growth with small molecule inhibitors of Bcl-2.

Keywords: DRUG DESIGN BCL-2 SCREENING