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Structure of the catalytic subunit of human protein kinase CK2alpha prime with a potent inhibitor

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Casein kinase ll (CK2) is a serine/threonine kinase and widely distributed in various tissues. CK2 predominantly exists in the form of heterotetramer composed of two catalytic subunits (CK2alpha or CK2alpha prime) and two regulatory subunits (CK2beta). Recently, the CK2alpha inhibition has been revealed to prevent the progression of glomerulonephritis. On the other hand, inhibition of CK2alpha prime in testis affect the spermatogenesis. In order to develop of novel CK2 inhibitor for nephritis, we determined the first structure of CK2alpha prime complexed with a potent inhibitor and compared with the structure of CK2alpha. The crystal structure of a C-terminal deletion mutant of human Ck2alpha prime was solved and refined to 3.2 Å resolution. Two isozymes, CK2alpha and CK2alpha prime, reveal the high similarity of the overall structure. The largest structural difference between CK2alpha prime and human Ck2alpha occurs at the loop connecting the strands beta 4 and beta 5. The corresponding region belongs on one hand to the CK2alpha/CK2beta interface in the holoenzyme and on the other hand to the catalytic core, which is structurally highly conserved among the eukaryotic protein kinases. This observation is consistent with the growing evidence that CK2alpha prime and CK2alpha may possess the difference of the relation in vivo with CK2beta and the substrate recognition.

Keywords: protein kinase CK2, structure-based drug design, X-ray crystallography

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Therepeutic antibodies target a locally misfolded region of tumour-specific EGFR

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Epidermal Growth Factor Receptor (EGFR) is a significant target for cancer therapy. In most normal tissues EGFR is expressed at low levels and is inactive but it stimulates growth in many human tumours. EGFR inhibitors have been developed as therapeutic agents but success has been limited by interference from the presence of EGFR on normal tissues. All current therapeutic antibodies bind both active and inactive EGFR. We have characterized antibodies mAb806 and mAb175 with the curious property of binding wild type EGFR on tumour cells but not wtEGFR expressed on normal cells. Fab structures with the EGFR epitope show that the epitope adopts a conformation similar to that in wild type receptor. However, binding is prohibited by significant steric clashes of the Fab with the CR1 domain in both observed conformations of the receptor. From the EGFR structure it appeared that breaking a disulfide bond just before the epitope should allow the CR1 domain to open up sufficiently for antibody binding. Mutant EGFRC271A/C283A binds mAb806 and mAb175 with significantly greater affinity than wtEGFR. While mAb806 fails to inhibit the in vitro growth of cells expressing wtEGFR, mAb806 completely inhibits ligand-associated stimulation of cells expressing EGFRC271A/C283A. Our results provide the first view of how an antibody can recognise a cryptic epitope in a cell surface receptor. The mechanisms of binding of antibodies mAb806 and mAb175 requires a form of the EGFR where the epitope is preferentially exposed during receptor activation. Detection of this locally misfolded form of EGFR associated with tumour cells suggests that it might be possible to produce therapeutics which target local misfolding when other cell surface proteins are overexpressed or activated on tumour cells.

Keywords: therapeutic antibody, cryptic epitope, cancer

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Crystal structures of SERCA in complex with inhibitors with potential as prostate cancer drugs

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Currently no treatment significantly prolongs the survival of men suffering from androgen insensitive prostate cancer. Treatment is complicated by a slow proliferation rate making the cells insensitive to standard chemotherapeutics. The cytotoxin thapsigargin (Tg) inhibits the membrane protein sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), thus obstructing the Ca^{2+} homeostasis and eventually invoking apoptosis. Conjugation of Tg with a specific peptide carrier produces an inert prodrug, which selectively is unmasked by the prostate-specific antigen (PSA) protease at prostate tissue sites, including prostate cancer cells. The structure of SERCA with the Boc-12-aminododecanoyl derivative of Tg was determined at 3.3 Å and revealed a highly unusual binding mode where the 12-aminododecanoyl group penetrates the transmembrane region of SERCA, reaching the surface on the opposite side of the protein, corresponding to one of the Ca^{2+} binding sites (Site II). Furthermore structures of complexes of SERCA with other inhibitors possessing a guaianolide nucleus esterfied with a long un-branched acyl goup have been obtained: i) nortrilobolide (Nor), ii) two additional thapsigargin-based compound (Hzl2308 and Hzl130407), and iii) a synthetic derivative of thapsigargin, in which the guaianolide nucleus has been hydrogenated (Tg2), have been determined at 2.65 Å, 2.65 Å, 2.85 Å and 3.1 Å resolution, respectively. The structures can now be compared to SERCA in the same functional state, but in the absence of inhibitors (Olesen et al. 2007), and give valuable new knowledge on the further development of Tg-derivatives and other SERCA-inhibitors as resources for drug design, both in cancer and

infectious diseases.

Keywords: ATPase, cancer drug design, membrane protein crystallization

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Human thymidylate synthase: Conformational stabilization and dimer asymmetry

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Loop 181-197 of human thymidylate synthase (hTS) populates two conformational states. In the first state, Cys195, a residue crucial for catalytic activity, is in the active site (active conformer); in the other conformation, it is about 10 Å away, outside the active site (inactive conformer). We have designed and expressed an hTS variant, R163K, in which the inactive conformation is destabilized. The activity of this mutant is 33% higher than that of wt hTS suggesting that at least 1/3 of hTS populates the inactive conformer. Crystal structures of R163K in three different crystal forms, with 6, 5 and 2 subunits per asymmetric part of the unit cells, have been determined. All subunits of this mutant are in the active conformation while wt hTS crystallizes as the inactive conformer in similar mother liquors. The structures show differences in the environment of catalytic Cys195, which correlate with Cys195 thiol reactivity, as judged by its oxidation state. One of the dimers is asymmetric with a phosphate ion bound in only one of the subunits. In the absence of the phosphate ion, that is in the inhibitor-free enzyme, the tip of loop 47-53 is about 11 Å away from the active site. The structures of crystals soaked in solutions with dUMP and FdUMP show variable occupancy of the active sites.

Keywords: thymidylate synthase, cancer drug design, inhibitor interactions

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Structure for an HIV-1 reverse transcriptase RNase H inhibitor bound at the active site

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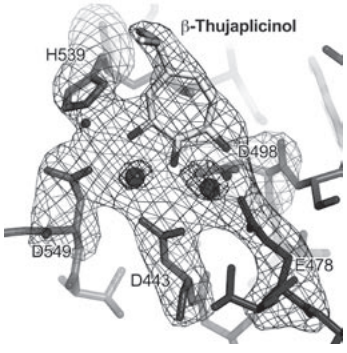
HIV-1 reverse transcriptase (RT) has been a key target of anti-AIDS drugs, because this enzyme is essential to the life cycle of HIV, the causative agent of AIDS. RT converts single-stranded viral RNA into double-stranded DNA suitable for integration into the host cell's genome. To do this, RT uses two enzymatic activities: (1) a DNA polymerase which can use either RNA or DNA as a template, and (2) an RNase H (RNH) that degrades the viral RNA after it is no longer needed as either a template for the first DNA strand or a primer for the second strand. RT inhibitors are typically included as part of a cocktail of therapeutic agents. The efficacy of these therapies has been reduced by the rapid emergence of drug-resistant viral strains. Antiretroviral inhibitors are therefore needed that target novel functions not affected by existing drugs. All clinically used RT inhibitors target the enzyme's polymerase activity, not its RNH activity. We have previously reported a structure for an RNH inhibitor (RNHI) that binds near the polymerase active site of RT. Here, we present a 2.8 Å resolution crystal structure for an RNHI, beta-thujaplicinol, that binds at the RNH active site.



Keywords: HIV-1 reverse transcriptase, inhibitor binding, X-ray crystal structure analysis

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Alanine racemase as a template for drug design against tuberculosis

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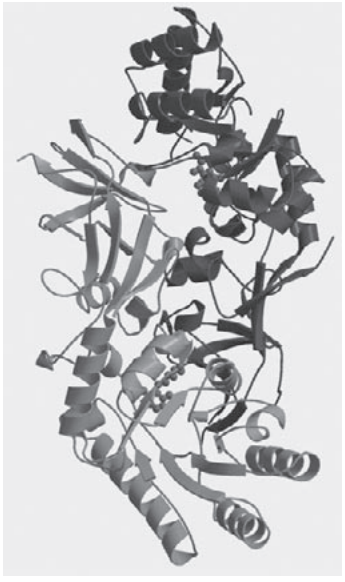
We present progress from an academic structure-aided drug design program aimed at developing new agents for the treatment of tuberculosis. We will present crystallographic data from five alanine racemase structure determinations, including M. tuberculosis and P. aeruginosa. We will analyze this data in terms of pharmacophore development, interface structure, and water conservation. We will review the creation of pharmacophore models that incorporate the results of molecular dynamics simulations. These pharmacophore models have been used to survey, in silico, chemical databases for racemase inhibitors. We will also present new results on inhibitor soaking experiments. From our most recent structures, including the alanine racemase from M. tuberculosis, we present evidence for a conserved substrate entryway that may result in improved pharmacophore development.



Keywords: alanine racemase, structure aided drug design, tuberculosis