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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