CRTSTAL STRUCTURE OF PORCINE PANCREATIC ELASTASE COMPLEX WITH NOVEL PEPTIDIYL TRIFLUOROMETHYL KETONE INHIBITOR OF HUMAN NEUTROPHIL ELASTASE

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Human neutrophil elastase (HNE) has been shown to contribute to the pathogenesis of destructive disease, such as acute respiratory distress syndrome, ischemia-reperfusion injury and multiple organ failure. Water-soluble HNE inhibitor that can be easily administrated intravenously would be desired for the treatment of such acute disorders. AE-3763 has potent *in vivo* activity (ED50=0.42 mg/kg/hr on lung hemorrhage induced by HNE in hamsters) and high solubility (1g/ml H_2O). We have determined the crystal structure of porcine pancreatic elastase complexed with AE-3763 at 1.55 Å resolution. A final complex model had crystallographic R-factor less than 0.19. A clear electron density was observed for the inhibitor molecule in the active site. In this presentation, we will describe the X-ray crystallographic study of the interaction of AE-3763 with the active site of PPE. AE-3763:S-[2-(3-carboxymethyl-2-oxo-1-imidazolidinyl)-acetyl]-L-valyl-N-(3,3,3,-trifluoro-1-isopropyl)-2-oxopropyl-L-prolinamide

Keywords: ELASTASE NOVEL PEPTIDIYL INHIBITOR DRUG DESIGN

AN X-RAY CRYSTALLOGRAPHIC ANALYSIS OF UDP-N-ACETYLGLUCOSAMINE 1-CARBOXYVINYL TRANSFERASE COMPLEXED WITH UDP-N-ACETYLGLUCOSAMINE FROM HAEMOPHILUS INFLUENZAE

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UDP-N-acetylglucosamine 1-carboxyvinyltransferase (murZ) is known to interfere with enzyme-catalyzed reactions in the early stage of the bacterial cell wall biosynthesis. We determined the three-dimensional structure of murZ from Haemophilus influenzae complexed with UDP-N-acetylglucosamine at 2.8 Å resolution by the molecular replacement method. In order to determine the structure of murZ, we have crystallized it by the hanging drop vapour-diffusion method using a protein solution, 6.5 mM UDP-N-acetylglucosamine, mixed with reservoir solution containing 2.0 M ammonium sulfate. It is found that the crystal of murZ is orthorhombic and belongs to the space group *I*222 with unit cell dimensions of a=63.9, b=127.0 and c=125.6 Å. One monomer of murZ is present in an asymmetric unit. It reveals the monomeric arrangement of subunits and an active site located between two domains. The domains have a very similar secondary structure, and the overall protein architecture is similar to that of UDP-N-acetylglucosamine enolpyruvyl transferase from E. coli. The present structural study is expected not only to confirm the mode of binding of the natural substrate UDP-N-acetylglucosamine, but also to provide a model for active-site residues.

Keywords: MURZ, CRYSTAL, HAMOPHILUS INFLUENZAE

STRUCTURE OF MISTLETOE LECTIN I FROM *VISCUM ALBUM* IN COMPLEX WITH GALACTOSE, LACTOSE AND ADENIN

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The crystal structure of the ribosome inactivating protein (RIP) Mistletoe lectin I (ML-I) from *Viscum album* was analysed and refined to about 2.0Å resolution. To analyse the specific features of sugar binding as well as the active site geometry ML-I was further investigated in complex with Galactose, Lactose and Adenine up to 1.9Å. The heterodimeric glycoprotein ML-I consists of a toxic A chain which depurinates specifically the ribosomal 23S/28S rRNA, which causes subsequently a total inactivation of proteinbiosynthesis in eukaryotic cells. The galactose-specific B chain shows distinct lectin activity required for a specific cell surface recognition. The overall protein fold of ML-I is to some extend homologous to ricin from *Ricinus communis* and some other known RIP type II proteins. However obvious structural features in the chain interface region, the overall glycosylation as well as for the sugar binding specificity characterise ML-I particularly, which is already medically applied in immunostimulating-applications as well as in cancer-therapy. The detailed analysis to high resolution and the comparison with other RIP type II proteins and to the so far identified ML-I isoforms provided new insights, about the active site architecture and the cytotoxic RIP reaction mechanism as well as substrate recognition.

Keywords: RIBOSOME INACTIVATING PROTEIN

THE 1.8 Å RESOLUTION CRYSTAL STRUCTURE OF THE S64C MUTANT DIMER OF FLAVODOXIN FROM D. VULGARIS BEARING A CYS64-S-S-CYS64 ENGINEERED DISULFIDE BRIDGE

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Flavodoxins are small flavoproteins isolated from sulphite reducing bacteria. They act as one-electron carriers in a variety of low potential reactions in which their prosthetic group, flavin mononucleotide (FMN), cycles between the semiquinone and the hydroquinone states, while the oxidized state does not participate in physiologically relevant redox reactions. Engineered flavodoxins in which a surface residue has been replaced by an exposed cysteine are useful models to link multidomain redox proteins, obtained by gene fusion, to electrode surfaces. The link between protein domains can be achieved by a peptide linker genetically engineered between the C-terminus of one domain and the N-terminus of the other or engineering a disulfide bridge between the two domains. The latter has been achieved adding Fe(CN) ~ in the crystallization medium of the S64C mutant monomer. The structure of the dimer that crystallizes in the $P4_12_12$ space group with a=b=55.23, c=121.84 Å has been refined to R=0.16 and Rfree=0.23. Data sets where collected with synchrotron radiation (ELETTRA beam-line, Trieste, ITALY). The engineered S64C mutation and the successive oxidation lead to the formation of the disulfide bridge that is forwarded by the position of Cys64 in the monomer in a zone of high accessibility to the solvent. The analysis of the stereochemical parameters characterizing the bridge with S-S bond distance of 2.02 (3) Å, indicates an absence of significant sterical strain.

Keywords: MULTIDOMAIN PROTEINS CRYSTAL STRUCTURE