1508 Fourier-Transform Infrared Spectroscopy (FTIR) is a rapid structural technique used for determining structural changes, enzyme-inhibitor interactions and enzyme activity. Although FTIR is more rapid than time-consuming detailed structural techniques, such as X-ray crystallography, and provides more structural information in terms of bond strengths and mobility than UV-visible spectroscopy, direct interpretation of FTIR data still remains difficult. Molecular modelling, molecular dynamics and quantum mechanical techniques have been used to further interpret FTIR data.

Acylated and native chymotrypsin have been used as a model system, since chymotrypsin is probably the best structurally characterised enzyme and extensive FTIR studies on acylated chymotrypsins have been undertaken. The data has been interpreted as indicating that most inhibitors adopt multiple conformations once covalently bound to the enzyme. We are undertaking X-ray crystallographic simulation and quantum chemical studies to directly link the observed FTIR frequencies with structure.

Molecular modelling and quantum chemical techniques, including Gaussian, Amber and MOPAC, have been utilised to produce models of the acylating agents N-trans-cinnamate and trans-O-hydroxymethylcinnamate, whilst molecular dynamics techniques are being used to provide models of the potential multiple conformations of these inhibitors bound to chymotrypsin.

Crystallographic techniques are being used to validate these models. Small crystals have been grown of α-chymotrypsin with and without the presence of the inhibitor N-trans-cinnamoyl-imidazole (NTCI). Predictions of the FTIR frequencies for specific bonds in the inhibitor are being made using both semiempirical and ab initio quantum chemical techniques and comparisons with the experimental results are being undertaken.

1509 Development and characterisation of novel E. coli expression vectors for the production of membrane proteins for structural studies

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The crystallisation of membrane proteins for structural studies represents the bottleneck in structure determination. Low levels of endogenous expression, high hydrophobicity and low stability in solution all combine to frustrate the membrane protein crystallographer. In many cases, even if enough pure protein can be obtained, it is impossible to grow crystals suitable for structural analysis, if they grow at all. Thus it is necessary to develop novel ways of both expressing and crystallising membrane proteins. It has been possible to obtain high resolution structures for a small number of membrane proteins including the multi subunit E. coli respiratory enzyme cytochrome bo3. We have developed an E. coli expression system involving the fusion of the gene coding for a small (<100 kDa) integral or membrane-associated protein of unknown structure to subunit IV (3 transmembrane spanning domains, intracellular N-terminus and extracellular C-terminus) of cytochrome bo3. In this way the cytochrome bo3 acts as a scaffold molecule supporting the fusion partner. We have fused Protein Z, a soluble protein, human apolipoprotein A-1 (apo A-1) and the G-protein coupled receptor, the human cannabinoid 2 (CB2) receptor to the 3' end of subunit IV of cytochrome bo3. All of the fusion constructs express, as assessed by Western blot analysis. Furthermore, the function of the cytochrome bo3 is maintained, demonstrating that it is possible to express a number of different proteins in this way. Crystals have been obtained for both the Protein Z (difficult to SA) and the apo A-1 fusion constructs (awaiting further analysis). It is hoped that this expression system can be used for a wide variety of membrane proteins.

1510 Bauhinia variegate Proteinase Inhibitor _Physical- Chemical Properties and Biological Activities

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Proteinase inhibitor from Bauhinia variegata seeds was purified by ion exchange chromatography, molecular exclusion chromatography and subsequent reverse phase chromatography. Three isoforms were detected (BvCTI-1, 2, and 3) with single polypeptide chain. The aminoacid analysis of the BvCTI-3 resulted in 167 aminoacids residues and the calculated molecular mass was 18529. Staining for trypsin inhibitors after isoelectric focusing showed the presence of inhibitors with isoelectric point about 4.85, 5.00 and 5.15. The primary structure sequence of BvCTI-3 was determined, confirming the inhibitor as Kunitz type. Extracts from seeds and purified inhibitor were tested for inhibitory activity against trypsin and chymotrypsin from cattle, pig and humans, as well as against plant pathogenic fungi and bacteria growth. The enzymes were strongly inhibited and the micro-organisms had their growth reduced.

1511 Studies of the Structures of Core Subunits of Escherichia coli DNA Polymerase III Holoenzyme


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The core of E. coliDNA polymerase III holoenzyme is comprised of three subunits, α, ε and θ. The polymerase (α) and 3'-5' proofreading exonuclease (ε) activities are the responsibilities of separate subunits. The function of the small θ subunit is not clear. The ε subunit is comprised of an N-terminal domain (ε(1-185)) that contains the active site and interacts with θ, and a small C-terminal domain that interacts with α. The limits of the structured core of ε(1-185) were defined by multidimensional NMR studies, and crystals have been grown for X-ray structure determination.

The solution structure of θ has been solved by NMR methods. Large segments of it appear to be quite flexible, but assume structure on interaction with ε(1-185). The structure of θ will be presented, and progress in determination of the structure of ε(1-185) will be described.