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P–A1–05

3D RECONSTRUCTION OF BOVINE PAPILLOMAVIRUS VISUALIZED AT 9Å

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Purpose: We have been using bovine papillomavirus (BPV) to extend the resolution of 3D density maps reconstructed from cryo-micrographs with a view to visualising protein domains and ultimately tertiary structure.BPV has a diameter of ~ 600 Å, a T number of 7, and two shell proteins (L1 and L2) in a ratio of 30 :1 (1).

Methods: Micrographs were recorded on a Philips EM400RT using a modified Gatan cooling holder, digitized on a Perkin Elmer 1010MG, preprocessed and reconstructed as described (2). Origin and orientational refinement ensued to high precision (3).

Results: Resolution of the 3D reconstruction from ~200 images was assessed by FRC coefficents (4) using the 2-sigma curve as the threshold for meaningful signal and a new method based on the spectral signal-to-noise ratio (5). Two independent reconstructions compared well by FRC (6) and DPR (7).

Conclusions: The 3D reconstruction was statistically significant to 9Å resolution by all criteria applied. Features are seen in greater detail at increasing resolution. The button of density at the center of the 5-fold pentons may mark the location of the minor capsid protein L2.

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P–A1–06

SECONDARY STRUCTURE OF HUMAN SEMINAL PLASMA PROSTATIC INHIBIN: BIOPHYSICAL STUDY

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Purpose: Prostatic Inhibin (HSPI) protein (M_r = 10.4 kDa) has been isolated from human seminal plasma to study the three-dimensional structure.

Methods: CD, fluorescence and 2D NMR experiments have been carried out in aqueous solution at different pH and temperature.

Results & Conclusions: CD measurements revealed that HSPI has a rigid and ordered structure. Fluorescence studies showed that both the tryptophans in the protein are in hydrophobic core. Analysis of NMR spectra has enabled us to identify and assign nearly all spin systems. NMR studies revealed that the protein predominantly adopts anti-parallel β-sheet conformation. No α-helical segments are present. The segments C42-Y43-E44-T45 and S88-V89-S90-E91 are found to adopt type II β-turns.

P–A1–07

SOLUTION CONFORMATION OF [PRO 7,13] αA-CONOTOXIN PIVA

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Purpose : An approach to the study of ligand-recpetor interactions is to investigate the high-resolution structures of various ligands which are molecules of less complexity than the receptor, while systematically collecting relevant information regarding ligand-receptor interactions.

Methods: NMR experiments were performed in a phase-sensitive mode using a Varian UNITY 500 spectrometer at 14°C and 25°C. Calculation of structures were done using distance-geometry and back-calculation of NOESY spectrum using 324 NOE restraints.

Results : The final set of the 12 best structures had an average backbone rms deviation of 0.95Å and back calculation of experimental NOE spectrum had provided 49 new NOE restraints and yielded the R-factors of R_a=0.641 and R_b=0.157.

Conclusions: The solution structure of [Pro 7,13] αA-conotoxin P_{IVA} should provide a framework for structure-function studies for the entire αA family of conotoxins and determin for acetylcholine receptor-targeted conotoxins.

P–A1–08

Solution Structure of E. coli enzyme IIB^{cellobiose} of the PEP-dependent PTS

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Purpose: Determination of the 3D solution structure of enzyme IIB^{cellobiose} (IIB^{cel}), the central catalytic domain in posphoenolpyruvate-dependent phosphotransferase system for cellobiose. Enzyme IIB^{cel} is phosphorylated at Cys10 by enzyme IIA^{cel} and in turn phosphorylates the carbohydrate cellobiose after transport through the membrane.
Methods: Heteronuclear triple-resonance 3D NMR experiment were performed in order to obtain the resonance assigniments and obtain NOE information. Distance geometry and molecular dynamics calculations were performed in to obtain a cluster of structures. In order to simplify the assignment of NOE resonances, we used ambiguous restraints during the MD calculation.
Results: Using app. 2000 NOE intensities a cluster of 32 structures was obtained with an rms deviation of C^{α} positions of 1.3 Å.
Conclusions: Enzyme IIB^{cel} consists of a central four-stranded parallel β-sheet, and 5 α-helices which flank the sheet on both sides. The catalytic residue Cys10 is positioned at the end of the first β-strand just before a flexible loop which probably constitutes the phophorylgroup binding loop.