P–A1–09

SOLUTION STRUCTURE OF EIIA^{mtl} AND INTERACTIONS OF EIIA^{mtl} WITH HPr DETERMINED BY HETERONUCLEAR NMR SPECTROSCOPY.

ILONA W. HANGYI, GERARD J.A. KROON, ESTHER R. THOLE, REMCO J. RENKEN, KLAAS DIJKSTRA, RUUD M. SCHEEK and GEORGE T. ROBILLARD.
GBB, University of Groningen (NL).

Enzyme-II mannitol (EII^{mtl}) of *E. coli* is part of a system that regulates the uptake and phosphorylation of specific carbohydrate substrates. Our group studies the structure/function relationship of the cytoplasmic domains of EII^{mtl} using multidimensional NMR techniques. The C-terminal domain of EII^{mtl} (EIIA^{mtl}, 148 residues, 16.4 kD) has been cloned and overexpressed in *E. coli*.
The low resolution structure was determined using 3D NMR techniques on ^{15}N enriched protein and $^{15}N/^{13}C$ labeled protein. EIIA^{mtl} consists of a two-stranded β-sheet surrounded by 5 α-helices.
Mutant EIIA^{mtl} (EIIAH65Q) that cannot be phosphorylated by phospho-HPr (P-HPr) was constructed. The binding interface between EIIAH65Q and HPr is compared to that of EIIAH65Q and P-HPr, using 2D ^{15}N-HSQC.

P–A1–10

STRUCTURE OF MARE LACTOFERRIN AT 4.0Å RESOLUTION

KAUR P, SHARMA AK, KARTHIKEYAN S, MITRA SN AND SINGH TP.
Department of Biophysics, All India Institute of Medical Sciences, New Delhi-110 029 (India)

Purpose: Lactoferrin an iron binding glycoprotein (MW≈80,000Da) has two structural lobes, each housing one Fe^{3+} and a synergistic CO_3^{2-} ion. Stucture determination has been carried out to understand the mechanism of action and the functional role played by lactoferrin.
Method: Purification from mare colostrum/milk by ion exchange and gel filteration. Crystallization by microdialysis method. Structure elucidation using Molecular Replacement.
Results: The protein crystallizes in orthorhombic space group $P2_12_12_1$ with a=79.8Å, b=103.5Å, c=112.0Å, Z=4 and a solvent content of 57%. The structure has been refined for 6474 reflections in the resolution range 10-4Å. The current R factor is 0.27. The model contains 690 amino acid residues and the resulting electron density is readily interpretable. At this stage of refinement the root mean square error in the coordinates is 0.48. The refinement is in progress.

P–A1–11

CRYSTAL STRUCTURE OF THE 13 SUBUNIT HEART CYTOCHROME C OXIDASE.

Shinzawa-Itoh, H,[1] Tsukihara, T,[2] Aoyama, H,[2] Yamashita, E,[2] Tomizaki, T,[2] Yamaguchi, H,[2] Nakashima, R,[1] Yaono, R,[1] Yoshikawa , S.[1]
[1]Himeji Institute of Technology (JP), [2]Institute for Protein Research, Osaka University (JP).

Purpose: For elucidation of the reaction mechanism of cytochrome oxidase, crystal structure of the enzyme was solved.
Methods: The fully oxidized bovine heart cytochrome c oxidase stabilized with decyl maltoside was crystallized and the crystal structure was solved with MIR method at 2.8 Å resolution with an R value of 20.4 % .
Results and conclusion: Each structure of all the residues of the monomer (1780 in total) except for 23 residues has been converged to a reasonable structure by structural refinement. A hydrogen bond system including an imidazole bound to Cu_A, a peptide unit and a propionate of heme a could be an effective electron transfer path between Cu_A and heme a. Two structures spanned from the cytosolic surface to the matrix , including hydrogen bonds and internal cavities likely to contain water molecules, could serve as proton pumping path. Possible channels, for chemical protons to produce H_2O, for removing the produced H_2O and for O2 were identified.

P–A1–12

STRUCTURE AND MODE OF ACTION OF A NEW POTASSIUM CHANNEL BLOCKER

NORTON RS,[1] TUDOR JE,[1] PALLAGHY PK,[1] PENNINGTON MW. [2]
[1] Biomolecular Research Institute, Parkville (AUS),
[2] Bachem Bioscience Inc, King of Prussia (USA).

Purpose: Kv1.3 potassium channels in T-lymphocytes are involved in lymphocyte proliferation and lymphokine production, and blockers of this channel are of interest as potential immunosuppressants. ShK toxin (1) is a potent blocker of this channel. Our aim is to define the structural basis for channel blockade by this polypeptide.
Methods: The structure was solved by 2D NMR.
Results: ShK toxin shows little sequence similarity to scorpion-derived potassium channel blockers, and its half-cystines are paired differently. Its structure in solution is also different, consisting of two short helices and a series of reverse turns. We are now mapping onto this structure the K^+ channel binding surface, using synthetic analogue data, which have identified several residues essential for binding (2). Based on these data, ShK toxin has also been docked into a model of the channel pore (3).
Conclusions: ShK toxin constitutes a novel protein fold capable of inhibiting potassium channel function.

1. O. Castañeda et al. *Toxicon* 33, 603-613 (1995)
2. M.W. Pennington et al. *Biochem. Biophys. Res. Commun.* 219, 696-701 (1996)
3. J. Aiyar et al. *Neuron* 15, 1169-1181 (1995)