PS04.17.29 A FORMAL ANALYSIS OF HINGING IN ACTIN. Rebecca Page, Princeton University, Uno Lindberg, Stockholm University and C.E.Schutt, Princeton University.

A formal analysis of the current atomic models of the protein actin reveals that changes in actin conformation are localized to only a few regions of the actin polypeptide. These regions function as hinges, about which the actin domains rotate as rigid bodies. Specifically, the crystal structures of the actin monomer, including the α-actin [2,3] and β-actin [4,5] structures, were systematically compared, using methods for studying alternative conformations of the same protein developed by Chothia, Lesk and Gerstein[5]. The results of this analysis indicate that the conformational changes observed in actin can be best described as a combination of small hinging and sheering motions between actin domains. The motions are due to structural changes in loops and helices connecting the actin domains. This analysis confirms predictions made about mechanisms of actin domain motions based on tertiary structure[5], and extends the observations made by Schutt *et al.* in 1993.

The mechanisms of conformational change observed crystallographically serve as strong constraints on plausible models of the actin filament. Thus, the above analysis was extended to include the present models of the actin filament [6,7]. Tertiary and secondary structural elements of the two f-actin models differ significantly from the conformations observed in the actin crystal structures. These changes require a higher energetic cost than those observed crystallographically, which are classified as low energy transitions[4].

The β-actin monomers form polymeric actin ribbons in the profilin:β-actin crystals. It has been proposed that the atomic of structure of the actin filament may be structurally related to the actin:actin contacts observed in the ribbon[2]. The results described above are being used as constrains in the development of an alternative model of f-actin. In this model, the actin:actin ribbon contacts are preserved and the conformation of the monomer is related to the crystallographic structures by changes in the hinges described above.

[1] Kabsch,*et al.* (1990) *Nature* , **347**, 37-44. [2] McLaughlin *et al.* (1993) *Nature* , **364**, 685-692. [3] Schutt *et al.* (1993) *Nature*, **365**, 810-816. [4] Chik *et al.* (1996) submitted. [5] Gersteinet *al.* (1994) *Biochemistry*, **33**, 6739-6749. [6] Lorenz *et al.* (1993) *J.Mol.Biol.*, **234**, 826-836. [7] Tirion *et al.* (1995) *Biophys.J.*, **68**, 5-12.

PS04.17.30 STRUCTURE OF HEVEIN, A LECTIN-LIKE FROM *HEVEA BRASILIENSIS* AT 1.9Å RESOLUTION. Kaliyamoorthy Panneerselvam and Manuel Soriano-García. Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Delegación Coyoacán 04510. México, D.F.

Hevein is a small, single-chain protein of 43 amino acids and rich in cysteine and glycine. Hevein is a lectin-like protein and experimental evidence indicates that this protein is involved in the coagulation of latex by bridging together rubber particles[1]. We solved the crystal structure of hevein by means of molecular replacement techniques using the PROTEIN program and refined the structure at 3.0Å resolution[2]. However, the resolution limit of the diffraction data collected could be improved and prompted us to remeasure a new native data-set up to 1.9 Å resolution. The crystals are orthorhombic with space group $P2_12_12_1$ with cell dimensions a= 21.59(2), b= 31.60(3) and c= 51.21(5)Å, V= 34931(1) Å^3 and Z= 4. The three-dimensional structure of hevein has been refined with XPLOR and SHELXL93 programs. A total of 324 protein atoms, 20 ordered water sites and 23 disorder water sites refined to a final R-factor of 0.16 at a resolution of 1.9Å and an average B-value of 15.1Å^2, using 73% (2326) of the total possible number of reflections in the range 10 to 1.9 Å with $I \geq 2\sigma(I)$. The final structure has r.m.s deviations from ideal bond distances and angles of 0.017Å and 2.7°, respectively. The standard deviation of atomic position estimated by Luzzzati plot was 0.15Å. The protein comprised of four-stranded ß-sheet, five turns and a short α-helix located at the C-terminal, all of these is required to accomplish the hevein-lectin like fold. The final structure of hevein in the solid state displays a folding similar to one determined by NMR techniques and also to that of domain C of wheat germ agglutinin.

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[1]Gidrol, X., et al., (1994). J. Biol. Chem. 269, 9278-9283.
[2]Rodríguez-Romero, A., et al., (1991)FEBS Lett.291,307-309.

PS04.17.31 ANALYSIS OF C-H...O HYDROGEN BONDS IN BETA SHEETS. Vasantha Pattabhi, G. Felcy Fabiola & S.Krishnaswamy+ Department of Biophysics, University of Madras, Guindy Campus, Madras-600025, INDIA and +Bioinformatics Centre, School of Biotechnology, Madurai Kamaraj University, Madurai-625021, INDIA

C-H...O hydrogen bonds stabilising organic structures have been thoroughly studied and well documented.However,the observation of contiguous C-H...O hydrogen bonds in the beta sheet region of one of our peptide structures prompted us to analyse the beta sheet regions of proteins whose high resolution structures are known.Our analysis shows that C-H...O hydrogen bond geometry calculated using the PDB coordinates is well within the acceptable limits. As for the sequence specificity, tryptophan is the major contributor (23%) to C-H...O hydrogen bonds in antiparallel region whereas Val, Leu and Lys contribute equally to the parallel sheet region.On the whole residues other than the nonpolar ones are poor contributors to C-H...O in parallel sheets,though lysine is an exception. In the side chain main chain interactions, charged residues like Arg,Gln,His have a major role as compared to others. Twist angle of the beta sheets do not seem to affect the formation of C-H...O hydrogen bonds in a systematic way.However C-H...O and N-H..O hydrogen bond strengths appear to be correlated.

PS04.17.32 HOW TO AVOID OVERFITTING IN REFINEMENT OF STRUCTURES WITH APPROXIMATE NON-CRYSTALLOGRAPHIC SYMMETRY. HIGH RESOLUTION STRUCTURE REFINEMENT OF GLUTAMINE SYNTHETASE REVEALS EVIDENCE FOR HOMOTROPIC COOPERATIVE BINDING. Gaston M.U. Pfluegl, Harindarpal Gill & David Eisenberg. Molecular Biology Institute, UCLA, Los Angeles, CA 90095-1570, USA.

Glutamine Synthetase (GS) is a key enzyme in nitrogen metabolism. GS from *S.typhimurium* is a stable complex of large size, consisting of 12 identical chains assembled into a dodecamer with 62 symmetry (Mr ~ 620 KDa; 12 times 468 amino acid residues). In the presence of ADP, GS crystallizes in space group C2 with 1 dodecamer per asymmetric unit. From the association constant of ADP for GS, about two ADP should be bound per dodecamer under crystallization conditions.

The previous 2.8 Å model for GS has been refined with strict non-crystallographic symmetry restraints (12-fold averaging). In this model, three regions were invisible and some of the active site residues showed disorder. No density was found for ADP in this model.

The present work is based on a 2.5 Å cryo synchrotron data set from a single crystal (98% complete; 200,000 reflections; 10 times redundancy). The new model was refined with 12 independent but constrained subunits and shows density for two bound ADP molecules in two adjacent active sites. This density was not