MS01 P01

Mutants of recombinant horse L-chain apoferritin co-crystallized with haemin Jean-Paul Declercq[a], Natalia de Val[b], Robert R. Crichton[b], [a]*Unit of Structural Chemistry,* [b]*Unit of Biochemistry, University of Louvain, 1 place Louis Pasteur, Louvain-la-Neuve, Belgium.*
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Keywords: apoferritin, haemin, demetallation

Structure similarities between both eukaryotic ferritins and prokaryotic ferritins have been extensively demonstrated. However, there is an essential difference between these two types of ferritins : whereas bacterioferritins binds haem, *in-vivo*, as Fe-protoporphyrin IX (this haem is located in a hydrophobic pocket along the 2-fold symmetry axes and is liganded by two axial Met residues), eukaryotic ferritins are known as non-haem iron proteins. However, it has been shown that horse spleen apoferritin is able to interact with haem. Studies of haemin (Fe(III)-PPIX) incorporation into horse spleen apoferritin have been carried out, which show that the metal free porphyrin is found in a corresponding pocket [1]. A mechanism of demetallation of haemin by L-chain apoferritins was subsequently proposed : this involved 4 Glu residues (E 53,56,57,60) situated at the entrance of the hydrophobic pocket and the Arg situated in position 59. This process appeared to be favoured by acidic conditions [2]. To verify this mechanism, we have determined the structures of wild type recombinant horse L chain apoferritin and of the mutant in which the cluster of acidic residues (Glu 53, 56, 57, 60) thought to be involved in demetallation of haemin have been mutated to Gln (quadruple mutant). We have prepared two further mutants in which the Arg at position 59 is mutated to Met, in both the wild-type and the quadruple mutant, and we have determined their three-dimensional structures when they are co-crystallised with haemin. This particular mutation reconstitutes the axial ligands found to be bound to iron within the haemin in bacterioferritins. All the crystallizations experiments were performed in acidic and basic conditions, leading to a total of eight crystal structures analyzed by X-ray diffraction. In several cases, both tetragonal and cubic crystals were obtained in the same crystallization drop and for an easier comparison of the results, the cubic form (space group F432) was always selected.

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MS01 P02

Why is Alanine Such a Good α-Helix Builder ?
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To answer this question several ab initio calculations have been made. However, it was found that the global minimum of L-N-acetyl-alanine-methylamide or L-N-formyl-alanineamide differs considerably in the φ,ψ angles from ideal α-helix values. I propose the hypothesis that an energy gain consisting of some increments listed below compensates for the energy gap if the structural fragment

–CONH–CH(CH3)–CONH–

is part of a protein strcture.

1. No sterical hinderance by the small methyl group
Energy consuming sterical interactions inside the protein are avoided placements with sterical restrictions are filled with alanine, because glycine would be to small to stabilize the α-helix (see point 2)

2. Enough space filling to stabilize the α-helix
The area of the Ramachandran plot around the ideal α-helix point should be a 'deep valley', spoken mathematically there should be a strong gradient of the energy map scalar field around the ideal α-helix point.

3. Hydrophobic interactions of the methyl group inside the protein
The non-polar methyl group of alanine fits well into the lipophile environment inside the protein

4. 2 hydrogen bonds
practically linear to the upper and lower helix turn. Of course this point applies to all amino acids and is not specific for alanine, listed only for completeness.

5. Van der Waals interaction
of the methyl group inside the protein,very small energy increment, could be neglected, listed only for completeness

Alanine should therefore have a high occurrence probability inside the protein. Outside, a methyl group pointing to bulk water, would be a less favoured placement of alanine because of disruption of the bulk water hydrogen bond pattern.

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Greetings to all paticipants of the ecm-24, and to the organizing committee in Marocco.

MS01 P03

Growing Chain Peptide Folding Hypothese Docteur Pierre Quellère Centre for hiv/AIDS Networking KwaZulu-Natal Member of the European Peptide Society
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A correct prediction of the native structure of a protein is only possible if the process of peptide chain folding is precisely simulated. The folding of the peptide chain is correlated with the biosynthesis of the peptide chain at the ribosome. The peptides chain folds during the biosynthesis, one domain after the other.
The growing chain peptide folding hypothesis has firstly been presented as virtual poster at the structbiol04, EMBL meeting, 2004, Heidelberg, Germany.

Hypothesis:
The molecular dynamics of protein folding are relatively fast in comparison to the slow process of peptide bond formation and place shifting of the growing peptide chain at the ribosome.
In procaryotic organisms the peptide chain leaves the ribosome to reach the bulk solution of the cytoplasm. As soon as the growing chain appears at the ribosomal exit region the peptide starts folding, first secondary structural elements, than the tertiary elements until a first domain is completed. In this partial chain folding process the surface structure of the ribosomal exit region is involved. An

empirical force field simulation should use the surface of the ribosomal exit region and bulk water.
In eucaryotic organisms the placement of protein synthesis is the rough endoplasmic reticulum. The growing peptide chain is channelled through the membrane into the matrix. The simulation should include the structure of the surface of the matrix sided channel protein. During folding molecular interactions with this region can be expected. After the last domain has been channelled and folded the domains rearrange and leave the synthisis/channelling exit side.

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Greetings to all paticipants of the ecm-24, and to the organizing committee in Marocco.

MS01 P04

Protein conformation families for automatic model building. Frantisek Pavelcik[a,b]. [a]*Department of Inorganic Chemistry, PRIF UK, Bratislava, Slovakia.* [b]*Department of Chemical Drugs, FaF VFU, Brno, Czech Republic.*
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Keywords: conformation, protein, model building

Conformation families were determined for di-peptides, tri-peptides, tetra-peptides and penta-peptides. These are related to model building fragments called AlphaD, AlphaT, AlphaQ, and AlphaP. The conformation family is a region of a conformation space highly populated with experimental conformations. The smoothed conformation density in this region should have a local or global maximum. The conformation space is an infinite periodic torsion angle space. The conformation families were determined by direct multidimensional mapping (2-D, 4-D, and 6-D). A method of Pavelcik & Vanco [1] was used. All PDB structures (Febr. 2007) with resolution better than 1.5 Å, and 90% homology criterion were selected for analysis. The number of calculated torsion angles was almost 500 000. The grid of mapping was 16. The search probe was variable: $R=R_D\sqrt{0.5N}$; R_D is empirically found radius for 2-D search, N is dimension of the conformation space. Penta-peptide conformations (8-D) were generated by a combination of two tri-peptide conformation families. Less populated families were removed. The number of conformation families for di-peptides is 6, for tri-peptides 24-26, and 130-140 for tetra-peptides.
The conformation families are used as search fragments in the model building program NUT [2], and will be used for automatic model building at lower resolutions.

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MS01 P05

RegX3 – a full-length response regulator that exhibits 3D-Domain swapping. J. King-Scott, E. Nowak, S. Panjikar, E. Mylonas, M. Roessle and P.A. Tucker *European Molecular Biology Laboratory, Hamburg Outstation, EMBL c/o DESY, Notkestr. 85, 22603 Hamburg, Germany.*
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Keywords: Two-component system, response regulator, *Mycobacterium tuberculosis*

RegX3, annotated as Rv0491 in the *Mycobacterium tuberculosis* (MTB) genome, is the response regulator (RR) from the SenX3-RegX3 two-component system (TCS). Two component systems are the predominant signalling systems found in prokaryotes. The SenX3-RegX3 TCS is one of the twelve TCS within the MTB genome [1,2]. These systems monitor environmental stresses and allow the bacteria to respond to the external threat by altering gene expression patterns. The sensor histidine kinase (SK), which is normally membrane anchored detects the external signal and passes it to the cytosolic response regulators (RR) [3]. The two components communicate through phosphotransfer between a histidine residue in the SK and an aspartic acid in the RR.
RRs are usually multi-domain proteins with the first domain being the characteristic receiver domain, which contains the conserved aspartic acid. The second domain can vary but is most commonly a DNA-binding domain. RRs are classified according to the C-terminal (effector) domain. Based on sequence comparisons, the RegX3 contains a winged-helix-turn-helix DNA-binding domain placing it in the OmpR/PhoB subfamily, the largest subfamily of Rrs [4-6]. RRs from this subfamily have been hypothesised to dimerise on a highly conserved interface in the receiver domain [7]. The crystal structure of RegX3 was solved in 2005 and revealed the first structure of a full-length RR exhibiting domain swapping on the proposed dimerisation interface. To support the high-resolution model, small-angle scattering measurements were made on the RR at various concentrations. According to the molecular mass estimate, RegX3 is monomeric in solution at low concentrations and becomes dimeric at higher concentrations. The molecular mass calculated from the scattering curve of RegX3 at the highest concentration agrees well with the molecular mass of the dimer.

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