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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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.

[1]Précigoux, G., Yariv, J., Gallois, B., Dautant, A., Courseille, C., Langlois d'Estaintot B., *Acta Crystallogr*. D50, 1994, 739.
[2]Crichton, R.R., Soruco, J.A., Roland, F., Michaux, M.A., Gallois, B., Précigoux, G., Mahy, J.P., Mansuy, D., *Biochemistry*, 1997, 36, 15049.

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