**PS-03.12.06** X-RAY STRUCTURAL STUDY OF APO-HAEMOFERRITIN. By G. Précigoux*, J. Yariv, A. Dautant, B. Gallois, and B. Langlois d'Estaintot, B. Busetta, Laboratoire de Cristallographie, Université de Bordeaux I, 33405 Talence, France.

Until recently, interest in ferritin structures was subservient to ferritin function in iron storage. This changed with the report of Kadir, F.H.A. and Moore, G.R. (FEBS Letters, 1990, 271, 81-84) who showed that horse spleen ferritin binds haem non covalently, with a stoechiometry of about 16 heams for 24 subunits.

Horse spleen ferritin thus affords for the first time the structure of an apo-haemoferritin.

Octahedral crystals of horse spleen apo-ferritin complexed to Sn-protoporphyrin IX were obtained by addition of stoechiometric amount of metalloporphyrin to a solution of apo-ferritin prior to crystallization. The crystals belong to the F432 space group with a parameter of 184.0 Å. The structure was solved by molecular replacement, starting with a horse spleen apo-ferritin model.

The structure of the complex has been refined to an R value of 18% with data collected to 2.6 Å (completeness 85%).

All the 174 residues were located. In the last cycles of refinement, the haem binding site was observed and 175 water molecules were positionned.

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**PS-03.12.07** STRUCTURAL STUDIES ON BOVINE PLASMA RETINOL BINDING PROTEIN (RBP). By G. Zanotti*, R. Berni+, P. Spadon and H.L. Monaco#, Biopolymer Research Center and Dept of Organic Chemistry, University of Padova, +Institute of Biochemical Sciences, University of Parma and #Dept of Genetics and Microbiology, University of Pavia, Italy.

Retinol transport, from its store to target cells, is performed by plasma Retinol binding protein (RBP), a single domain protein of about 21 kDa (Goodman, D. S., 1984,in "*The Retinoids*", Academic Press, New York). RBP, sinthesized in epathocites, is secreted in the blood, where it interacts with thyroxine binding protein (TTR). Upon delivery of retinol to cell surface receptors, the resulting *apo*RBP possesses low affinity for TTR and can be selectively filtered through kidney glomeruly.
The three-dimensional structures of human and bovine RBP, in the complexed and uncomplexed forms with retinol, were determined (Cowan, S.W., Newcomer, M.E. and Jones, T.A., 1990, *Proteins: struct. Funct Genet.* **8**, 44-61; Zanotti, G., Ottonello, S., Berni, R. and Monaco, H.L., 1993, *J. Mol. Biol.*, in press; Zanotti, G., Berni, R. and Monaco, H.L., 1993, *J. Biol. Chem.*, in press). It was shown that a limited conformational change is present in the unliganded protein prepared *in vitro* by extracting retinol during the protein purification or with ethyl ether. The crystal structure of TTR has also been determined (Blake, C.C.F., Geisow, M.J., Oatley, S.J., Rerat, B and Rerat, C., 1978, *J. Mol. Biol.* **121**, 339-356). On the contrary, crystals of the complex RBP-TTR suitable for x-ray diffraction studies have not yet been obtained.
In order to explain some differences in the kinetic behavior of unliganded RBP produced using other organic solvents, the crystal structure determination of the unliganded protein produced extracting the vitamin with esanol was undertaken. The structure, isomorphous with the previous unliganded forms of RBP, was refined to a resolution of 1.6 Å with a final R factor of 0.18. Differences observed in the molecular models will be described.
It was also observed that the replacement of retinol with its analogs in the RBP molecule affects the interaction between RBP and TTR. To study the effect of the binding of modified retinoids on the formation of the RBP-TTR complex, the structure of the N-ethyl retinamide-RBP complex was determined. The final crystallographic R factor was 0.17 for 11261 observed reflections between 9.0 and 1.9 Å. Features of the complex and implications for the binding to TTR will be discussed.

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