PS04.18.01 \textbf{STRUCTURE OF THE R121D MUTANT N-TERMINAL LOBE OF HUMAN LACTOFERRIN.} B.F. Anderson, W. Breyer, R.L. Kingson, H.R. Faber, C.L. Day and E.N. Baker, Department of Biochemistry, Massey University, Palmerston North, New Zealand.

The three dimensional structure of the iron free, R121D mutant N-terminal half molecule of human lactoferrin is currently being investigated by molecular replacement methods. Small colourless crystals were obtained by microdialysis from low-salt Tris/Isopropanol solutions and a data set complete to 3Å was collected on image plates with CuKα radiation. Although the diffraction pattern appears hexagonal the crystals are trigonal P3₁ with a=b=151.3Å, c=48.6Å, γ=120°.

The program AMORE, using the two domains of the recombinant N-terminal lobe of human lactoferrin, LF₅₆ (Day et al., J. Mol. Biol. (1995) 232, 1084-1100) independently, located two independent molecules in the assumed P₆₃ asymmetric unit (correlation coefficients > 0.5). One molecule however seriously overlaps with its symmetry mates about the screw axis. A possible expansion of this is that there is merohedral twinning about a two fold axis parallel to the unique direction of a P₃₁ crystal. Statistical tests on the observed data confirm that twinning is present with an approximate twin fraction of 0.4. Further rotation/translation searches with AMORE in space group P₃₂₁, and using as search model one of the full molecules found previously gives four strong sites which can be divided into pairs related by a diad coincident with the 3₁ axis. Since this model gives a V₀ of 4.4, our present efforts are directed toward locating the rest of the molecules and is refined to 1.6 Å resolution.

Comparison with (Ca²⁺)₂-Calbindin has two EF-hands with a conformation very similar to that shown by the N lobe of apolactoferrin. Comparison with Lf₅₆ shows that there has been a rotational movement of the N₂ domain with respect to N₁ of approximately 52°. Our present efforts are directed toward locating the rest of the molecules and is refined to 1.6 Å resolution. Further refinement and structure analysis is in progress.

PS04.18.02 \textbf{CRYSTAL STRUCTURE OF CALBINDIN D₉K BINDING MG²⁺.} Maria Andersson¹, L. Anders Svensson¹, Sara Linse¹. ¹Molecular Biophysics and ²Physical Chemistry 2 at the University of Lund, Box 124, S-221 00 Lund, Sweden

Calbindin D₉K is thought to transport Ca²⁺/Mg²⁺ in mammalian² and is present in bone and in the intestine. Calbindin D₉K has two EF-hand subunits, binding calcium cooperatively³. In this structure only one of two EF-hand loops binds Mg²⁺ and the other is devoid of metal ions. Significant structural differences exist between the (Ca²⁺)₂-calbindin structure⁴ and the Mg²⁺-calbindin structure. Due to these structural differences molecular replacement with (Ca²⁺)₂-calbindin as a model failed to solve the structure and instead isomorphous replacement techniques had to be used. The final structure includes all 75 aminoacids, Mg²⁺, 50 water molecules and is refined to 1.6 Å (87% completeness). The final R-factor is 19.6 %. The Mg²⁺-calbindin structure further supports the idea of cooperativity. As Ca²⁺ levels increase intracellularly, Ca²⁺ goes into the empty site, inducing a conformational change which releases Mg²⁺. Thereafter Ca²⁺ binds to the second site.

²Henningsen C., Stuun M., Olgaard K. \textit{Miner Electrolyte Metab} 20, 265-273, (1994)