s8a.m7.p9 **Magnesium-dependent Serratia marcescens endonuclease: three-dimensional structure at 1.1 Å resolution and molecular mechanism of enzyme action.**
V.V. Lunin[a], S.V. Shlyapnikov[b], M. Perbandt[c], Ch. Betzel[c], A.M. Mikhailov[a], *[a]Institute of Crystallography of RAS, Moscow, Russia, [b]Engelhardt Institute of Molecular Biology of RAS, Moscow, Russia, [c]Institute of Physiological Chemistry, University Hospital, Macromolecular Structure Unit, c/o DESY, Hamburg, Germany.*
Keywords: *Serratia marcescens*, endonuclease, X-ray structure.

The three-dimensional crystal structure of *Serratia marcescens* endonuclease has been refined at 1.1 Å resolution to R-factor of 12.9% and R-free 15.6% with use of anisotropic temperature factors. The model contains 3694 non-hydrogen atoms, 715 water molecules, four sulfate ions and two Mg^{2+}-binding sites at the active site of homodimeric protein. The magnesium ion linked to the active site Asn-119 of each monomer is surrounded by five water molecules and shows an octahedral co-ordination geometry. The temperature factors for the bound Mg^{2+}-ions in A and B subunit are 7.08 and 4.60 $Å^2$, and the average temperature factors for surrounding water molecules are 12.13 and 10.3 $Å^2$, respectively. In comparison with earlier structures, alternative conformations of side-chains are defined for 51 residues of dimer. Due to high resolution such details of structure as protonated atoms of active site residues became visible. Based on the high resolution *Serratia* nuclease structure, functional characteristics of the natural and mutational forms of enzyme and considering its structure analogy with homing endonuclease I-*Ppo*I, a plausible mechanism of enzyme functioning is proposed.

s8a.m7.p10 **T_6 and T_3R_3 Human Insulin at Atomic Resolution**. R.H. Blessing,[1] G. D. Smith,[1,2] and W.A. Pangborn.[1] *[1]Hauptman Woodward Institute, 73 High Street, Buffalo, New York 14203, USA. [2]Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, New York 14203, USA.*
Keywords: metalloproteins.

Crystals of human insulin in both the T_6 and T_3R_3 conformational states of its hexameric zinc complex, $Zn[(AB)_2]_3Zn$, were grown under microgravity on NASA space shuttle flight STS-95, and X-ray data from cryofrozen crystals of both types have been measured at beamline X8c at NSLS at BNL.

The T_6 data to d_{min} = 0.9 Å resolution merged to 59,760 unique reflections with $\langle n \rangle$ = 24.4 and R_{merge} = 0.051. At the present stage of cns_solve refinement, $R(|F|)$ = 0.231 and R_{free} = 0.242 to 1.0 Å resolution, and the model for the crystal chemical asymmetric unit consists of an $(AB)_2$ dimer of two-chain insulin AB monomers (with hydrogen atoms), two zinc ions, 12 disordered side chains, and 140 water molecules in fully occupied sites. As a result of freezing, the N-terminus of the B-chain of monomer 2 is displaced 7.9 Å, which eliminates two salt bridges present in the room temperature structure and places the phenyl ring of B1 Phe directly under and parallel to a guanidinium group of B22 Arg at 4.3 Å plane-to-plane.

The T_3R_3 data to d_{min} = 1.2 Å resolution merged to 53,700 unique reflections with $\langle n \rangle$ = 8.6 and R_{merge} = 0.049. At the present stage of cns_solve refinement, $R(|F|)$ = 0.172 and R_{free} = 0.193. As a result of freezing, the hexagonal c axis of the space group R3 crystal doubled; the doubled asymmetric unit contains two independent $(AB)_2$ insulin dimers, and each TR-dimer contains one T-AB and one R-AB monomer conformation. One T-state zinc has octahedral coordination, and the other has tetrahedral; both R-state zincs are tetrahedrally coordinated. Features not observed in the room temperature structure include an extra zinc ion in one of the hexamer's phenolic-additive binding pockets, tetrahedrally coordinated by B5 and B10 His side chains and two water molecules. The second phenolic binding pocket contains only water. Two other, partially occupied zinc sites are located between the two independent dimers. One zinc is tetrahedrally coordinated by a B5 His side chain and three chlorides, while the second zinc is tetrahedrally coordinated by the same B5 His side chain in an alternate conformation, a B5 His side chain from a symmetry related dimer, and two chlorides.

Research supported by a grant from Eli Lilly Research Laboratories and by USDHHS PHS NIH grant no. GM56829.