# Crystallization of Insulin Derivatives

*Ivana Pulić, Biserka Prugovečki, Adela Jurković, Dubravka Matković-Čalogović*

***Department of Chemistry, Faculty of Science, University of Zagreb, Croatia***
ipulic@chem.pmf.hr

Every protein is unique, so the parameters for crystallization have to be determined for each protein. The influence of salt concentration (NaCl, NaBr, NaI) and protein concentration on crystallization of the human insulin hexamer derivative stabilised by Co or Zn ions was examined. In addition, the crystal structure of the insulin derivatives was determined by the single X-ray crystallographic method. Therefore, the conditions in which single crystals of the Co(II) chloro-, Zn(II) bromo- and Zn(II) iodo- derivatives of human insulin are formed have been determined using the hanging drop vapour diffusion method[1].

Biosynthetic human insulin used for this method was Zn-free. The initial concentration of human insulin was 7.5 mg $cm^{-3}$ for the bromo insulin derivative. In the case of Co(II) chloro- and Zn(II) iodo-insulin derivatives initial concentrations that resulted in crystals were both 3.5 and 7.5 mg $cm^{-3}$. For a successful crystallization of the cobalt(II) chloro-derivative of human insulin the concentration of sodium chloride in the precipitant solution varied between 1.00 and 2.00 mol $dm^{-3}$ while the concentration of cobalt(II) acetate tetrahydrate was between 0.022 and 0.045 mol $dm^{-3}$. Furthermore, the optimal concentration of sodium iodide varied between 0.60 and 1.05 mol $dm^{-3}$ for crystal growth of zinc(II) iodo-derivative of human insulin and for the zinc(II) bromo- insulin derivative the concentration of sodium bromide was 1.75 mol $dm^{-3}$. The optimal concentration of zinc(II) acetate dihydrate was 0.022 mol $dm^{-3}$ for Zn(II) bromo- and Zn(II) iodo-derivatives. The temperature of crystallization was 293(2) K.

All investigated derivatives crystallize in the trigonal system, space group *R*3. The conformation of the cobalt(II) chloro-insulin hexamer was determined as $T_6$ (similarly to the Ni-derivative)[2] while the bromo- and iodo-insulin derivatives exhibit the $T_3R_3$ form of insulin (as in the Zn-chloro derivative)[3]. Due to that a difference in coordination of metal ion was observed. In the chloro-insulin derivative both Co ions lie on the three-fold axis and are octahedrally coordinated by three symmetrically related His B10/His D10 and three water molecules. In the I-derivative both coordination sites are disordered with the coordination being tetrahedral (3His, one I) and octahedral (3 His, 3 water molecules). In the bromo-insulin derivative one Zn ion lies on the three-fold axis and is tetrahedrally coordinated by three symmetrically related His B10 and one bromide ion while the other is off-axial and is tetrahedrally coordinated by His D5 and His D10 and two bromide ions.

[1] Cutfield, S. M. (1975). D.Phil Thesis, University of Oxford.
[2] Prugovečki, B. *et al.* (2009). *Croat. Chem. Acta*. **82** (2), 433-438.
[3] Ciszak, E. & Smith, G. D. (1994). *Biochemistry*. **33**, 1512-1517.