ESRF	**Experiment title:** The crystal structure of a native insulin monomer, and its relevance to receptor binding and fibre formation.	**Experiment number**: LS-1811
Beamline: ID14-3	**Date of experiment**: from: 06/12/00 to: 08/12/00	**Date of report**: 30/08/01
Shifts: 1	**Local contact(s)**: Steffi Arzt	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Zhang Youshang, Jean L. Whittingham, Johan P. Turkenburg, Eleanor J. Dodson, Jens Brange and G. Guy Dodson.

Chemistry Department, University of York, YO10 5DD, U.K.

Report:

Introduction

The peptide hormone insulin carries out its *in vivo* activities as a monomer yet one of its most striking characteristics is its ability to adopt different association states including dimers, tetramers and hexamers. Hexamer formation leads to the burial of hydrophobic surfaces on the molecule, which might otherwise render insulin vulnerable to random aggregation interactions. It is not surprising therefore that conditions which promote the monomerisation of the molecule often give rise to fibril formation. Studies on insulin fibres have shown that acid pH, heat and agitation all promote their formation. To learn more about the process of fibrillation, the present crystallographic study is focussed the effect of low pH on the structure of the insulin molecule.

Materials and methods

Human insulin, donated by Novo Nordisk A/S, Denmark, was crystallised at pH 2.0 in the presence of sulphate ions. The crystals grew in spacegroup I422 (cell dimensions a = b = 58.31 Å, c = 54.69 Å), with one molecule in the asymmetric unit. A 1.6 Å data set was collected on station ID14-3. The data were processed using DENZO and scaled and merged in SCALEPACK (Otwinowski & Minor, 1997). The structure was solved with the molecular replacement package AMoRe (Navaza, 1994), using the coordinate for the monomer of the 2-zinc insulin dimer as a starting model (Baker et al., 1988).

Refinement was then carried out using REFMAC (Murshudov *et al*., 1997). Whilst most of the structure refined very well, the B chain C-terminus was highly disordered, especially close to the crystallographic 4-fold axis. The appearance of the electron density maps suggested that the 4-fold symmetry might be breaking down close to the axis. Therefore, another data collection experiment was undertaken, this time treating the crystal as triclinic. It was hoped that this would remove any averaging effects which might be occurring as a result of treating the crystal as tetragonal. Refinement of the triclinic structure is now in process.

Results

At low pH, insulin crystallised as a monomer. This is the first time that native insulin has been prevented from aggregating either as a dimer or a hexamer in a crystalline state. Hence, this structure may give some more insight into the conformation of insulin when it binds to its cell surface receptor, and may also reveal information about insulin fibre formation. In the refined structure the great majority of the protein is the same as that in dimeric or hexameric insulin. There are differences in the B chain C-terminal residues B21-B30, which are disordered in all but one of the eight molecules of the asymmetric unit. In the more ordered molecule, the positions of residues B21-B24 are more clear, having been displaced slightly from their usual positions (as they would appear in dimeric insulin). Residue B25 Phe, which is know to be very important for insulin activity, is particularly will ordered, its aromatic ring packing against the main chain atoms of residue B19 Cys. Residues B26-B30 are disordered, consistent with the idea that the B chain C-terminus moves during receptor binding.

Hence, the reindexing of the crystal structure from I422 into P1 appears to have helped solve some ambiguities at the B chain C-terminus of this insulin structure.

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