PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 518th Meeting of the Society was held at the University of Sussex, Falmer, Brighton, on Thursday and Friday, 2 and 3 September 1971, when the following papers were presented:

JOINT COLLOQUIUM WITH THE PROTEIN GROUP OF THE CHEMICAL SOCIETY ON 'POLYPEPTIDE HORMONES: STRUCTURE AND FUNCTION'

The Conversion of Proinsulin into Insulin in the β -Cells of Mammals and Fishes

By P. T. GRANT. (*Natural Environment Research Council Institute of Marine Biochemistry, St Fittick's Road, Aberdeen AB1 3RA, U.K.*)

It has now been established in several species of mammals and fishes that the single-chain polypeptide proinsulin is the biosynthetic precursor of the two-chain molecule of insulin. This specific hydrolysis of a precursor polypeptide to yield a biologically active product of a lower molecular weight may be an intracellular event peculiar to the formation of insulin in the β -cells of islet tissue. The possibility remains, however, that this could be a general mechanism common to other cells that also specialize in the controlled formation and export of small polypeptide hormones such as glucagon (Rigopoulou, Valverde, Marco, Faloona & Unger, 1970), vasopressin (Pickup & Hope, 1971) and calcitonin.

Membrane-limited secretory granules of β -cells fuse with the cell membrane and discharge approximately equimolar amounts of insulin and a smaller peptide (C-peptide) that together account for the cleavage of proinsulin with the loss of four basic amino acid residues (Steiner *et al.* 1971) by a process that cannot involve a non-specific digestion by acid cathepsins. Indeed the only role that has so far been determined for lysosomes in β -cells and other secretory cells is concerned with the disposal of secretory granules produced in excess of demand (Creutzfeldt, Creutzfeldt, Frerichs, Perings & Sickinger, 1969).

Experiments involving pulse-labelling followed by either high-resolution radioautography of rat islets (Howell, Fink & Lacy, 1969) or the determination of the radioactivity in proinsulin and insulin isolated from subcellular fractions of rat (Sorensen, Steffes & Lindall, 1970) and fish β -cell preparations (Grant, Reid, Coombs, Youngson & Thomas, 1970; Grant, Coombs, Thomas & Sargent, 1971) indicate that there is a rapid synthesis and translocation of proinsulin from the endoplasmic reticulum into membrane-limited vesicles or granules with a concomitant conversion into insulin.

Both the microsomal and the vesicle-granule fraction from fish β -cells contain a firmly membrane-bound precursor of an enzyme with a specificity corresponding to that of trypsin (EC 3.4.4.4). This activity of this enzyme is inhibited by di-isopropyl phosphorofluoridate (Grant *et al.* 1971) and this compound also minimizes the conversion of proinsulin in intact fish β -cells (Grant & Reid, 1968). Although this enzyme cannot be specifically identified as a proinsulinase, it may be pointed out that the β -cell may, with advantage to its internal economy, synthesize both an active hormone and a potent endopeptidase in the form of their inactive precursors. This would enable both precursors to be packaged and contained in a membrane-limited vesicle before an activation mechanism results in the eventual formation of the active hormone.

The conversion of proinsulin into insulin in rat islets is not sensitive to di-isopropyl phosphorofluoridate (D. F. Steiner, personal communication), and the major product of a tryptic digest of either pig (Chance, Ellis & Bromer, 1968) or ox proinsulin (Steiner *et al.* 1971) is the corresponding des-alanine-insulin (insulin minus the C-terminal alanine residue of the B-chain, which is normally terminated by the sequence -Lys-Ala). In fish insulins the B-chain is terminated by a lysine residue. For these reasons proinsulinase(s) of mammals must differ in some respects from those of fish, but an enzyme with a specificity similar to that of trypsin must be involved in the conversion process since the C-peptide obtained by tryptic digestion of ox proinsulin can also be isolated in equimolar amount with insulin from the ox pancreas (Steiner *et al.* 1971).

Although it has not been tested experimentally, it is possible that zinc may have a role in minimizing the further digestion of insulin in the membrane-limited vesicle of the β -cell. Unlike proinsulin, the corresponding insulin is readily precipitated in the presence of excess of Zn^{2+} at neutral pH (Coombs, Grant & Frank, 1971). The β -cells of most animals are rich in zinc, and it is suggested that nascent insulin would complex with the metal ion and polymerize to yield a close-packed insoluble polymer

that is resistant to further digestion by proinsulinase or other digestive enzymes.

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The Structure and Biology of Insulin

By T. L. BLUNDELL, J. F. CUTFIELD, G. G. DODSON, E. DODSON, D. C. HODGKIN and D. MERCOLA.
(Laboratory of Molecular Biophysics, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, U.K.)

Recently the three-dimensional structure of insulin has been determined by X-ray analysis of rhombohedral 2Zn insulin crystals. The electron-density maps at 2.8 Å resolution and the structure of the zinc insulin hexamers based on these have been described previously (Blundell *et al.* 1971a). We present here some features of the structure that appear relevant to an understanding of the chemistry and biology of insulin (Blundell, Hodgkin, Dodson, Dodson & Vijayan, 1971b).

There is now a considerable body of evidence for the view that in solutions approximating physiological conditions insulin retains an ordered and stable structure resembling that of crystalline insulin.

The insulin hexamer has a circular 'doughnut' shape with a fairly compact non-polar core from which solvent is excluded and which is stabilized by disulphide bridges. Many of the surface residues are hydrophilic and most are surrounded by solvent, which comprises about 30% of the crystal lattice. These features are also characteristic of enzymes

where the results of X-ray analyses have been used extensively to rationalize the solution properties, and they suggest that the zinc insulin hexamer will also be similar in solution. Comparative tritium-exchange experiments in crystals and solution are in agreement with this conclusion.

Structure studies in solution give evidence not only of the availability of various functional groups (i.e. whether they are buried or exposed) but also of the geometric relations between parts of the molecule. The beautiful studies by Arquilla, Lindsay, Shall, Bradenbury, Zahn, Carpenter and their co-workers indicate a close correlation of the solution structure with the crystal structure. However, we consider that the general agreement does not preclude small changes of conformation, particularly on dissociation of the hexamers to dimers, the species often studied in solution. In particular, the N-terminal residues of the B-chain, B1-B3, and the side group of tyrosine-A14, which are fixed in the hexamers, may become flexible in the dimers. Nevertheless there is good evidence that the general tertiary structure seen in the crystals is maintained in all states of aggregation in solution.

The invariant residues of the 20 insulins whose amino acid sequences have been determined to date include the non-polar core and some surface residues that are involved in stabilizing interactions. This suggests that these insulins may have the same tertiary structure as the pig insulin studied by X-ray analysis. However, the changes of sequence at residues B10, B14, B17 and B20, which are restricted to coypu and guinea-pig insulins, must interfere with hexamerization. This is consistent with the recent observation by C. Yip (personal communication) that guinea-pig insulin does not hexamerize in the presence of Zn^{2+} , and the report by Falkmer (1968) that guinea-pig and coypu insulins alone among those whose amino acid sequences have been determined have no zinc in the β -granules, which are also unusually amorphous. This exceptional behaviour is no doubt related to the unusually high rate of mutation of these insulins. There is good evidence that other insulins are stored as zinc insulin hexamers, and it is interesting that many aspects of the β -granules of these species resemble the zinc insulin crystals studied by X-ray analysis.

The structure makes intelligible the resistance of the hexamer to digestion by proteolytic enzymes such as leucine aminopeptidase and trypsin, and this may be an important aspect of storage as zinc insulin hexamers. It would not be relevant to the guinea-pig and coypu insulins, but these have further unique sequence changes in the C-terminal B-chain residues, which may confer stability with respect to tryptic-like digestion.