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.

The close juxtaposition of residues A1 and B30 in the insulin structure shows that only a few residues of the rather long and species-variable connecting peptide of proinsulin are required to connect these groups. The position of residues A1 and B30 on the surface of the hexamer is consistent with the reported dimerization and hexamerization of proinsulin.

Most of the species-variable residues are on the surface of the insulin hexamer, and have been implicated by studies on modified insulins in the binding of antibodies. In general, residues implicated in the same antibody-binding site occupy adjacent positions on the surface of the insulin molecule.

Residues in a region of the hexamer surface, including glycine-A1, glutamine-A5, tyrosine-A19 and residue B25 (aromatic amino acid), are invariant. Chemical and spectral studies indicate that modification of some of these residues usually gives rise to changes of conformation and aggregation of the insulin molecules. Further, the biological activity is extremely sensitive to these modifications. In particular, the striking loss of biological activity associated with deamination of residue A1 or removal of residue A21 may imply that subtle changes of conformation on binding are an integral part of the insulin mechanism. Until reliable information about the state of aggregation of the active species is obtained it is impossible to say whether the invariant non-polar residues such as valine-B12 and phenylalanine-B24 that are involved in dimerization are also important to the activity. If so, the region involved in binding to the receptor may include all these groups. In this case the interactions would be largely hydrophobic, perhaps with hydrogen bonds involving the peptide groups of residues B24 and B26 (usually involved in the antiparallel sheet structure of the dimer). Whether the mechanism of insulin action at the receptor is simply a 'lock-and-key' fit or a more subtle induced fit involving changes of structure of the insulin molecule and receptor, the initial integrity of the general tertiary structure of much of the insulin molecule, and certainly this complex region, appears to be necessary for activity.

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Semisynthetic Insulin Analogues

By D. BRANDENBURG, H. G. GATTNER, L. HERBERTZ, G. KRAIL, M. WEINERT and H. ZAHN. (*Deutsches Wollforschungsinstitut an der Technischen Hochschule Aachen, D-51 Aachen, German Federal Republic*)

One approach to the preparation of insulin analogues is to start with insulin and to carry out a partial degradation (e.g. the preparation of des-B30-alanine-des-A21-asparagine-insulin; Nicol, 1960; Slobin & Carpenter, 1963) or to effect chain lengthening by peptide synthesis, yielding aminoacyl-insulins (Levy & Carpenter, 1966, 1967).

One other possibility is the transformation of the isolated insulin chains by partial degradation or partial synthesis into chain analogues that are subsequently combined to give insulin analogues. Bovine insulin A-chain-S-sulphonate was treated with phenyl isothiocyanate and the resulting phenylthiocarbamoyl derivative treated with trifluoroacetic acid to remove the N-terminal glycine residue. The des-glycine-A-chain-S-sulphonate contained less than 2% of glycine and was combined with bovine insulin B-chain-S-sulphonate by following the procedure of Du, Yang & Tsou (1965). The electrophoretically homogeneous des-A1-glycine-insulin, obtained on gel filtration [Sephadex G-50 (fine grade), with 0.05M ammonium bicarbonate pH 8.2] and ion-exchange chromatography (CM-Sephadex, with 0.5-5M-acetic acid gradient), gave the expected amino acid analysis. It did not crystallize, and the biological activity as determined in the isolated-fat-cell assay was 2-10% (Gliemann, 1965).

The immunoreactivity in the double-antibody radioimmunoassay (Morgan, Sorenson & Lazarow, 1964) was 20-30% as compared with that of bovine insulin at the point of 50% displacement of labelled insulin from the antibodies. Des-glycine-A-chain-S-sulphonate was also used for reaction with *p*-nitrophenyl [¹⁴C]acetate. The acetylated chain was isolated by gel filtration (at pH 8.2) and ion-exchange chromatography (Shimonishi & Zahn, 1968), and combined with B-chain-S-sulphonate to yield non-crystalline des-A1-amino-insulin, showing 15% of insulin's biological activity and 40% of its immunoreactivity. Crystalline A0-arginyl-insulin (Weinert, 1971; Weinert, Kircher, Brandenburg & Zahn, 1971) with 59% (mouse-convulsion assay) and 68% (fat-pad assay) biological activity was prepared starting with A-chain-S-sulphonate, which was acylated with the N-carboxyanhydride of arginine hydrobromide. [Shimonishi (1970) has also reported on the preparation of semisynthetic insulin A-chain-S-sulphonates.] The resulting