Meeting Report

CHEMISTRY, STRUCTURE AND FUNCTION OF INSULIN AND RELATED HORMONES

A report on the 2nd International Insulin Symposium, Aachen, Germany, 4–7 September 1979

Tom BLUNDELL

Laboratory of Molecular Biology, Department of Crystallography, Birkbeck College, University of London, Malet Street, London WC1E 7HX, England

Received 9 November 1979

Many biochemists would consider it unfashionable to concentrate all their efforts on the detailed study of one pharmaceutically important molecule. Rather they would argue for a more fundamental approach in their work in the hope that this might have a practical application in the treatment of disease. Nevertheless, the enormous industrial production of insulin for the treatment of diabetes has stimulated a wide range of chemical, biophysical and pharmacological studies, leading to important developments in fundamental biochemistry.

Progress in this area has resulted from the fact that interest in insulin has brought together an interdisciplinary group of research workers, including chemists, X-ray crystallographers, immunologists, pharmacologists and physicians. Much of this collaborative activity has been due to the foresight and personality of Helmut Zahn in Aachen, in whose laboratory many hundreds of insulin analogues have been tailored to the requirements of the international scientific community concerned with the study of insulin. It was, therefore, quite appropriate that Aachen should be the meeting place for discussion of new developments in this area and the location for an international symposium, organised by Dietrich Brandenburg, on the chemistry, structure and function of insulin and related hormones.

Several laboratories have developed the ability to synthesise insulin; new ideas in the synthesis of polypeptides have come as a result and were described at the symposium. The most difficult step is the correct pairing of the disulphide bridges. To this end Birr and coworkers have protected the cysteinyl mercapto functions of the A and B chains using three differing reagents which allow selective liberation of the SH-groups and formation of the correct disulphide bridges. An alternative method involves an enzyme catalysed semisynthesis from porcine insulin which differs from human insulin by the replacement of threonine by alanine at the B chain C-terminus. The methods developed by Carpenter and Canuva-Davis in Berkeley and Gattner et al. in Aachen depend on the reversibility of trypsin digestion at either B_{22}–B_{23} or B_{29}–B_{30} in the presence of excess peptides of the human sequence.

Certainly the most exciting new development has been the achievement of K. Itakura and his colleagues of the City of Hope National Medical Centre, California, in expressing the synthetic insulin gene in *Escherichia coli*. They have designed human insulin A and B genes from overlapping fragments of 29 oligodeoxy ribonucleotides which are joined using T_4 DNA ligase. The synthetic genes were fused on a plasmid within the *E. coli* β-galactosidase gene and preceded by a methionine codon. Transformation of *E. coli* with the plasmid allowed the synthesis of hybrid polypeptides including the A and B chains, which could be easily excised with cyanogen bromide treatment, purified and combined to give insulin with immunological and receptor activity.

Much has been learnt of the action of insulin from the study of sequence variants from a wide variety of vertebrates. The cyclostome, the atlantic hagfish, is the most primitive species whose insulin has been studied in detail. Although this insulin does not form zinc hexamers, the dimeric molecule closely resembles that of porcine insulin. Falkmer and Emdin have shown that hagfish insulin release is stimulated by 3 nM glucose but not by amino acids. Exogenous hagfish insulin stimulates incorporation of [^{14}C]-

glucose and [^{14}C]leucine into skeletal muscle protein and glycogen, but not in the liver. These observations indicate that only small changes in the regulatory mechanism of insulin have occurred during the 500-million year span of vertebrate evolution. Study of the relative affinity of hagfish insulin for the mammalian receptor based on competition of the unlabelled species for ^{125}I-labelled insulin has led to divergent reports. Emdin et al. previously reported a binding potency (23%) higher than the biological potency (5%) whereas Muggeo et al. reported a binding potency of 5–10%. De Meyts has now shown that this discrepancy most probably results from the unusual kinetic properties of hagfish insulin receptor binding, which may not have been at equilibrium in the studies showing lower binding potency. Studies on lymphocyte receptors indicate a 25% relative binding potency.

Similar unusual kinetics are found in another highly substituted insulin from the hystricomorph, casiragua. Horuk has shown that casiragua insulin is monomeric, has low biological potency, and is substituted at B_{25} and B_{26}, residues thought to be involved in receptor binding. Histricomorphs such as guinea pig and casiragua have insulins which have a relatively low receptor affinity even on their own tissues indicating that the receptor has been fairly conserved in evolution relative to the hormone itself. However, Lazarus et al. have found evidence from chemically modified insulins for some changes in the insulin receptor of hystricomorphs. Even the insulin of the old world hystrix, the porcupine, has an unusual structure which resembles that of guinea pig insulin in having an aspartate at B_{22} instead of arginine.

Abnormal insulins may also be found in certain diabetic humans. Olefsky and his collaborators from Colorado and Chicago have demonstrated the existence of an insulin component (60% of the total) in the pancreas obtained during abdominal surgery of a patient with severe endogenous hyperinsulinemia. Although this abnormal insulin appears to have a leucine substituted for a phenylalanine at B_{24} or B_{25}, many characteristics, including its apparent ability to antagonise the action of normal insulin, need to be elucidated; but this is the first unequivocal demonstration that an abnormal insulin may play a role in diabetes.

Variations in the sequences of insulins complicate insulin therapy by giving rise to immunological reactions, especially insulin allergy. However, the genetic constitution of the individual plays an important role in the immune response. In fact, Keck and Momayesi have shown that in mice the immune response is initiated by a set of immunogenic determinants on insulin and a corresponding set of immune response genes present in the major histocompatibility complex and in the region coding for the immunoglobulins. Rosenthal et al. have shown that T cell response in strain 2 guinea pigs varies with the sequence A8–A10 whereas in strain 13 guinea pigs the response is to residues around B_{10}. However, no specific determinants unique to the insulin used for immunization were detected in the antibodies or B cells in these animals. In a survey of 115 patients with insulin allergy proliferative responses were found to vary. Some patients responded to the B chain C-terminus of beef and pork insulins, others to their C-peptides and some to the bovine sequence A8–A10. Protamine, used in an insulin complex for the treatment of diabetics, also appears to potentiate insulin allergy.

In certain syndromes of severe insulin resistance circulating antibodies to the receptor are found. Kahn et al. have shown that these antibodies block insulin binding to their receptors, and have insulin-like effects on both muscle and adipose tissue but not the growth promoting effects such as thymidine incorporation into DNA in human fibroblasts. The monovalent F_{ab} fragments are not active although they are competitive antagonists. The biological activities of the anti-receptor antibody require bivalence indicating the need for receptor aggregation in insulin action.

Work on the characterisation and isolation of the insulin receptor has moved slowly. However, recent developments reported at the symposium in the use of analogues containing photosensitive groups such as 4-azido-phenylacetyl are stimulating interest in this area again. Three groups have shown that photoreactive insulin analogues can be labelled with ^{125}I and retain biological potency. The collaborative project of Brandenburg and Sonsken and their co-workers has shown that when activated by ultraviolet irradiation the photoprobes are bound covalently to insulin binding sites in rat liver plasma membrane fractions and isolated rat hepatocytes. SDS–polyacrylamide gel electrophoresis, in the presence of mercaptoethanol, of either liver plasma membrane fractions or isolated hepatocytes showed that a polypeptide of app. mol. wt 130 000 was specifically labelled. Similar results were obtained by Yip and by Rees. The photolabelling is abolished by trypsin

treatment of the membranes whereas neuraminidase increased the electrophoretic mobility of the receptor proteins.

The isolation and characterisation of two insulin-like growth factors (IGF I and II) by Froesch, Humbel and coworkers in Zurich has given a great stimulation to the study of receptors specifically concerned with aspects of insulin-like activity such as glucose transport and stimulation of proliferation of fibroblasts. IGF I and IGF II have been isolated from a Cohn fraction of human plasma in homogenous form. The amino acid sequences are homologous to that of proinsulin, and Bedarkar and colleagues in collaboration with Humbel have shown that they may have an insulin-like fold, with a distribution of the surface residues consistent with the inability of IGF to bind anti-insulin antibodies. The models also predict that both IGF I and IGF II might bind the insulin receptor, IGF II with the higher affinity. Indeed both IGF I and II share biological properties with insulin: on fat cells and lymphocytes, IGF competes with insulin for the insulin receptor; on fibroblasts and chondrocytes, insulin competes with IGF for the IGF receptor. De Meyts et al. have shown that on the lymphocyte insulin receptor IGF II has 10% of the potency of insulin in competing for ^{125}I-labelled insulin binding and IGF I only 2%, and both factors induce negative cooperativity.

Humbel et al. have shown that in serum IGF is bound to a mol. wt 40 000 glycoprotein which like IGF I is under growth hormone control. Preliminary evidence indicates that IGF may be produced in the liver. IGF I levels are raised in acromegalics, and very low in Laron dwarfs, hypopituitarism and liver cirrhosis. These observations and radioimmunoassays comparing IGF I and II with somatomedins indicate that somatomedin C may be identical to IGF I whereas somatomedin A may be a mixture of the two insulin-like growth factors.

Relaxin, an ovarian polypeptide hormone, has also proved to be structurally related to insulin; with substantial sequence homology and very probably, a similar three-dimensional structure. The two chain structure of relaxin has led to the suggestion that it may have a precursor prorelaxin. Preliminary results obtained by Niall et al. translating luteal mRNA from pregnant sow ovaries in an ascites tumour cell system indicate the presence of a mol. wt 23 polypeptide which crossreacts immunologically with relaxin. Although this molecule is probably a preprorelaxin it is certainly rather larger than would be expected from a direct comparison with proinsulin. The heterogeneity of relaxin-like polypeptides indicates that the converting enzymes are certainly different and possibly less specific than those involved in processing proinsulin.

Schwabe has extended his studies on porcine relaxin to that of shark; this relaxin is larger than porcine relaxin, it has some amino acids, tyrosine, histidine and proline, not found in porcine relaxin, and it appears to have only two disulphide bridges. Shark relaxin resembles some peptides found in the purification of porcine relaxin and indicates that the evolution of relaxins may have involved greater variability than that of insulin.

The symposium also provided the opportunity to compare and contrast other pancreatic hormones with insulin. Epand reported the most recent experiments from his laboratory in Ontario which demonstrate that all regions of the glucagon molecule are important for receptor binding. Studies of the ability of chemically modified glucagons to activate adenylate cyclase and to displace ^{125}I-labeled glucagon from specific membrane binding sites indicate that loss of ability to activate adenylate cyclase was at least as great as loss in receptor binding. However, for analogues modified towards the N-terminus, for example N^{α}-trinitrophenyl glucagon, the ability to activate cyclase is very much more impaired than the binding potency. Epand suggests that glucagon may bind the receptor through a helical conformer, similar to that suggested previously by Sasaki et al. on the basis of X-ray data. However, Epand considers that the interaction may be relatively unspecific and involve hydrophobic interactions with lipids. Clearly glucagon receptor binding is more complicated than that for insulin as the glucagon molecule is more flexible and the active conformer is almost certainly induced at the receptor.

The least studied of the pancreatic hormones is the 36 amino acid PP (pancreatic polypeptide) which is released into circulation after feeding and may act as a satiety factor. Recent crystallographic studies by Pitts et al. at Birkbeck College, London, of the avian polypeptide has shown that this molecule appears to have a stable conformation involving a polyproline II-like helix involving residues 1–9 including prolines at 2, 5 and 8 close-packed against an α-helical region of residues 14–32. Like insulin, the molecule forms dimers and further polymerises

in the presence of zinc ions.

In many ways the pancreatic polypeptides are the most carefully described of all polypeptide hormones, although we are still some way from producing a proper oral insulin or a useful glucagon antagonist both of which would transform the treatment of diabetes. But clearly this is still an active and productive research area so that there may be hope for the future.