(Sieler, 1970) in the eluted glucagon (60 μ g), indicating that the hormone was essentially pure. After affinity chromatography of ileum extracts, more than 90% of the glucagon-like immunoreactivity had a molecular weight of approx. 12000 on gel filtration at pH 8.8. Thus the antibody-Sephadex complex, though not discriminating between pancreatic glucagon and glucagon-like immunoreactivity of gut, can be used in association with gel filtration for the purification of the gut glucagon-like immunoreactivity in amounts that will facilitate determination of structure and physiological properties.

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- Akanuma, Y., Kuzuya, T., Hayashi, M., Ide, T. & Kuzuya, N. (1970). *Biochem. biophys. Res. Commun.* **38**, 947.
- Buchanan, K. D. & McCarroll, A. M. (1971). In *Radioimmunoassay Methods*, p. 266. Ed. by Kirkham, K. E. & Hunter, W. M. Edinburgh: E. and S. Livingstone.
- Cuatrecasas, P. (1970). *J. biol. chem.* **245**, 3059.
- Kenny, A. J. & Say, R. R. (1962). *J. Endocr.* **25**, 1.
- Moody, A. J., Markussen, J., Schaich-Fries, A., Steenstrup C., Sundby, F., Malaisse, W. & Malaisse-Lagae, F. (1970). *Diabetologia*, **6**, 135.
- Sieler, N. (1970). In *Methods of Biochemical Analysis*, vol. 18, p. 259. Ed. by Glick, D. New York: Interscience Publishers Inc.
- Valverde, I., Rigopoulou, D., Marco, J., Faloona, G. R. & Unger, R. H. (1970). *Diabetes*, **19**, 614.
- Weintraub, B. D. (1970). *Biochem. biophys. Res. Commun.* **39**, 83.

Conformational Studies of some Hormonal Peptides in Solution by using Nuclear-Magnetic-Resonance Spectroscopy

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N.m.r. can provide detailed information about the conformation of small peptides in solution. The basis for such studies is that the coupling constant between the α -proton and its adjacent NH proton (J_{NC}) is related to the ϕ dihedral angle. Side-chain conformational information can be similarly obtained by studying the vicinal coupling constants between the α -CH and β -CH₂ protons. It is also possible to detect the presence of intramolecular hydrogen bonds in peptides by considering the temperature-dependence of the NH chemical shifts. We have used these methods to study oxytocin, [8-lysine]-vasopressin and component peptides of gastrin in aqueous solution.

Oxytocin and vasopressin are found to have similar conformations and it is possible to indicate the range of ϕ , ψ values consistent with the n.m.r. data and the requirement of ring closure. There is no evidence for intramolecular hydrogen-bonding, nor for assuming that the Pro-Leu(Lys)-Gly-NH₂ fragment is folded over the rings.

For the component peptides of gastrin, Asp-Phe-NH₂, Met-Asp-Phe-NH₂, Trp-Met-Asp-Phe-NH₂ and β -Ala-Trp-Met-Asp-Phe-NH₂, the aspartic acid and phenylalanine side-chain conformations were determined: significant but small differences in rotamer population between the inactive tripeptide and active pentapeptide were found. From the observed J_{NC} values and from consideration of peptide potential-energy maps, the most populated backbone conformations have ϕ angles centred around -95° .

Differences in the Binding of Zinc Ions by Insulin and Proinsulin

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In agreement with previous observations on ox, pig and cod proinsulins (Grant & Coombs, 1970), it has been shown by equilibrium dialysis (ultra-filtration) and by sedimentation-equilibrium and velocity studies that pig proinsulin bound more than 5 mol of Zn²⁺/mol and aggregated to form a mixture of soluble polymers (mainly $s_{20,w}$ 5.1S) in the presence of excess of Zn²⁺. In contrast, pig insulin bound only 1.0 mol of Zn²⁺/mol and was almost completely precipitated from solution. These differences were only manifested when more than 0.3 mol of Zn²⁺ was bound/mol of protein.

Binding data from equilibrium mixtures containing insulin or proinsulin (0.1 mM), free ⁶⁵Zn²⁺ (0.001–1.0 mM), tris-HCl buffer (50 mM) and sodium chloride (0.1M) at pH 7 at 20°C indicate that each protein contained two main orders of Zn²⁺ binding.

In equilibria containing 0.001–0.01 mM free Zn²⁺ both insulin and proinsulin aggregated to form soluble hexameric polymers ($s_{20,w}$ 3.2S and 3.4S respectively; see also Frank & Veros, 1970) and bound essentially the same amount of Zn²⁺. The bound Zn²⁺ was only slowly removed by ultra-filtration with Zn²⁺-free buffer. The capacity of this site was calculated from a Scatchard plot (Hughes & Klotz, 1963) to be about 0.33 mol of Zn²⁺/mol and the apparent intrinsic association constant was $1.9 \times 10^6 \text{ M}^{-1}$. These results imply

that this site of higher affinity is similar if not identical in both proteins. Presumably, two zinc atoms are co-ordinated with six histidine residues, as shown for the insulin hexamer (Adams *et al.* 1969).

In contrast, as the concentration of free Zn^{2+} was increased above 0.01mM, the binding, aggregation and solubility properties of insulin and proinsulin complexes became progressively different. The Zn^{2+} bound to these sites of lower affinity in both proteins was, however, readily removed by ultra-filtration with Zn^{2+} -free buffer and precipitates of zinc-insulin redissolved. The fivefold difference in Zn^{2+} binding between insulin and proinsulin in equilibrium with 1.0mM- Zn^{2+} must be related to the four γ -carboxyl groups of glutamic acid residues present in the connecting peptide of pro-insulin. These sites of lower affinity for Zn^{2+} that are additional to those present in insulin would facilitate weak inter- and intra-molecular cross-linking, but it may be presumed that the general configuration of the zinc-proinsulin complex must preclude the formation of very large and close-packed insoluble aggregates.

Adams, M. J., Blundell, T. L., Dodson, E. J., Dodson, G. G., Vijayan, M., Baker, E. N., Hardine, M. M., Hodgkin, D. C., Rimmer, B. & Sheat, S. (1969). *Nature, Lond.* **224**, 491.

Frank, B. H. & Veros, A. J. (1970). *Biochem. biophys. Res. Commun.* **38**, 284.

Grant, P. T. & Coombs, T. L. (1970). In *Essays in Biochemistry*, vol. 6, p. 69. Ed. by Campbell, P. N. & Dickens, F. London: Academic Press (Inc.) Ltd.

Hughes, T. R. & Klotz, I. M. (1963). In *Methods of Biochemical Analysis*, vol. 3, p. 265. Ed. by Glick, D. New York: Interscience Publishers Inc.

The Behaviour of Insulin at Non-Polar Surfaces

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Cecil & Louis (1970) studied the behaviour of a number of proteins, in particular insulin, lysozyme and ribonuclease, at hydrocarbon/water interfaces. They established that there is an equilibrium between protein in solution in the aqueous phase and protein adsorbed on the interface. These proteins did not appear to undergo any irreversible conformational change during adsorption; lysozyme and ribonuclease lost their activity when adsorbed, but recovered it completely when returned to the aqueous phase.

This system has been used to study the behaviour of insulin at non-polar surfaces in more detail, in

an attempt to throw some light on its behaviour in cell membranes. The immediate questions to be answered are (i) is the insulin molecule adsorbed in a unique way at the interface, (ii) if so, which part of the molecule is in contact with the surface, and (iii) is the insulin adsorbed as a monomer, dimer or hexamer?

The technique used was to add to the hydrocarbon phase a reagent that would react with that part of the insulin molecule that is in contact with the interface. Dilute aqueous solutions of insulin (approx. 10mM) at pH7 were shaken gently for several hours with small volumes of a solution of DNS chloride in decane. The DNS chloride is sufficiently non-polar to remain in the hydrocarbon phase, so that the sulphonyl chloride group can only react with insulin adsorbed on the interface.

Under these conditions only the B1 phenylalanine residue reacts with the DNS chloride. Purification of the adsorbed insulin yields three products: unchanged insulin, insulin with one phenylalanine residue per dimer labelled and insulin with both phenylalanine residues per dimer labelled. The mono-labelled insulin has approx. 50% of the normal activity (mouse-convulsion method) and the bis-labelled insulin is inactive. When zinc was removed from the insulin before reaction the yield of DNS-insulin was increased threefold.

If insulin was adsorbed as a monomer it would be expected that the hydrophobic region that forms the area of contact between the monomers would be in contact with the interface and that the B26 tyrosine residue would react with the DNS chloride. Since only the B1 phenylalanine residue reacts it would appear that insulin is adsorbed either as a dimer or a hexamer. Removal of zinc from the insulin will increase the proportion of dimer present, and, since this increased the yield of B1-phenylalanine-labelled DNS-insulin, it may be assumed that the dimer is the reactive species.

Results obtained by other workers (e.g. Lindsay & Shall, 1971) with smaller groups on the B1 phenylalanine residue show only a small loss of activity by mouse convulsion but a large decrease by immunoassay. It is probable therefore that the B1-phenylalanine end is the antigenic part of the molecule but not the 'active site'. Since it is apparently the part of the dimer with the greatest affinity for a non-polar surface it may be inferred that the receptor area of the cell membrane is not hydrophobic.

Cecil, R. & Louis, C. F. (1970). *Biochem. J.* **117**, 139.

Lindsay, D. G. & Shall, S. (1971). *Biochem. J.* **121**, 737.