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.

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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.

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