# INTERACTION OF ZINC WITH PROINSULIN

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Proinsulin exhibits self-association behavior in solution which is quite similar to that of insulin over a wide range of pH (Frank and Veros, 1968, Zühle and Behlke, 1969). Insulin solutions in the absence of zinc are a mixture of even-aggregate states (Jeffrey and Coates, 1966); in the presence of zinc a single species is formed which consists of a complex of two zinc ions and six insulin molecules (Cunningham et al., 1955; Frank and Veros, 1967). The structure of this complex probably is analogous to that established for the hexamer unit of crystalline zinc-insulin by Hodgkin and co-workers (Dodson, 1969). It is therefore of considerable interest to determine whether proinsulin interacts with zinc ions and forms the same type of hexameric complex in solution. In addition, if such a complex exists, it may have some implications for the mechanism of conversion of proinsulin to insulin in the β-cell.

We have found that proinsulin does interact with zinc to form a zinc-proinsulin complex with a molecular weight of 55,000, which corresponds to a hexamer of proinsulin. This complex appears to require a minimum of two zinc ions per proinsulin hexamer.

## EXPERIMENTAL

The porcine proinsulin used in these studies was kindly provided by Dr. R. E. Chance of the Lilly Research Laboratories.

The ultracentrifugal studies were performed on a Beckman Model E ultracentrifuge equipped with a photoelectric scanning system. The ultraviolet spectra were recorded on a Cary 14R spectrophotometer. The zinc contents of the protein solutions were determined by atomic absorption spectroscopy.

## RESULTS

The effect of zinc on the sedimentation-velocity behavior of proinsulin in 0.03M phosphate, pH 7.3 buffer is shown in Figure 1. The sharpening of the peak in the schlieren patterns in the presence of zinc indicates an interaction of zinc with proinsulin and a decreased degree of heterogeneity in the proinsulin solutions. Figure 2 depicts the $s_{20,w}$ as a function of protein concentration both in the presence and absence of zinc. The marked difference in the concentration dependency of the $s_{20,w}$ in these two systems again indicates interaction of zinc with proinsulin.

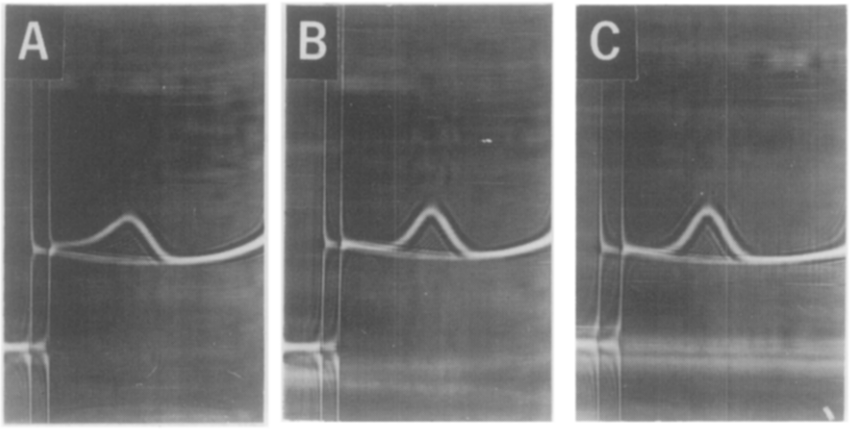


Figure 1. Sedimentation of proinsulin at pH 7.3 in 0.03M phosphate, 0.10M NaCl buffer at a proinsulin concentration of 3.5 mg/ml. (A) no zinc present, (B) one mole zinc per three moles proinsulin, (C) one mole zinc per mole of proinsulin.

Sedimentation equilibrium studies have been performed in order to clarify the effect of zinc on proinsulin self-association. In a 0.003M phosphate, 0.10M NaCl, pH 7.3 buffer with a minimum of one mole of zinc per three moles of proinsulin, a homogeneous entity of molecular weight 55,000 ± 5000 is observed. A range of 0.08 to 1.5 mg protein per ml has been examined; in the absence of zinc, the proinsulin solutions are quite heterogeneous with molecular weights ranging from 22,000 to 58,000. The data clearly indicate that proinsulin forms hexamers in the presence of zinc.

Ultraviolet difference spectroscopy also has been employed to characterize the interaction of zinc with proinsulin. Concentration difference spectra of insulin have been shown to be dependent in large measure on the monomer-dimer transition (Rupley et al., 1967). We have observed that when zinc is added to insulin solutions, a difference spectrum is obtained which

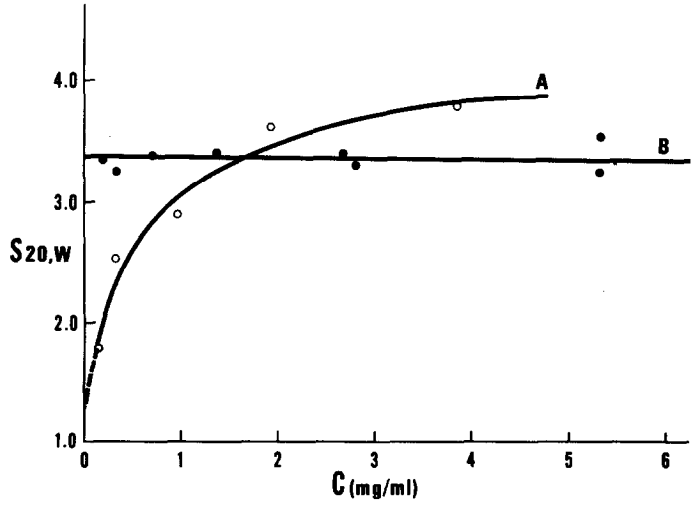


Figure 2. Sedimentation coefficient as a function of proinsulin concentration in 0.03M phosphate, 0.10M NaCl, pH 7.3: (A) no zinc, (B) 1 mole zinc per 3 moles proinsulin.

is virtually identical to insulin concentration difference spectra, suggesting that the zinc-induced spectral changes ai also associated with monomer-dimer equilibria. Addition of zinc to proinsulin solutions yields an ultraviolet difference spectrum exactly like that obtained upon zinc addition to insulin solutions (See Figure 3). Thus, the zinc-induced spectral change in proinsulin solutions is most likely associated with proinsulin monomer-dimer equilibria. More important is the fact that the $\Delta O.D.^{286}$ observed in the zinc-insulin difference spectra reaches a maximum when the zinc to insulin mole ratio is about 1 zinc per 3 insulins. This value corresponds very well to the minimum molar ratio necessary to form the zinc-insulin 35,000 molecular weight complex found in zinc-insulin solutions. In the zinc-proinsulin system, the $\Delta O.D.^{286}$ is also maximizing at zinc to proinsulin mole ratios of about 1 to 3. Furthermore

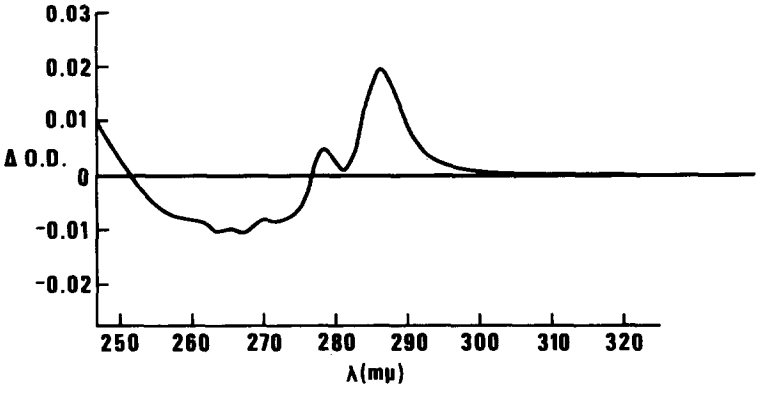


Figure 3. Ultraviolet difference spectra of zinc-proinsulin versus proinsulin in 0.03M phosphate, pH 7.3. Path length 10 cm, 25°C. Sample cell contains 1 mole zinc per 3 moles proinsulin. Reference cell contains no zinc. Proinsulin concentration of 0.22 mg/ml.

no appreciable change in the boundary in sedimentation velocity studies can be observed at mole ratios above 1 to 3; sedimentation equilibrium studies on zinc-proinsulin solutions with metal to

protein molar ratios of 1 to 3 and 1 to 1 in both cases yield a molecular weight of 55,000 for the zinc-proinsulin complex. Thus the zinc-proinsulin complex contains in all probability a minimum of 2 zinc ions.

## DISCUSSION

Previous studies (Frank and Veros, 1968) on the physical properties of proinsulin have led to the proposal that the insulin moiety of the proinsulin molecule exists in essentially the same conformation as the free insulin molecule. Thus the 33 amino acid connecting peptide segment is thought to exert no discernible effect on the conformation or physical properties of the insulin moiety. The demonstration of the same type of interaction of zinc with both proinsulin and insulin is in agreement with this interpretation of previous studies. Furthermore, the existence of such a zinc-proinsulin complex suggests that the crystallization of proinsulin may be facilitated by zinc.

The present studies provide a basis for consideration of the possible physiologic role of the zinc-proinsulin complex. One mechanism which has been proposed for the conversion of proinsulin to insulin (Chance et al.,1968 and Steiner et al., 1968) involves the combined action of enzymes with specificities like trypsin and carboxypeptidase B, resulting in the cleavage of the proper peptide linkages in the proinsulin molecule. There are, however, additional peptide bonds in the insulin moiety of proinsulin which can also be cleaved by these enzymes under normal conditions. If the zinc-proinsulin complex is formed under physiologic conditions, the conformation of the complex may be such that the peptide bonds in the insulin moiety are inaccessible to attack by these enzymes. The effect of zinc on the trans-

formation of proinsulin to insulin is presently under investigation in this laboratory.

REFERENCES

Chance, R. E., Ellis, R. M., and Bromer, W. W., Science 161, 165 (1968).
Cunningham, L. W., Fischer, R. L. and Vestling, C. S. JACS 74, 5703 (1955).
Dodson, G., New Scientist 43, 370 (1969).
Frank, B. H. and Veros, J. A., 154th Meeting Amer. Chem. Soc., Chicago, Abst. C248, (1967).
Frank, B. H. and Veros, A. J., Biochem. Biophys. Res. Commun. 32, 155 (1968).
Jeffrey, P. D. and Coates, J. H., Biochemistry 5, 489 (1966).
Steiner, D. F., Hallund, O., Rubenstein, A., Cho, S. and Bayliss, C., Diabetes 17, 725 (1968).
Zühle, H. and Behlke, J., FEBS Letters 2, 130 (1968).