PHYSICAL STUDIES ON PROINSULIN - ASSOCIATION BEHAVIOR AND CONFORMATION IN SOLUTION

B. H. Frank and A. J. Veros

The Lilly Research Laboratories
Indianapolis, Indiana

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The demonstration of a single chain biological precursor of insulin by Steiner et al. (1967a,b) and the isolation and subsequent elucidation of the amino acid sequence of porcine proinsulin by Chance et al. (1968) have led to this investigation of the physical properties of this molecule. The optical rotatory dispersion (ORD) and circular dichroic (CD) properties of proinsulin and insulin have been compared in order to gain some knowledge of the relative conformations of these proteins in solution. In addition the monomer molecular weight and association behavior of proinsulin in acid solution have been determined. The results have been interpreted in terms of a model in which the insulin moiety of the proinsulin molecule is postulated to exist in the same conformation as insulin itself.

EXPERIMENTAL

Porcine proinsulin was prepared and supplied by Dr. R. E. Chance of this laboratory and was shown to be better than 98% single component material as judged by polyacrylamide gel electrophoresis. All other reagents were of analytical grade. All protein solutions used in these studies were dialyzed to equilibrium before use in either the centrifugal or spectral studies. The molecular weight studies were performed on a Model E

analytical ultracentrifuge equipped with interference and ultraviolet absorption scanning optical systems. Standard sedimentation equilibrium techniques were used (Van Holde and Baldwin, 1958). The partial specific volume of proinsulin was calculated (McMeekin and Marshall, 1952) using the amino acid composition. A Cary-60 spectropolarimeter equipped with a CD unit was used to record the ORD and CD spectra.

RESULTS

Proinsulin was found to exhibit strong self-association properties over a wide range of pH. The results of the sedimentation-equilibrium studies at pH 2 are shown in Figure 1. The lowest experimental value for the molecular weight was 9150 at a concentration of 50 μg/ml, while the highest molecular weight observed was 18,600, a value slightly higher than twice the monomer molecular weight. The extrapolated monomer molecular weight of 9000 is in excellent agreement with the value of 9082 obtained from the amino acid composition.

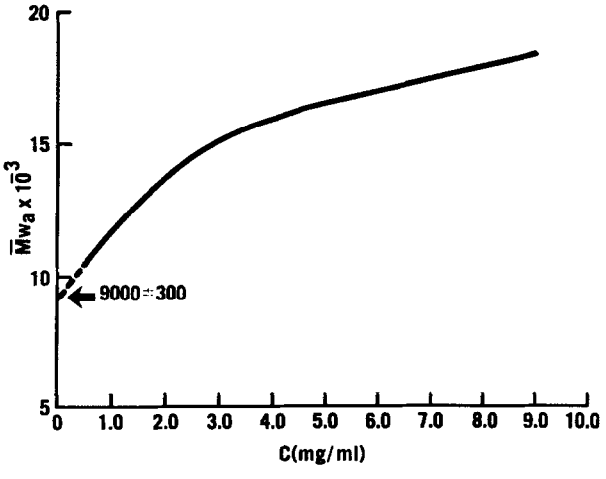

Figure 1. Computer fit of data of apparent weight average molecular weight of proinsulin as a function of concentration at 26°C. Solvent 0.05M glycine - 0.05M NaCl, pH 2.0

In order to compare the type as well as the strength of the self-association of insulin and proinsulin, the results have been analyzed by a method originally devised by Steiner (1952) and later applied to insulin association by Jeffrey and Coates (1966). They described insulin behavior in terms of a monomer-dimer-tetramer type of association. The proinsulin self-association can also be described in this manner. Moreover the values of the free energies of association are nearly the same for both proteins and are about -5 kcal/mole for dimer formation and -4 kcal/mole for tetramer formation. Though the insulin association appears to be slightly stronger (~-0.5 kcal/mole) at pH 2, some preliminary equilibrium studies at pH 7.0 indicate that the proinsulin association may be slightly stronger than that of insulin under neutral pH conditions. Thus on the basis of these results it would appear that the primary sites of self-association are the same for proinsulin and insulin.

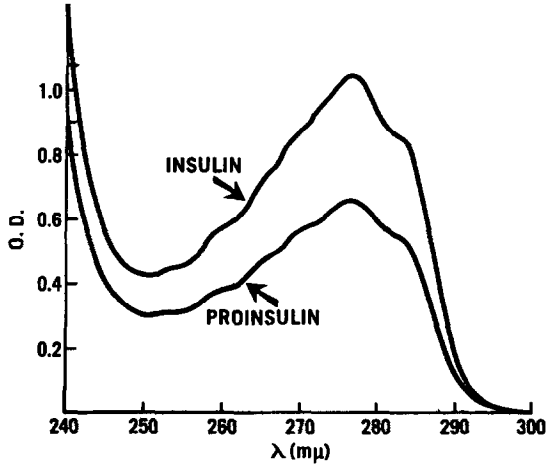

Figure 2. Ultraviolet spectra of insulin and proinsulin in 0.03M phosphate buffer, pH 7.02, 25°C, 1 cm path, 1 mg/ml concentration, $O.D._{276}$ insulin=1.052; proinsulin=0.667

The ultraviolet absorption spectra of proinsulin and insulin are shown in Figure 2. Apparent in the 250-275 mμ region of the spectra of both proteins is the same fine structure, which is due primarily to the phenylalanine residues. Using the fact that the phenylalanine and tyrosine content is the same for both proteins, and assuming that these residues are in similar environments in both molecules, a molecular weight of 9100 for proinsulin was calculated from the extinction coefficients.

However, UV spectra are not very sensitive criteria for subtle environmental differences. Therefore, the ORD and CD spectra of the two proteins were determined, since these parameters are directly related to conformational factors. The results are shown in Figures 3 and 4. Conformational differences between the two molecules are clearly indicated. Values of 14 and 25% α-helix for proinsulin and insulin respectively, were calculated using the 223 mμ ellipticity values (Holzworth and Doty, 1965).

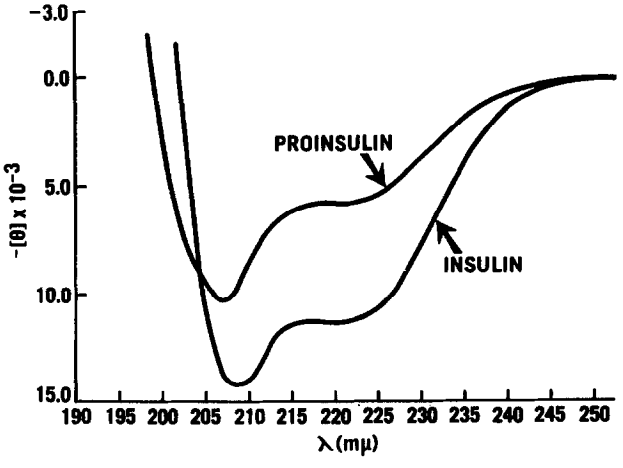

Figure 3. Circular dichroic spectra of insulin and proinsulin in 0.03M phosphate buffer, pH 7.02, 25°C at protein concentrations of 0.1 to 0.3 mg/ml.

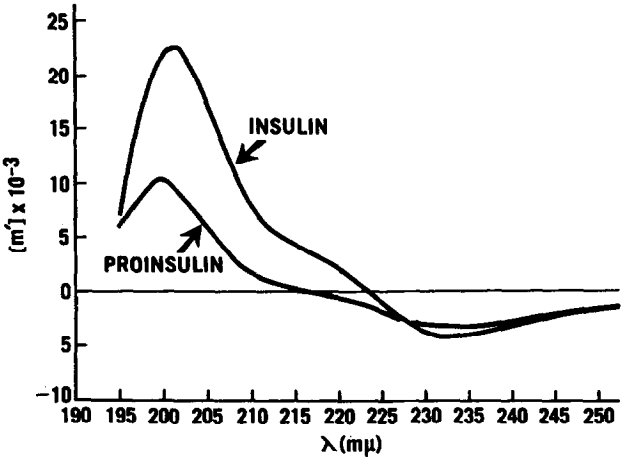

Figure 4. Optical rotatory dispersion spectra of insulin and proinsulin in 0.03M phosphate buffer, pH 7.02, 25°C at protein concentrations of 0.1 to 0.3 mg/ml.

Approximately the same values for per cent α-helix were obtained using the depth of the 233 mμ ORD trough (Simmons et al., 1961).

DISCUSSION

The chemical and physical evidence gained thus far does not lead to a clear picture of the relative conformations of insulin and proinsulin in solution. From the centrifugal data it is apparent that proinsulin exhibits strong self-association behavior very similar to that of insulin. The most reasonable interpretation of this behavior is that the portion of the molecule responsible for the association behavior is in nearly the same conformation in both the insulin and proinsulin molecules. Based on this interpretation a model is proposed in which the insulin moiety of proinsulin exists in the same conformation as insulin itself. Although the ORD-CD data indicate some differences in α-helical content between the two molecules, one can account for the difference by assuming that the connecting polypeptide chain

exists in a random coil conformation. This is a distinct possibility, since of the 33 amino acid residues in the connecting peptide of porcine proinsulin, 3 are proline residues which will not allow α-helix formation, and 7 are glycine residues which cannot contribute to stabilization of an α-helix by side chain interaction. In addition, of the 30 residues present in the connecting peptide chain of bovine proinsulin, 4 are proline and 8 are glycine residues (Chance, 1968); thus, a random coil conformation may be characteristic of the connecting polypeptide chain in proinsulin from other species.

Further work will be necessary to test this model. A study of the effect of the connecting polypeptide chain of proinsulin on the conformation of insulin is now in progress. With this information it may be possible to determine more definitively the conformation of the insulin moiety of proinsulin relative to that of insulin itself, and thus gain an understanding of the overall conformation of the proinsulin molecule in solution.

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