AMINO ACID COMPOSITION OF BOVINE 'PROINSULIN'

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The structure of insulin being composed of two polypeptide chains and the differentiation of the endocrine and the exocrine pancreas from the same embryonic cell type have made the existence of a 'proinsulin' in the endocrine pancreas an attractive hypothesis to a number of investigators (Givol et al, 1965). However, the works by Humbel (1965) on fish islets and by Wang and Carpenter (1965) on bovine pancreatic extract have failed to establish the existence of such a protein. In this laboratory this hypothesis has been explored during the past three years, using ligated rabbit pancreas and fetal calf pancreas as the source materials. Results so obtained during this period show the existence of such a protein and will be reported in details in a separate communication. Recently, Steiner et al (1967a, 1967b) have reported on the formation of 'proinsulin' from the incubation in vitro of human islet adenoma tissues and isolated rat islets. In this communication, the existence of a 'proinsulin' in crystallized bovine insulin and the amino acid composition of this purified protein are presented.

METHODS AND MATERIALS

Crystallized bovine insulin was a gift from Dr. A.M. Fisher

of Connaught Medical Research Laboratories. Preparative column chromatography was performed on a Sephadex (G-50, fine) column (2.5 x 90 cm) equilibrated to 1M acetic acid. A smaller column (1.0 x 50 cm) was used for analytical purpose. Elution was done with 1M acetic acid. The eluate was monitored at 275 mμ with a Cary-15 recording spectrophotometer. Insulin activity was assayed by the method of Yarlow and Berson (1964). Amino acid analysis was performed in a Model 120 amino acid analyzer (Spackman et al. (1958).

RESULTS

Analytical column chromatography of 2 mgm of a 4 times recrystallized bovine insulin is shown in Figure 1A. It may be seen that a significant amount of protein material emerged ahead of monomeric insulin in 1M acetic acid. Re-chromatography of this heavier material is shown in Figure 1B showing clearly the absence of u.v. absorbing material in the insulin position and the homogeneity of the pooled material. Limited trypsinization (6μg/ml for 1 hr at 37°) of this protein resulted in the formation of two smaller species of protein, one of which was similar to insulin in its elution volume (Figure 1c). Immuno-electrophoresis assays of this product and of the original material, both at 1/200,000 dilution, showed that they were strongly immunoreactive (Figure 2).

Results of amino acids analysis of the purified heavier protein after hydrolysis are shown in Table I in comparison to bovine insulin.

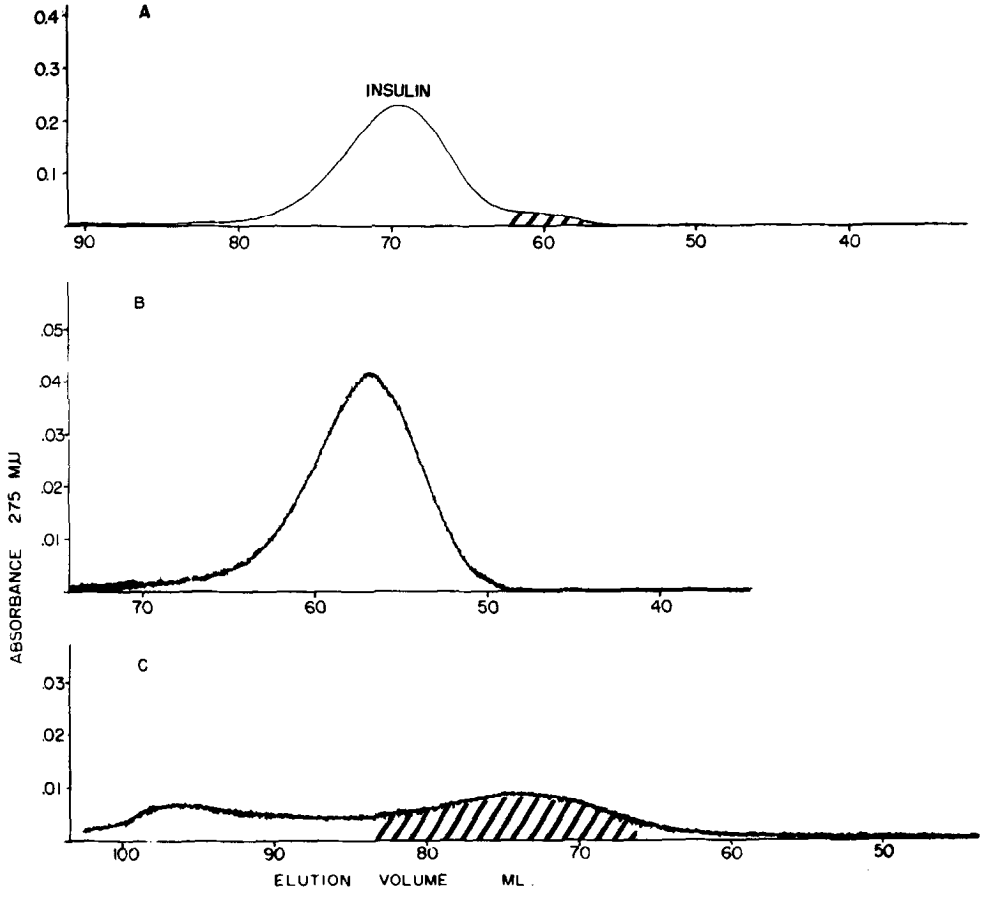

Figure 1 - Column chromatography on Sephadex G-50 (1.0 x 50 cm) of crystalline bovine insulin. The area indicated by the stripes is 'proinsulin'. B. Re-chromatography of 'proinsulin'from A on the same Sephadex column. C. Chromatography of 'proinsulin' after limited trypsin treatment on the same Sephadex column. The fractions marked by the stripes were combined and assayed for insulin immunoactivity.

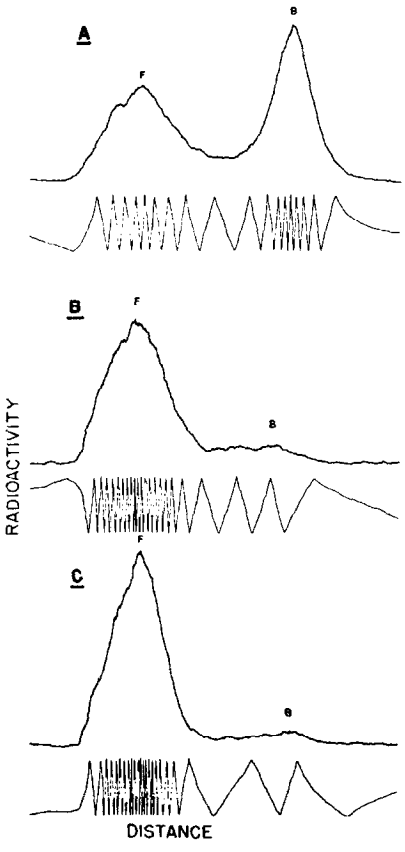

Figure 2 -

Immunoelectrophoresis assay of insulin activity. The immuno-assay was performed by the method of Yalow and Berson (1964). The tracings represent the scanning of the electrophoresis strips for I^{131} radioactivity. A. Contained only I^{131}-insulin (Porcin) and guinea pig anti-insulin (Porcin) antibody; B. Addition of 1/200,000 diluted 'proinsulin' material shown in Figure 1B; C. Addition of 1/200,000 diluted trypsinized 'proinsulin' material shown in 1C. (F-free insulin, B-antibody-bound insulin).

DISCUSSION AND CONCLUSION

It may be concluded from the data that a protein, larger than monomeric insulin, is present in crystallized bovine insulin. This protein is most likely not an aggregate of insulin in view of the acidity of the elution medium and of its amino acid composition which is neither identical to nor a multiple of monomeric insulin. It behaves immunologically like insulin and can be converted to a smaller molecule, the size of insulin, which is also immunologically reactive. Amino acid analysis shows that it does not contain any amino acid residues absent in bovine insulin, and it is apparent that the amino acid composition

Table I. Amino acid composition of bovine 'proinsulin'*

Amino Acid	moles/100,000 g protein	
	'Proinsulin'	Bovine Insulin**
Lys	14.5 ± 2.1	14.5
Hist	26.6 ± 1.2	34.5
Arg	30.8 ± 1.8	16.3
Asp	50.4 ± 1.1	53.0
Thr	17.0 ± 0.4	17.3
Ser	47.1 ± 1.2	53.0
Glu	151.8 ± 1.2	115.8
Pro	42.8 ± 0	16.7
Gly	117.1 ± 0.3	70.5
Ala	68.0 ± 2.0	44.8
Half-Cyst	83.9 ± 2.6	52.0
Val	86.9 ± 1.6	71.0
***Ileu	11.6 ± 1.6	10.0
Leu	116.5 ± 0.1	101.0
Tyr	61.5 ± 0.2	66.0
Phe	51.3 ± 4.2	49.8
Met	0	0
Try	not determined	0

* 22-hr hydrolysis (duplicate)

** Corfield, M.C. and Robson, A., Biochem. J. 84, 146 (1962), cited in the Proteins, Vol. 1, p.48. ed. Neurath, H., Academic Press, New York (1963).

*** 70-hr hydrolysis

of the 'proinsulin' is different from that of insulin. The significance of these differences cannot be adequately discussed before the exact molecular weight of the 'proinsulin' is determined. However, it is apparent from Table I that the 'proinsulin' contains an excess of glutamic, glycine and proline residues. The value of Leu/Phe is about 2 in this protein and it is of interest to note that the incorporation of these two amino acids into the 'proinsulin' fraction of isolated rat islets also showed a ratio of approximately 2 (Steiner et al., 1967). In summary, it may be stated that a protein is present in crystallized bovine insulin, which appears to meet the criteria of a 'proinsulin'.

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