SYNTHESIS OF PREPROINSULIN WITH RNA PREPARATION OF STREPTOZOTOCIN–NICOTINAMIDE INDUCED B-CELL TUMOR

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1. Introduction

It has been recently reported that a larger peptide than proinsulin was synthesized in a heterologous cell-free protein synthesizing system of wheat germ in the presence of nucleic acid or poly(A) containing RNA obtained from total pancreas [1], from islets of *Langerhans* [2], and from an X-ray induced islet tumor [3]. Chan et al. have further shown that the major cell-free translation product directed by rat pancreatic islet nucleic acid was a polypeptide with approx. mol. wt 11 500 consisting of proinsulin plus a 23 amino acid sequence covalently linked to the NH_2-terminus of proinsulin [4]. They have named this larger product preproinsulin, based on the assumption that it is a biosynthetic precursor of proinsulin.

In the present study, using both total RNA and poly(A) containing RNA prepared from streptozotocin–nicotinamide-induced B-cell tumors of rats, we have demonstrated that the major cell-free translation product of the RNA preparations is preproinsulin. However, in additional experiments in which isolated pancreatic islets of rats were exposed for short times (1–10 min) to [^{3}H]leucine, we failed to demonstrate preproinsulin.

Abbreviations: SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid

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2. Experimental

2.1. *Materials*

Streptozotocin was purchased from Upjohn, Kalamazoo, Michigan; L-[^{3}H]leucine (57.4 Ci/mmol) from New England Nuclear, Boston; guinea pig antiserum to bovine insulin and rabbit antiserum to guinea pig IgG from Miles-Yeda, Israel; horse heart cytochrome *c* from Boehringer Mann., GmbH, FRG; collagenase (CLS IV, 160 U/mg) from Worthington Biochem. NJ; oligo-(dT)-cellulose from P-L Biochem. Inc., Milwaukee; bovine pancreatic RNAase A from Sigma, St. Louis.

Rat proinsulin was prepared by the method of Steiner et al. [5].

2.2. *Preparation of B-cell tumor RNA*

Rat B-cell tumors were induced with streptozotocin and nicotinamide according to the method of Rakieten et al. [6]. The tumors were produced in 82.3% (82/99) of the male Wistar rats treated with these two agents. Total RNA was extracted from the B-cell tumors by the method of Chan et al. [4]. The yield was approx. 2 mg total RNA/5 tumor rats. Poly-(A)-containing RNA was prepared from the total RNA by the oligo-(dT)-cellulose chromatography method of Duguid et al. [3]. Yield: 20 μg from 2 mg total RNA.

2.3. *Cell-free translation experiments*

Translation experiments in the presence of total RNA or poly(A)-containing RNA were carried out at 25°C for 90 min in an incubation mixture (50 μl) containing 1.5 mM ATP, 0.3 mM GTP, 16.5 mM creatine phosphate, 0.4 mg/ml creatine kinase, 3 mM dithiothreitol, 15 mM Hepes-KOH (pH) 7.6), 20 mM

KCl, 0.12 mM each of unlabeled amino acids (without leucine), 0.8 mM spermidine phosphate, 5 μCi of [^{3}H]leucine and 15 μl of wheat-germ extract prepared by the method of Roberts and Paterson [7]. The translation was stopped by adding 20 μl 1 mg/ml RNAase A to the incubation mixture followed by incubation at 37°C for 20 min. An aliquot of the incubated mixture was treated with trichloroacetic acid (TCA) or with antiserum to insulin as described by Chan et al. [4] and the precipitates were analyzed by SDS–polyacrylamide gel electrophoresis according to the method of Maizel [8]. The gels were sliced, and radioactivity was measured by scintillation spectrometry after extraction of gels with NCS tissue solubilizer (Amersham/Searle).

2.4. *Experiments with isolated pancreatic islets*

Fifteen islets, prepared from normal male Wistar rat pancreas by the collagenase digestion method [9], were preincubated at 37°C for 45 min in 25 μl Krebs-Ringer bicarbonate solution [10,11] supplemented with 20 mM glucose. To this preincubated mixture 25 μl Krebs-Ringer bicarbonate solution, containing 5 μCi [^{3}H]leucine, was added and the incubation was further continued at 37°C for 1.25–10 min. The incubation was stopped by adding 2 ml ice-cold Hanks' buffer to the incubation mixture. After centrifugation at 2000 × *g* for 30 s, the supernatant was discarded, and the islets were washed once with the same buffer. The islets were resuspended in 40 μl 0.12 M Tris-phosphate buffer (pH 7.6) containing 2% SDS, and all the material of the islets was dissolved by boiling for 1 min. The dissolved material was analyzed by SDS–polyacrylamide gel electrophoresis.

3. Results

When added to a cell-free protein synthesizing system of wheat germ, total RNA of rat B-cell tumor caused a stimulation of incorporation of [^{3}H]leucine into the TCA-insoluble product with increasing time of incubation up to 90 min (fig.1.A). The stimulation of incorporation was proportional to the amount of RNA added (fig.1.B).

The TCA-insoluble or immunoprecipitated translation product was further analyzed by SDS–polyacrylamide gel electrophoresis. As shown in fig.2.A, the TCA-insoluble product gave a major peak that was clearly

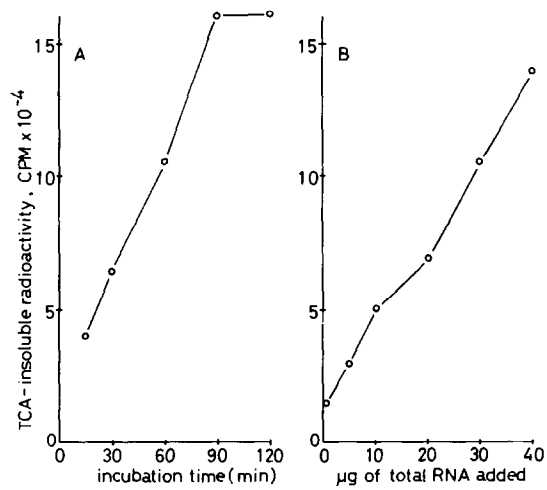

Fig.1. Translation of total RNA of B-cell tumor in the cell-free system of wheat-germ. (A) Incubation was performed at 25°C for the indicated time in the incubation mixture containing 40 μg total RNA. (B) Incubation was performed at 25°C for 90 min in the incubation mixture containing the indicated amount of total RNA. After the addition of 20 μl 1 mg/ml RNAase A, a 10 μl aliquot of the incubated mixture was removed and assayed for TCA-insoluble radio-activity by the filter paper method [12]. CPM represents TCA-insoluble radioactivity in 70 μl incubated mixture.

larger than proinsulin and a minor peak smaller than proinsulin. The immunoprecipitated material gave a single peak which corresponded to the major TCA-insoluble peak. Since the immunoprecipitation reaction was almost completely inhibited by insulin (data not shown), it is reasonable to assume that the structure of the immunoprecipitated material was closely related to that of insulin. The TCA-insoluble translation product directed by B-cell tumor poly(A) containing RNA gave a major peak which corresponded to the above immunoprecipitated peak (fig.2.B). The molecular weight of the major peak was estimated to be at approx. 11 500 on the gel.

In the light of the above results obtained in the experiments with the use of a cell-free protein synthesizing system of wheat-germ, we then undertook a series of experiments with the use of isolated pancreatic islets of normal rats. After incubation for a short time (1.25–10 min) of the isolated islets in the presence of [^{3}H]leucine, all the labeled product in the islets was analyzed by SDS–polyacrylamide gel electrophoresis.

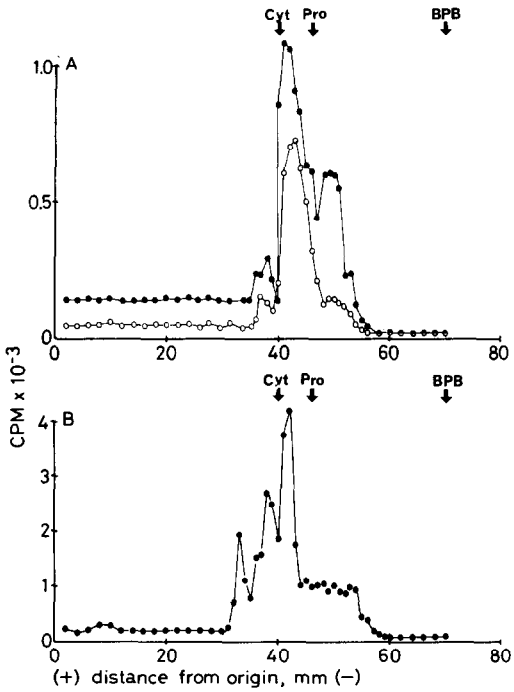

Fig.2. SDS–Polyacrylamide gel electrophoresis of the cell-free translation product. Conditions are as in fig.1.B, supplemented with 40 μg total RNA (A) or 2 μg poly(A)-containing RNA (B). [^{3}H]Leucine-labeled TCA-insoluble or immunoprecipitated translation product from a 10 μl aliquot of the incubated mixture was analyzed by SDS–polyacrylamide (15%) gel electrophoresis. (•———•) TCA-insoluble. (○———○) Immunoprecipitated. Arrows indicate relative migration of: Cyt, horse heart cytochrome *c* (Mol. wt 13 400); Pro, rat proinsulin (Mol wt 9000); and BPB, bromophenol blue, run on parallel gels.

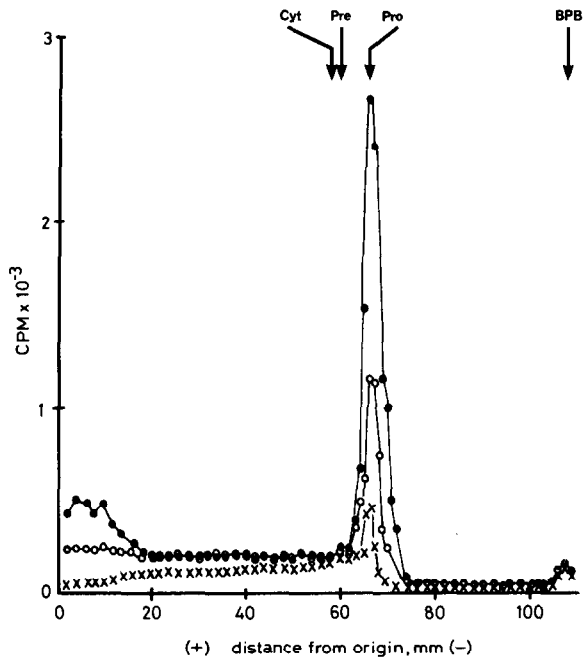

Fig.3. SDS–Polyacrylamide gel electrophoresis of all the labeled product in isolated pancreatic islets. Pancreatic islets were incubated as described in Experimental and all the [^{3}H]-leucine-labeled product in 15 islets was analyzed by SDS–polyacrylamide (15%) gel electrophoresis. (X———X) Incubated for 1.25 min. (○———○) Incubated for 5 min. (•———•) Incubated for 10 min. Cyt, horse heart cytochrome *c* (Mol wt 13 400); Pre, preproinsulin synthesized in the cell-free system of wheat-germ; Pro, rat proinsulin (Mol. wt 9000); BPB, bromophenol blue.

The results are shown in fig.3. It is seen that the labeled product gave only a major peak corresponding to proinsulin; neither peak of radioactivity for preproinsulin nor any labeled product smaller than proinsulin was observed. Here, the radioactivity comigrated with the dye is obviously due to free [^{3}H]leucine.

4. Discussion

In the present work, we have translated total RNA as well as poly-(A)-containing RNA from rat B-cell tumors induced by streptozotocin and nicotinamide in a cell-free protein synthesizing system of wheat germ. The major translation product was found to be preproinsulin from its molecular weight and immunoprecipitability with the antiserum to insulin.

Therefore, it may be said that the B-cell tumor induced by streptozotocin and nicotinamide can be used as a suitable material for isolation and purification of (pre)proinsulin mRNA, since the RNA from the tumor was found to be highly active in stimulating the incorporation of [^{3}H]leucine into preproinsulin in the wheat germ cell-free system, and since the tumor can be easily produced in rats.

In contrast to the synthesis of preproinsulin in a heterologous cell-free protein synthesizing system of wheat-germ, [^{3}H]leucine-labeled product in pancreatic islets was shown to be exclusively proinsulin. Therefore, if preproinsulin is the precursor of proinsulin in B-cells

of pancreatic islets, preproinsulin must be converted into proinsulin in a short time (within 1 min) or before the chain is completed. According to Chan et al., preproinsulin is composed of a pre-peptide, a NH_2-terminal portion of 23 amino acids, having at least 7 residues of leucine, and a proinsulin portion having 9 residues of leucine [4]. If proinsulin is formed via preproinsulin in B-cells of pancreatic islets, [^{3}H]leucine should be incorporated into leucine residues in the pre-peptide as well as in proinsulin. However, in the present experiment with isolated pancreatic islets, neither preproinsulin nor cleaved pre-peptide could be detected. Therefore, one possible explanation might be that preproinsulin is a product resulting from faulty translation of proinsulin mRNA in a heterologous cell-free protein synthesizing system of wheat-germ.

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