PROINSULIN PRECURSORS IN ISOLATED RAT PANCREATIC ISLETS

M. Alan Permutt , and Alan Routman

Department of Medicine, Washington University
School of Medicine, St. Louis, Missouri 63110

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SUMMARY

Two proteins larger than proinsulin (estimated molecular weight 11,000 and 10,000 daltons), were observed when labeled rat islet proteins were electrophoresed on sodium dodecyl sulfate gels. The proteins are synthesized before proinsulin, turn over more rapidly than proinsulin, their synthesis is stimulated by glucose, and they are specifically bound by anti-insulin antibodies.

INTRODUCTION

Biosynthesis of proinsulin, the single-chain polypeptide precursor of insulin, has been studied in a number of animal islet tissues (1-3). Using pulse-chase techniques, the half-time of conversion of proinsulin to insulin was estimated to be 1 hour in isolated rat pancreatic islets (2). No peptide larger than proinsulin was detected in incubations as short as 1 minute (4). In contrast, cell-free translation of islet mRNA has yielded preproinsulin, a peptide with 23-25 extra amino acids on the amino-terminus of proinsulin (5-8). Since this proinsulin precursor has not been observed in cells, it has been hypothesized that the precursor is cleaved to proinsulin while still a nascent chain (8).

Recently we have described a proinsulin precursor in fish islet cells (9). To determine whether precursors exist in mammalian islet cells, we investigated rat pancreatic islets. With the improved resolution of $NaDodSO_4$-urea slab gel electrophoresis, we now report detection of 2 rapidly synthesized peptides slightly larger than proinsulin.

METHODS

A. Rat Islet Incubations: Rat islets were isolated and incubated in Kreb's

Abbreviations: mRNA, messenger ribonucleic acid: $NaDodSO_4$, sodium dodecyl sulfate; M.W., molecular weight; PPO, 2,5'diphenyloxazole, AIS, anti-insulin serum.

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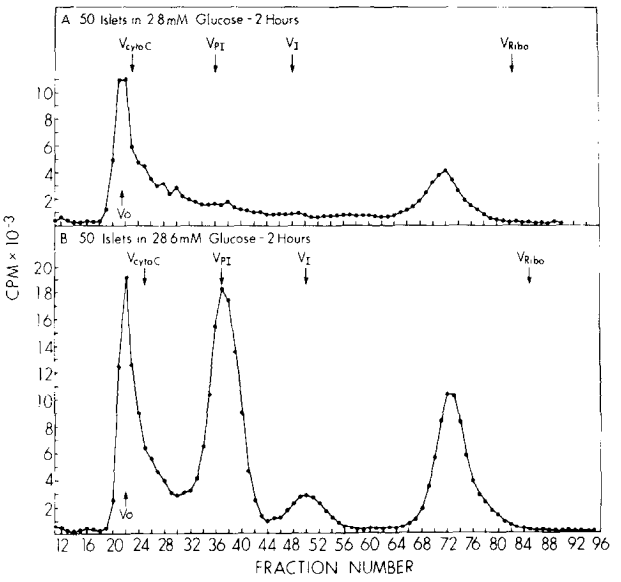

Figure 1: Gel-filtration chromatography (Biogel P-30) of rat islet proteins synthesized in 2.8 mM glucose (A) compared to 28 mM glucose (B). Rat islets were incubated with [^{3}H]leucine (100 µCi/ml) and chromatographed as indicated in Methods V_o, PI, and I refer to the void volume, and migration of bovine proinsulin and insulin, respectively.

bicarbonate-buffered media (0.1-0.5 ml) (10), with [^{14}C]L-leucine (0.3 Ci/mmol) or [^{3}H]L-leucine (70 Ci/mmol) (New England Nuclear, Boston, MA) from 2-240 minutes as indicated in the figure legends. After incubation, islets were either extracted in acid-alcohol and chromatographed on Biogel P-30 (7) or centrifuged at 3000 rpm (2 minutes), heated to 100°C (2 minutes) in 100 µl of NaDodSO4 extraction buffer for electrophoresis.

B. NaDodSO4 Slab Gel Electrophoresis: NaDodSO4 polyacrylamide slab gel electrophoresis was performed by a modification of the method of Swank and Munkres (11) using 17.5% acrylamide, 8M urea with the discontinuous buffer system of Laemmli (12), as previously described (5). Porcine proinsulin standard was kindly supplied by Dr. Ronald Chance, Eli Lilly Co. Radioactive proteins labeled with [^{3}H]leucine were detected by impregnating the gels with PPO, drying the gel (slab gel dryer - Hoefer Scientific Instruments, San Francisco), and exposing it to Kodak RP/R540/X-Omat film at -70°C according to the method of Bonner and Laskey (13). Exposure was from 2-30 days.

C. Immunoprecipitation Studies: Labeled rat islets were sonicated in phosphate buffered saline (0.15M sodium chloride, 0.015M sodium phosphate, pH 7.4, 0.5% Triton X-100), reacted with an excess of guinea pig anti-insulin serum (lot #526, Dr. Peter Wright, Indiana University) at 37°C for one-half hour, and precipitated with goat anti-guinea pig serum as previously described (10). The immune precipitates were extracted with acid-alcohol, evaporated to dryness and boiled in 10-25 µl of NaDodSO4 electrophoresis buffer prior to electrophoresis.

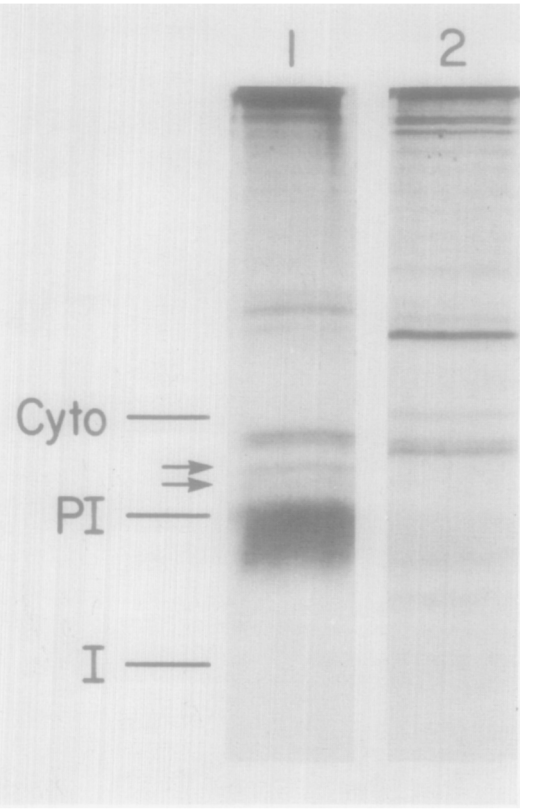

Figure 2: $NaDodSO_4$-urea polyacrylamide gel electrophoresis of labeled rat islet proteins. Isolated rat islets (20) were incubated with 28 mM glucose and [^{14}C] leucine (100 μCi/ml) for 1 hour (lane 1), or 1 hour with [^{14}C]leucine followed by 3 hours in 5 mM unlabeled leucine (lane 2), electrophoresed and autoradiographs of the dried gel made as in Methods (the autoradiograph was developed 28 days).

RESULTS

A. Analysis of Rat Islet Proteins by Gel-Filtration Chromatography and $NaDodSO_4$-urea Slab Gel Electrophoresis: [^{3}H]leucine labeled rat islet proteins from islets incubated in either 2.8 mM or 28 mM glucose were chromatographed on a Biogel P-30 column (Figure 1). Islets incubated in the higher glucose synthesize proteins which comigrate with carrier bovine proinsulin and insulin as previously noted (2,10). With $NaDodSO_4$ slab gel electrophoresis, a major band comigrating wit proinsulin, as well as 4 distinct proteins larger than proinsulin but smaller than cytochrome C (12,300 M.W.), were observed (Figure 2). To determine whether any of

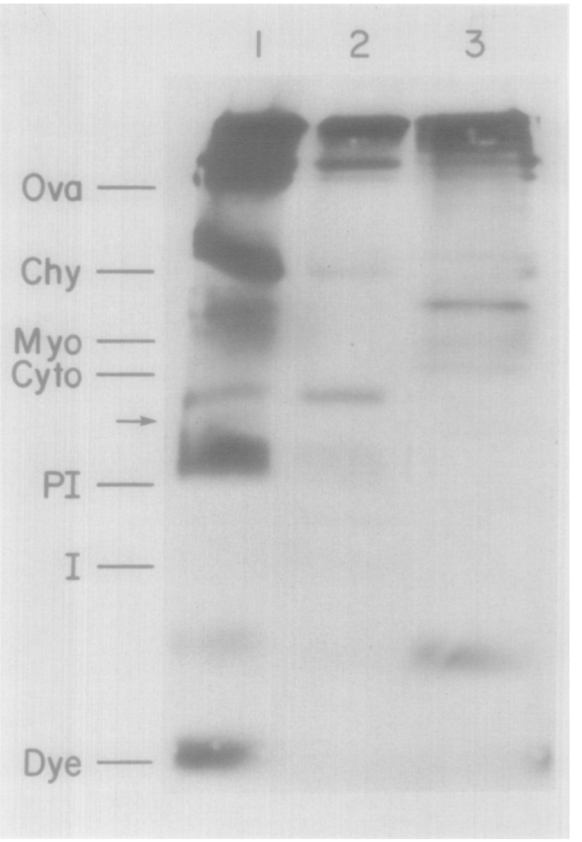

Figure 3: [^{3}H]leucine labeled islet proteins on NaDodSO$_4$-urea slab gels. Lane 1 - 1 hour, 28 mM glucose; lane 2 - 1 hour, 28 mM glucose, then 3 hours in 5 mM unlabeled leucine; lane 3 - 4 hour, 2.8 mM glucose. These proteins were detected by autofluorography (13).

these bands larger than proinsulin could potentially be a proinsulin precursor, the islets were pulsed with [^{14}C]leucine for 1 hour and chased with cold leucine for 3 hours. While the 2 proteins just below cytochrome C are stable, the 2 proteins just larger than proinsulin have disappeared (Figure 2, lane 2, arrows). The proinsulin band diminished in intensity, and 2 new bands below proinsulin appeared, one comigrating with insulin. To determine whether the proteins larger than proinsulin, whic turn over faster than proinsulin, were also glucose-stimulated, islets were incubated in [^{3}H]leucine and 28 mM glucose (Figure 3, lanes 1 and 2), or 2.8 mM glucose (lane 3). [^{3}H]protein was detected by autofluorography which resolved

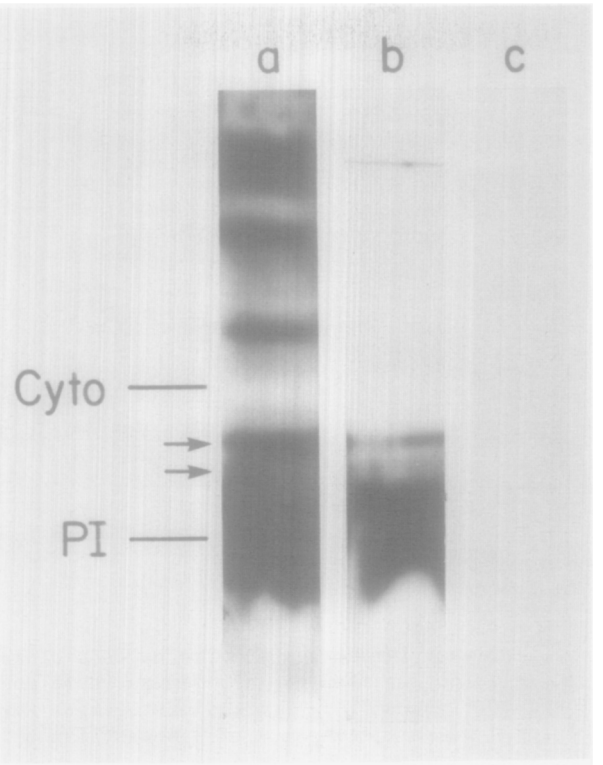

Figure 4: Immunoprecipitation and $NaDodSO_4$ gel electrophoresis of [^{3}H]leucine labeled islet proteins. Islets were incubated for 1 hour in [^{3}H]leucine and total protein (a), immunoprecipitated with AIS (b), or normal serum (c) electrophoresed as in Methods.

only 2 bands larger than proinsulin, rather than 4 seen in Figure 2. The band just larger than proinsulin (Figure 3, lane 1, arrow) is chased away with unlabeled leucine chase (lane 2). In contrast to the proteins synthesized in high glucose, none of the bands in the cytochrome C, proinsulin, or insulin region are observed with low glucose incubations (lane 3). The glucose sensitive bands were partially inhibited in the presence of 2-deoxyglucose (28 mM), and completely inhibited by mannoheptulose (28 mM) (not shown).

B. Immunoprecipitation Studies: Specific binding with anti-insulin serum of the 2 proteins larger than proinsulin (arrows), as well as proinsulin, is demonstrated in Figure 4.

C. Timed Incubations: To obtain enough labeled protein during short incuba-

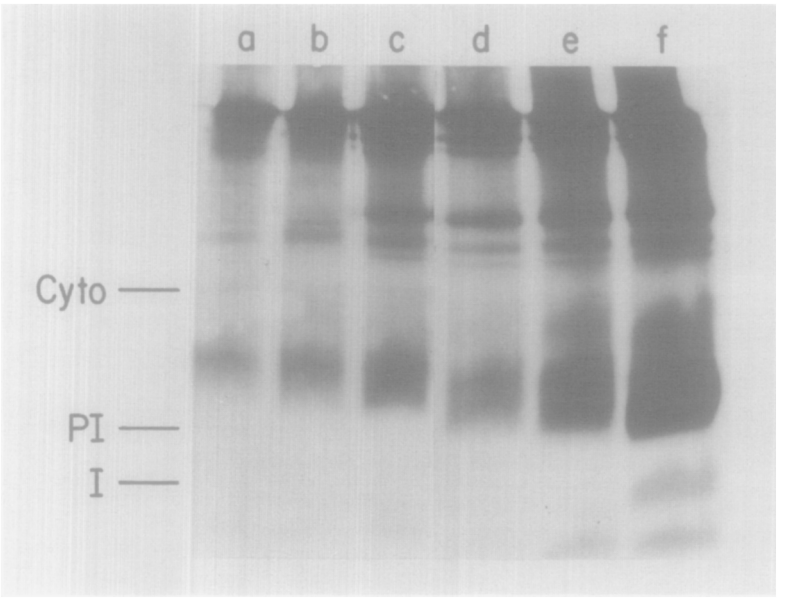

Figure 5: NaDodSO4-urea polyacrylamide gel electrophoresis of islet proteins labeled with [^{3}H]leucine (1 mCi/ml) for 2.5 minutes (a), 5 minutes (b and c), 15 minutes (d), 30 minutes (e), and 60 minutes (f). The radioautographs were developed by autofluorography (13) for 4 weeks (lanes a-c) or 2 weeks (lanes d-f).

tions, [^{3}H]leucine at 1 mCi/ml was used and proteins detected by autofluorography, with less resolution than the [^{14}C] autoradiographs. The pattern of incorporation is seen in Figure 5. At 2.5 minutes, 1 band was observed clearly larger than proinsulin. By 15 minutes, a band comigrating with proinsulin is seen, and by 60 minutes most of the labeled protein comigrates with proinsulin. Insulin is also visible at 60 minutes.

D. Conversion of Proinsulin Precursors: Various inhibitors of post-translational processing have been used to demonstrate processing of large viral polypeptide precursors (14,15). Neither Zn^{++} (1 mM), TLCK (0.1 mM), nor canavinine (3 mM) inhibited conversion of proinsulin precursors to proinsulin. Canavinine, an arginine analog, did inhibit conversion of proinsulin to insulin (not shown).

DISCUSSION

By the improved resolution of NaDodSO4-urea slab gel electrophoresis, what appeared to be 1 protein comigrating with proinsulin standard on Biogel P-30, was

resolved into 5 proteins; a major proinsulin band plus 4 proteins between 9000 and 12,000 daltons. The 2 largest of these proteins are stable, whereas the 2 proteins in the 10,000-11,000 M.W. range are turning over rapidly. Synthesis of these proteins is stimulated by glucose and inhibited by inhibitors of glucose oxidation. Since these proteins are specifically bound by anti-insulin antibody, and are synthesized before proinsulin, it was concluded that proinsulin precursors exist in cells.

The nature of these proinsulin precursors in cells has yet to be defined. The cell-free products have been shown to have 23-25 additional amino acids on the amino-terminus of the B-chain (7,8). The proinsulin precursors reported here appear somewhat smaller than fish (8,9) or rat cell-free products (7), and may represent intermediates in the conversion of preproinsulin to proinsulin. Alternatively, they may be proinsulin molecules with carboxyl extensions. Ullrich, et al (16) observed 55 nucleotides at the 3'-end of rat preproinsulin mRNA, enough nucleotides to code for 18 additional amino acids. The chemical nature of the rat as well as fish proinsulin precursors in cells is currently being evaluated.

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