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Insulin secretory granule biogenesis

Co-ordinate regulation of the biosynthesis of the majority of constituent proteins

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Two-dimensional gel-electrophoretic analysis combined with fluorography and densitometric quantification was used to examine the effects of glucose on the biosynthesis of rat pancreatic islet proteins. An increase in the medium glucose concentration from 2.8 to 16.7 mM produced a 10–20-fold stimulation in the synthesis of 10 out of 260 detected islet proteins, as judged by incorporation of [^{35}S]methionine during a 20 min incubation. The synthetic rates of the majority of the remaining proteins were stimulated by 2–4-fold. Greater resolution achieved by pulse–chase labelling and subcellular fractionation showed that, of 32 major proteins localized to insulin secretory granules, the biosynthesis of 25 were stimulated 15–30-fold by glucose. By contrast, only eight of 160 proteins in the soluble fraction showed a response of similar magnitude. It is concluded that there is a major and co-ordinated activation of the biosyntheses of proteins destined for secretory granules, which most likely occurs at the level of translational initiation and signal-recognition-particle-mediated translocation into the endoplasmic reticulum lumen. However, it is clear that not all granule proteins, or the majority of proteins translocated across the endoplasmic reticulum membrane, are affected in an equivalent manner. In addition, the synthesis of a small number of cytosolic proteins may be increased markedly by insulinotropic stimuli.

INTRODUCTION

The secretory granule of the pancreatic B cell performs a specialized subcellular function in the storage and secretion of the hormone insulin (for review, see Docherty & Steiner, 1982). In addition to insulin and connecting (C-)peptide, the insulin granule contains more than 150 polypeptides (Hutton *et al.*, 1982). These include the proteinases involved in proinsulin-to-insulin conversion, intermediates of the conversion process, minor co-secreted peptides, membrane proteins involved in intracellular granule movement and exocytosis, and ion-translocating proteins involved in regulation of the intragranular environment. Many of these proteins are probably essential to the segregation and storage of insulin within the granule, and it is clear that the granule constituents must be synthesized and assembled in a co-ordinate manner to ensure the integrated function of the organelle.

Insulin biosynthesis is regulated by many circulating nutrients and hormones, with glucose being the predominant stimulus of physiological importance (for review, see Hedeskov, 1980). The biosynthetic control exerted by glucose, which occurs primarily at the level of translation of pre-formed insulin mRNA (Permutt & Kipnis, 1972*a*), appears to extend to an insulin granule membrane constituent, SGM 110 (Grimaldi *et al.*, 1987), and the granule matrix protein chromogranin A (Guest *et al.*, 1989*a*). However, it is not known what proportion of insulin secretory granule proteins is affected in this way, or whether proteins localized in other islet cell compartments respond to glucose in a similar manner. We have addressed these questions through an investigation of the effects of glucose on total islet proteins by using two-dimensional gel-electrophoretic analyses of subcellular fractions prepared from [^{35}S]methionine-labelled rat islets.

EXPERIMENTAL

Materials

Analytical-grade biochemicals were obtained from Sigma Chemical Co. or BDH Chemicals (both of Poole, Dorset, U.K.), unless specified otherwise.

Preparation of islets

Islets of Langerhans were isolated from 10–12-week-old New England Deaconess Hospital rats by a collagenase digestion technique, as described previously (Guest *et al.*, 1989*a*). Islets were collected into an incubation medium consisting of modified Krebs bicarbonate buffer (120 mM-NaCl, 5 mM-KCl, 1 mM-$MgSO_4$, 2.5 mM-$CaCl_2$, 24 mM-$NaHCO_3$) containing 20 mM-Hepes (pH 7.4), 0.1 % BSA and 2.8 mM-glucose.

Radioisotopic labelling

Batches of 400 islets were preincubated in 500 μl of incubation medium containing either 2.8 or 16.7 mM-glucose for 1 h at 37 °C under O_2/CO_2 (19:1) in 1.8 ml capacity sealed plastic tubes (Nunc Cryotubes; Gibco, Paisley, Scotland, U.K.). Islets were recovered by centrifugation for 10 s at 800 *g* (MSE bench-top centrifuge) and resuspended in 200 μl of the same pre-warmed (37 °C) medium containing 200 μCi of [^{35}S]methionine (1400 Ci/mmol; SJ235; Amersham International, Amersham, Bucks., U.K.). Islets were recovered after 1 h by centrifugation as above and were then incubated for a further 3 h in 500 μl of pre-warmed (37 °C) modified tissue culture medium (Dulbecco's modified Eagle's medium; Gibco) containing 10 % (v/v) newborn calf serum and 2.8 mM-glucose. Incubations were terminated by addition of 1 ml of ice-cold Krebs incubation medium followed by centrifugation for 10 s at 3300 *g* (MSE Microcentaur microcentrifuge). Islets were washed by two further cycles of resuspension and centrifugation (as above) and either stored at −70 °C awaiting two-dimensional electrophoretic analysis or subjected immediately to subcellular fractionation.

Subcellular fractionation

In order to facilitate efficient homogenization and recovery of subcellular fractions, radiolabelled islet homogenates were combined with unlabelled rat insulinoma tissue before density-gradient centrifugation. Immediately before completion of the islet incubation, a post-nuclear supernatant fraction (0.5 g of tissue) was prepared from rat insulinoma tissue according to the method of Hutton *et al.* (1982) using a homogenization medium consisting of 10 mM-Mes (potassium salt, pH 6.5) containing 0.3 M-sucrose, 1 mM-$MgSO_4$ and 1 mM-EGTA. All fractionation

procedures were performed at 4 °C. Islets were combined with the insulinoma fraction and then homogenized in a 1 ml capacity glass tube homogenizer (Jencons Scientific, Leighton Buzzard, Beds., U.K.) using 10 strokes of a Teflon pestle driven at 600 rev./min. Homogenates were centrifuged for 5 min at 1700 *g* (MSE Coolspin) to remove unbroken cells and nuclei. The supernatants were transferred to 1.2 cm × 5.0 cm polypropylene centrifuge tubes (Beckman Instruments, Palo Alto, CA, U.S.A.) and underlayered with 1.3 ml portions of each of 4.4, 8.8 and 17.7% (w/v) Nycodenz (Nyegaard Diagnostica, Oslo, Norway) in homogenization medium. The samples were centrifuged for 1 h at 100000 *g* in a Beckman SW 50.1 rotor. The material not entering the gradient (soluble fraction) was reduced to a volume of 40 μl using a Centricon 10 concentrator (Amicon) and stored at −70 °C. The material at each interface in the gradient and the pellet were suspended in 10 mM-Mes containing 0.25 M-sucrose and centrifuged for 20 min at 50000 *g* (SW 50.1 rotor). The particulate material which sedimented was subjected to an additional cycle of resuspension and centrifugation and the final pellets were stored at −70 °C.

Highly purified insulin secretory granules were prepared from rat insulinoma tissue by Percoll density-gradient centrifugation according to the method of Hutton *et al.* (1982).

Two-dimensional electrophoretic analysis

Islets and subcellular fractions were mixed for 1 min with 100 μl of 9.5 M-urea containing 5% (v/v) 2-mercaptoethanol, 0.4% pH 3–10 range Pharmalyte (Pharmacia Fine Chemicals, Uppsala, Sweden), 1.6% pH 5–7 range Ampholines (Pharmacia LKB Biotechnology, Bromma, Sweden) and 0.001% Bromophenol Blue, and then centrifuged for 5 min at 13000 *g* (MSE Microcentaur microcentrifuge). The supernatants were subjected to two-dimensional gel-electrophoretic analysis by isoelectric focusing in the first dimension on polyacrylamide gels containing 8 M-urea, 2% pH 3–10 range Pharmalyte and 1% each of pH 3.5–5.0, 5–7 and 7–9 range Ampholines, and in the second dimension by SDS/PAGE on linear 5–20% (w/v) gradient gels run in Tris/HCl-based buffer (Anderson *et al.*, 1979). Gels were subsequently impregnated for fluorography, vacuum-dried and exposed to Cronex 4 X-ray film (Dupont, Stevenage, Herts., U.K.) at −70 °C as described previously (Guest *et al.*, 1989*a*). Incorporation of radioactivity into specific protein spots was determined by densitometric scanning (Chromoscan III; Joyce–Loebl, Gateshead, U.K.). Both pre-flashed and non-flashed films were used with no differences in the fluorographic images of intense or faint spots over exposure periods of 3–28 days. Responses in incorporation in the range 2–14-fold could be established by direct comparison of the peak absorbances of spots identified on fluorographs exposed for the same time period. Responses were classified as 10–30-fold on the basis that they were below the limit of detection (absorbance < 0.05) in the control situation (2.8 mM-glucose), and increased to absorbance in the range 0.5–1.5 at high glucose (16.7 mM). The results represent single experiments as dictated by the mode of analysis. Qualitatively and quantitatively similar data were obtained on at least two separate occasions. Molecular size calibration was achieved using ^{14}C-labelled lysozyme, β-lactoglobulin, α-chymotrypsinogen, ovalbumin, BSA, phosphorylase *b* and myosin heavy chain (BRL, Paisley, Scotland, U.K.) run in the second dimension.

Assay procedures

Insulin was determined by a two-site immunoradiometric method as described previously (Guest *et al.*, 1989*b*), carboxypeptidase H by the radiometric method of Rossier *et al.* (1989), and β-*N*-acetylglucosaminidase by the fluorimetric method of Barrett & Heath (1977). Spectrophotometric assays were performed for cytochrome oxidase (Cooperstein & Lazarow, 1951), NADPH–cytochrome *c* reductase (Sottocasa *et al.*, 1967), lactate dehydrogenase (Nielands, 1955) and alkaline phosphatase (Bowers & McComb, 1975). Protein was determined by the dye-binding method of Bradford (1976), with BSA as standard. Incorporation of [^{35}S]methionine into islet proteins was determined by trichloroacetic acid precipitation as described previously (Guest *et al.*, 1989*a*).

RESULTS

Incorporation of radioactivity into islet proteins

Islets incubated in the presence of 2.8 mM-glucose for 20 min incorporated 6250 d.p.m. of [^{35}S]methionine/islet, as determined by trichloroacetic acid precipitation. This was increased to 21500 d.p.m./islet in the presence of 16.7 mM-glucose. Two-dimensional electrophoretic analysis of these islets revealed that the majority of the proteins detected exhibited a 2–4-fold biosynthetic induction in response to the increased glucose concentration (Fig. 1), consistent with the results on total

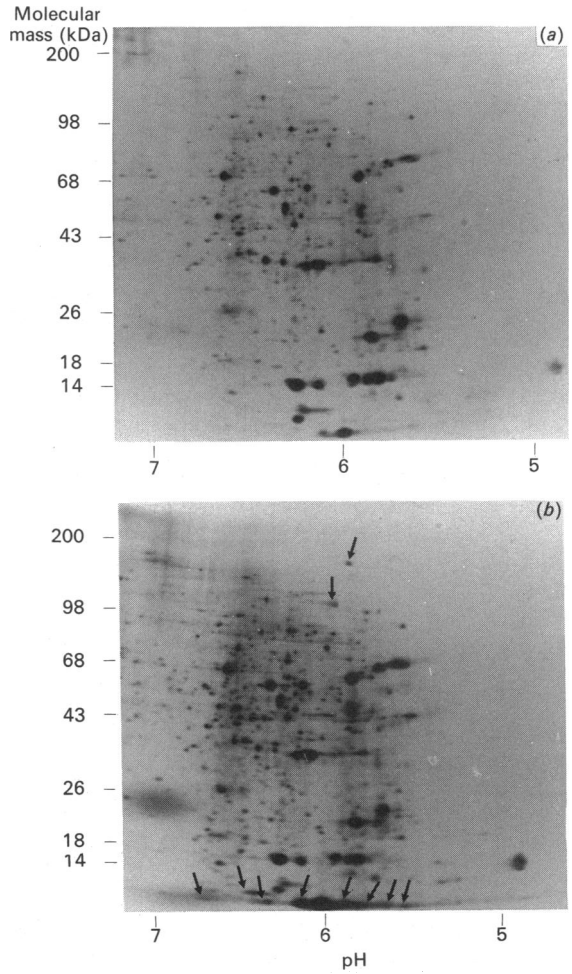

Fig. 1. Effect of glucose on the biosynthesis of pancreatic islet proteins

Islets were preincubated for 40 min and then labelled for 20 min with [^{35}S]methionine. The concentration of glucose in the medium was maintained throughout at either 2.8 mM-glucose (*a*) or 16.7 mM-glucose (*b*). Islet proteins were subjected to two-dimensional electrophoretic analysis followed by fluorography (3 days exposure; see the Experimental section for further details). Spots exhibiting a 10–20-fold increase in incorporation are indicated (→). The approximate pH and molecular mass scales were derived from gels run in parallel.

incorporation. Among the 260 spots amenable to analysis, 10 proteins showed an increased response of 10–20-fold. Most of the high-responding proteins were of low molecular mass.

Subcellular fraction of [^{35}S]methionine-labelled islet proteins

In order to increase the sensitivity and resolution of the detection method and to determine the localization of glucose-stimulated proteins, radiolabelled islets were subjected to subcellular fractionation before electrophoretic analysis. Biochemical (Sorenson *et al.*, 1970) and morphological (Orci *et al.*, 1985) studies have shown previously that newly synthesized insulin secretory granule proteins are delivered to the granules after a delay of 30–90 min. Therefore, to allow newly synthesized secretory proteins sufficient time to reach their cellular destinations, and to ensure that post-translational modifications were completed, a 1 h radiolabelling period and 3 h chase incubation were employed before fractionation.

Marker protein analyses were performed using fractions produced from insulinoma tissue in separate experiments. Discontinuous Nycodenz density-gradient centrifugation of these homogenates yielded five fractions designated (in order of increasing density): soluble (96.0 mg), A (0.4 mg), B (1.1 mg), C (20.5 mg) and pellet (11.9 mg). Analysis of selected marker proteins revealed that the soluble fraction contained cytosolic constituents, fraction A was enriched in plasma membranes, fraction B was composed of a mixture of plasma membranes and secretory granules, fraction C was enriched in secretory granules, and the pellet was a mixture of lysosomes, mitochondria and endoplasmic reticulum (Table 1).

In labelled (16.7 mM-glucose) islet fractions, 58% of the total trichloroacetic acid-precipitable radioactivity (5.4×10^6 d.p.m./400 islets) appeared in the soluble fraction, with 0.9% found in fraction A, 1.1% in fraction B, 7.9% in fraction C and 32.1% in the pellet. The total proportion of insulin granules is underestimated in these fractions when using [^{35}S]methionine as the radiolabel, since only one of the two rat insulins (II) contains methionine, and then only a single residue (Smith, 1966; Clark & Steiner, 1969). The distribution of immunoprecipitable labelled insulin in these fractions paralleled that of the total insulin determined by immunoradiometric assay (results not shown). This indicated that the newly synthesized islet-derived material was in a vesicular compartment of the same buoyant density as mature granules, and thus the assignments based upon marker protein analyses were most likely valid.

Table 1. Distribution of marker proteins in subcellular fractions

Subcellular fractions prepared from rat insulinoma tissue were assayed for marker proteins as follows: (1) secretory granules (insulin and carboxypeptidase H), (2) lysosomes (*β*-*N*-acetylglucosaminidase), (3) mitochondria (cytochrome *c* oxidase), (4) endoplasmic reticulum (NADPH–cytochrome *c* reductase), (5) plasma membranes (alkaline phosphatase), and (6) cytosol (lactate dehydrogenase), as described in the Experimental section: ND, not detected.

	Specific activity				
Fraction...	Supernatant	A	B	C	Pellet
Insulin (units/mg)	0.08	0.78	1.90	3.25	2.35
Carboxypeptidase H (nmol/min per mg)	8.9	54.8	341.3	403.0	249.9
Alkaline phosphatase (nmol/min per mg)	28.8	720.7	488.6	156.4	62.7
Lactate dehydrogenase (μmol/min per mg)	688.2	169.3	89.5	61.9	ND
β-*N*-Acetylglucosaminidase (arbitrary units)	0.7	26.6	27.4	29.1	100.0
Cytochrome *c* oxidase (nmol/min per mg)	ND	35.0	60.0	135.7	460.0
NADPH–cytochrome *c* reductase (nmol/min per mg)	0.5	26.3	30.0	27.8	94.0

Two-dimensional electrophoretic analysis of subcellular fractions

Two-dimensional electrophoretic analysis of ^{35}S-labelled proteins in secretory-granule-enriched fraction C revealed the presence of up to 240 polypeptides (28 days of fluorograph exposure) (Fig. 2). Exposure of islets to 16.7 mM-glucose enhanced the incorporation of [^{35}S]methionine into more than 60 of these proteins by 10–30-fold compared with proteins in islets preincubated in 2.8 mM-glucose. Most of the remaining proteins either exhibited a 2–4-fold increase in response to the higher glucose concentration or showed no response. Of 80 designated major radioactive spots (see Fig. 2*c*), 32 were distributed predominantly in this fraction and co-migrated with proteins in highly purified insulinoma granules identified by Coomassie Blue

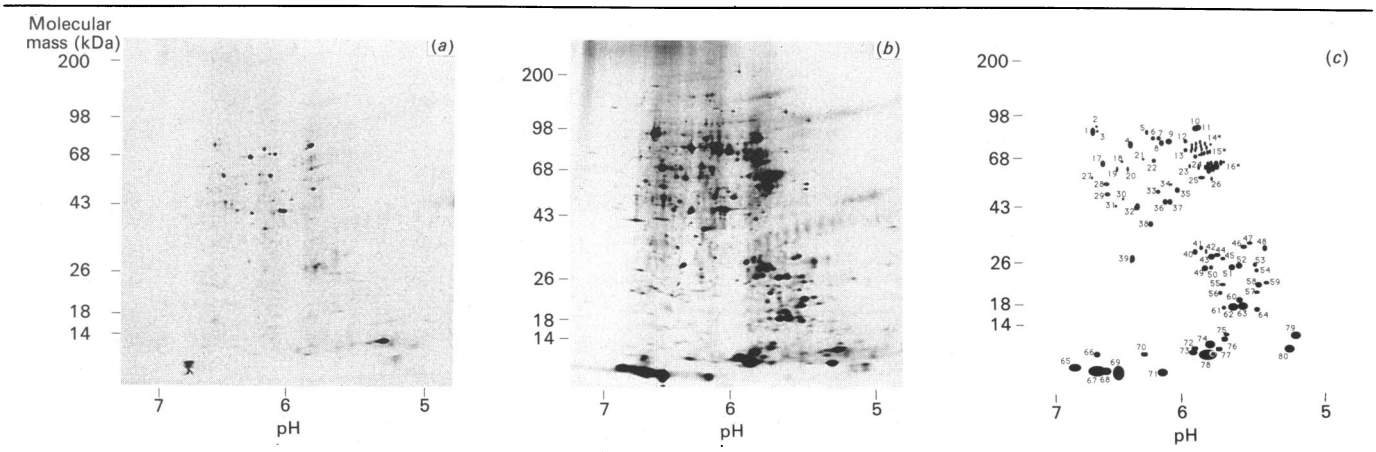

Fig. 2. Effect of glucose on the biosynthesis of islet secretory granule proteins

Islets were labelled with [^{35}S]methionine for 1 h in either 2.8 mM-glucose (*a*) or 16.7 mM-glucose (*b*), and then incubated for a further 3 h in non-radioactive medium containing 2.8 mM-glucose. Subcellular fractions were prepared (as described in the Experimental section), and those enriched in secretory granules were subjected to two-dimensional electrophoretic analysis followed by fluorography (28 days exposure). A diagrammatic representation of the predominant labelled proteins is shown in (*c*): * indicates large clusters of spots which are not clearly resolved.

Table 2. Classification of labelled proteins in the secretory granule fraction

Spots on two-dimensional gel fluorographs of secretory granule fraction C were classified as follows: (1) presence of equivalent spots on Coomassie Brilliant Blue-stained electrophoretograms of highly purified insulin secretory granules (I.S.G. equiv.); (2) predominant location in the labelled islet fractions S (supernatant), A, B, C or P (pellet); and (3) biosynthetic response to an increase in the medium glucose concentration from 2.8 to 16.7 mM (responses designated as 0, 1, 2 or 3, representing fold increases of 0, 2–4, 5–14 and 15–30 respectively).

Spot no.	I.S.G. equiv.	Location	Glucose response	Spot no.	I.S.G. equiv.	Location	Glucose response
1	–	P	2	41	–	C	2
2	–	C	3	42	–	C	2
3	–	P	3	43	+	C	3
4	+	S,P	1	44	+	C	3
5	+	P	1	45	+	C	3
6	+	C	2	46	–	C	2
7	+	S,P	2	47	–	P	2
8	+	C	3	48	+	C	3
9	+	C	3	49	–	C	3
10	+	P	2	50	+	C	3
11	+	P	2	51	–	C	3
12	+	P	3	52	–	C	3
13	–	C	3	53	+	C	3
14	+	C	3	54	+	C	3
15	+	C	2	55	–	C	3
16	+	C	3	56	–	C	3
17	+	P	2	57	–	C	3
18	+	C	3	58	–	C	3
19	+	C	3	59	–	C	3
20	+	C	2	60	–	C	3
21	+	C	2	61	–	C	3
22	+	P	1	62	+	C	3
23	–	C	2	63	+	C	3
24	–	P	2	64	+	C	3
25	+	C	1	65	+	P	3
26	–	C	3	66	+	C	3
27	–	C	2	67	?	C	3
28	–	C	2	68	?	C	3
29	–	C	1	69	+	C	3
30	+	C	3	70	–	C	3
31	+	C	3	71	?	C	3
32	+	P	3	72	–	C	3
33	+	C	2	73	–	C	3
34	–	C	1	74	+	C	3
35	+	P	2	75	+	C	3
36	+	B	1	76	–	C	3
37	+	B	1	77	+	C	3
38	+	C	2	78	+	C	3
39	+	C	3	79	–	P	0
40	–	C	3	80	–	C	3

staining (Table 2 and Fig. 3). Of this number, 25 proteins (78 %) were stimulated 15–30-fold by glucose, whereas the remaining seven proteins showed moderate or low glucose responses (Table 2).

Out of approx. 60 polypeptides detected (28 days exposure) in plasma-membrane-enriched fraction A, none were stimulated to a large extent by glucose (Fig. 4). In contrast, incorporation into three acidic proteins (pI 5.5–5.8) of 5–10 kDa was decreased by 10–20-fold at the higher glucose concentrations. Most of the remaining proteins in this fraction showed no response to the increased glucose concentration, although three components appeared to be stimulated by 2–3-fold. By comparison of the arrays of proteins in each fraction, including fraction B and the pellet, which represented mixed components (results not shown), it was demonstrated that none of the plasma membrane

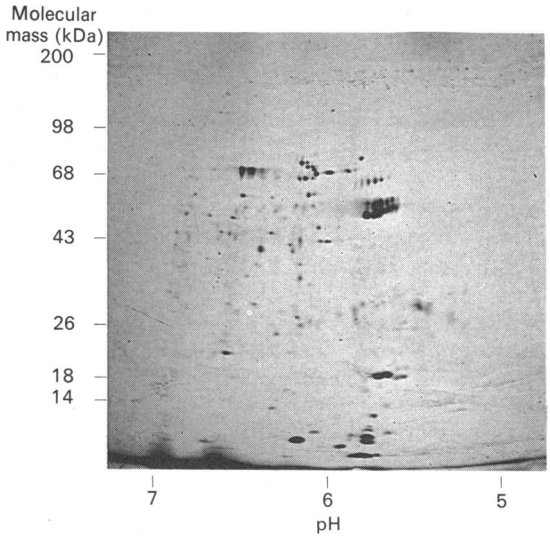

Fig. 3. Protein staining of a two-dimensional gel electrophoretogram of highly purified insulin secretory granules

Highly purified insulinoma secretory granules (200 μg) were subjected to two-dimensional electrophoretic analysis and subsequently stained with Coomassie Brilliant Blue-R.

components were attributable to contamination by other fractions.

Two-dimensional electrophoresis of the soluble protein fraction revealed the presence of more than 160 proteins (3 days exposure) (Fig. 5). Approx. 15 of these were stimulated by 10–20-fold at 16.7 mM-glucose compared with 2.8 mM-glucose. These included two abundant components of approx. 100 kDa (pI 6.1–6.3) and other prominent proteins of 43, 30 and 22 kDa (pI 6.0–6.6). However, seven of these high-responding proteins were found in significant amounts in the pellet (results not shown), indicating that the soluble fraction may contain significant quantities of proteins released from the endoplasmic reticulum during homogenization. Therefore, eight at most of the soluble glucose-responsive constituents are likely to be of cytosolic origin. Also present in the cytosolic fraction was a small cluster of acidic proteins (pI 5.0–5.7) of 3–5 kDa which were decreased in response to glucose by a factor of 10–20-fold, whereas most of the remaining proteins were stimulated 2–4-fold or not affected by glucose.

Due to the mixed nature of the pellet material and of fraction B, it was not possible to unequivocally identify glucose-responsive proteins in these fractions.

DISCUSSION

Translational regulation of insulin biosynthesis in the pancreatic B cell is thought to occur at the levels of insulin mRNA initiation (Morris & Korner, 1970; Permutt & Kipnis, 1972*b*), signal-recognition-particle-mediated translocation of initiated insulin mRNA to microsomal membranes (Welsh & Welsh, 1986), and preproinsulin elongation (Welsh *et al.*, 1986). The combined effects of these mechanisms can result in an increase in insulin biosynthesis of up to 20-fold within 1 h of stimulation by glucose (Howell & Taylor, 1966; Morris & Korner, 1970; Lin *et al.*, 1972; Ashcroft *et al.*, 1978).

Previous studies using antisera raised against specific insulin secretory granule proteins have demonstrated that glucose also increases the biosynthesis of a secretory granule membrane protein, SGM 110 (Grimaldi *et al.*, 1987), and of a granule matrix constituent, chromogranin A (Guest *et al.*, 1989*a*), to an

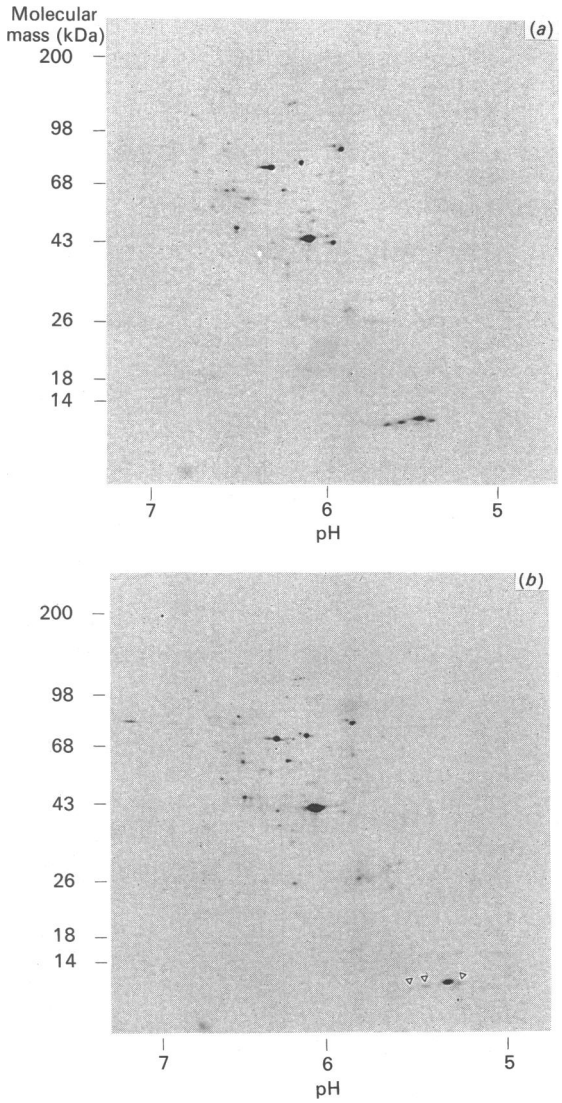

Fig. 4. Effect of glucose on the biosynthesis of islet plasma membrane proteins

Islets were labelled with [^{35}S]methionine in either 2.8 mM-glucose (*a*) or 16.7 mM-glucose (*b*) and subjected to subcellular fractionation as described in the legend to Fig. 2. Fractions enriched in plasma membranes were analysed by two-dimensional electrophoresis and fluorography (28 days exposure). Spots exhibiting a 10–20-fold decrease in incorporation are indicated (▷).

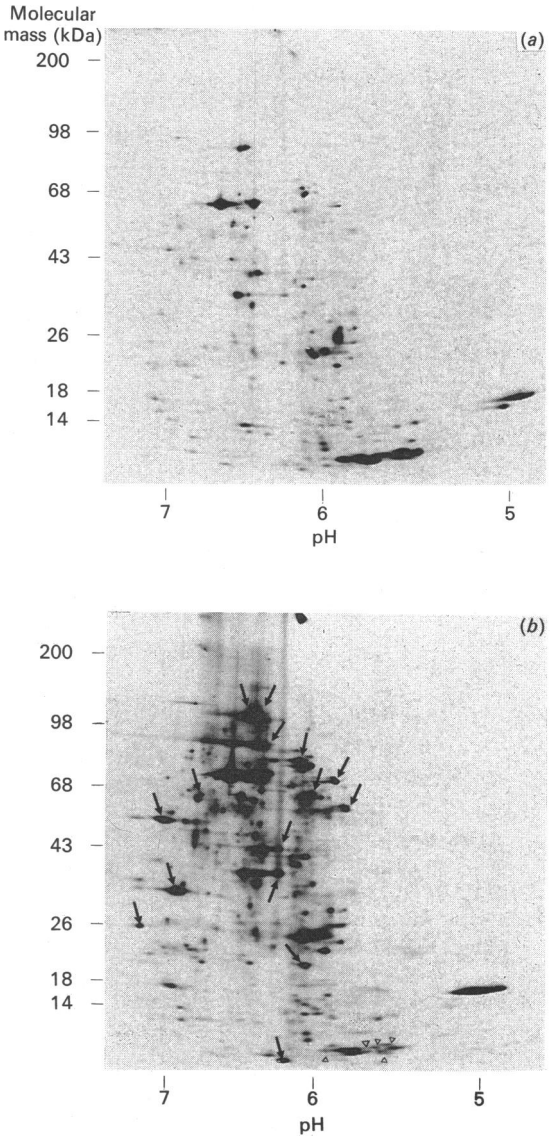

Fig. 5. Effect of glucose on the biosynthesis of islet cytosolic proteins

Islets were labelled with [^{35}S]methionine in either 2.8 mM-glucose (*a*) or 16.7 mM-glucose (*b*) and subjected to subcellular fractionation as described in the legend to Fig. 2. Fractions enriched in cytosol were analysed by two-dimensional electrophoretic analysis and fluorography (3 days exposure). → indicates spots exhibiting a 10–20-fold increase in incorporation, and ▷ indicates decreased incorporation.

extent similar to that observed for insulin. This indicates that translational control may extend to other insulin granule constituents or to proteins that are translocated into the lumen of the endoplasmic reticulum through their possession of an *N*-terminal signal sequence. We have investigated this possibility using a combination of subcellular fractionation and two-dimensional electrophoretic analysis of radiolabelled rat islets.

The Nycodenz density-gradient centrifugation subcellular fractionation technique used in this study afforded rapid separation and quantitative recovery of fractions, and for these reasons were used in preference to the Percoll density-gradient method described previously (Hutton *et al.*, 1982). However, the purity of secretory granules prepared on these gradients was less than half that of Percoll-purified granules, as judged by insulin specific activity measurements. By comparison of the two-dimensional gel profiles of each of the subcellular fractions, it proved relatively easy to distinguish genuine secretory granule components from contaminants. The granule components thus identified for the most part co-migrated with mature proteins in Coomassie Blue-stained two-dimensional gel electrophoretograms of highly purified insulin granules. This finding also indicated that the pulse–chase protocol adopted in this study permitted complete post-translational modifications of the proteins under consideration.

Another factor which should be considered is that the fractionated proteins under study were derived from a heterogeneous endocrine cell population. Besides the insulin-secreting B cells, which comprise 66–74% of the islet cell number, islets also contain glucagon-secreting A cells, somatostatin-releasing D cells and pancreatic-polypeptide-secreting PP cells (Baetens *et al.*, 1979). Therefore some of the proteins in the secretory granule fraction could be of non-B-cell origin. This may apply to a cluster of peptides of 20–30 kDa which were not identifiable as

either insulin granule peptides or contaminants from other fractions.

In agreement with previous investigations on islet protein synthesis (Morris & Korner, 1970; Permutt & Kipnis, 1972*a*), the majority of islet proteins in the present study exhibited a 2–4-fold increase in biosynthesis in response to glucose. Studies on the subcellular distribution of total islet mRNA indicate that this phenomenon could be accounted for by a widespread effect of glucose to increase translational initiation rates (Permutt & Kipnis, 1972*b*). Glucose exerted a marked stimulatory effect (10–30-fold) on the biosynthesis of a large number of islet proteins, the majority of which were localized to secretory granules. A smaller proportion of glucose-activated proteins were present in cytosolic fractions but, curiously, none were found in fractions enriched in plasma membranes.

Since the biosynthetic response of the highly stimulated secretory granule proteins was of the same magnitude as that of insulin, and was achieved over the same short time scale (less than 2 h), it is likely that translational control is operational. One possible mechanism by which this may be achieved is if the mRNAs encoding these proteins contain common structural elements which allow their selective recognition by the translational machinery. However, such elements, if they exist, are not obvious by direct comparison of the linear cDNA sequences encoding insulin and chromogranin A, which are known to respond in this manner (Hutton *et al.*, 1990). Another possible mechanism involves an increase in the rate of signal-recognition-particle-mediated translocation of nascent peptides into the endoplasmic reticulum lumen, as has been proposed previously for insulin synthesis (Welsh *et al.*, 1986). Although possibly operative, this mechanism is not sufficient to account for the observed biosynthetic response, since not all secretory granule and plasma membrane proteins responded to glucose in an equivalent manner. Furthermore, it could not account for the small number of glucose-responsive cytoplasmic proteins, since there are presumably synthesized on free ribosomes.

The biogenesis of the insulin secretory granule requires the co-ordinated synthesis and assembly of a large number of different proteins. It is conceivable that aberrations in the biosynthesis of one or more of these proteins might result in the defective storage or secretion of insulin. For example, the presence of increased circulating levels of proinsulin and processing intermediates in type 2 (non-insulin-dependent) diabetics and non-diabetic twins of type 1 (insulin-dependent) diabetics (Ward *et al.*, 1987; Heaton *et al.*, 1987) might reflect alterations in the biosynthesis or activation of the processing enzymes involved in proinsulin conversion. Similarly, a perturbation in the biosynthesis of the insulin granule islet amyloid polypeptide may lead to increased amyloid deposition in the islets of type 2 diabetics (Westermark *et al.*, 1986; Cooper *et al.*, 1987).

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