Biosynthesis of insulin secretory granule membrane proteins

Control by glucose

Keith A. GRIMALDI,* Kenneth SIDDLE and John C. HUTTON†

Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QR, U.K.

The biosynthesis of a component SGM 110, specifically localized to the membrane of insulin secretory granules, was studied in rat insulinoma cells and in normal islets of Langerhans. Cells or islets were labelled with [³⁵S]methionine or [³H]mannose and SGM 110 was immunoprecipitated by using a monoclonal antibody. Pulse-chase experiments demonstrated that the nascent polypeptide was cotranslationally glycosylated to form a 97000 Da peptide which in turn was processed to the mature 110000 Da form. A 50000 Da form detected by immunoblotting with the same antibody was not conspicuously labelled even after a 20 h chase incubation, suggesting that it represented late processing of SGM 110 in lysosomes. With insulinoma cells, an increase in medium glucose concentration from 3 mM to 20 mM was without effect on the secretion of insulin or on the biosynthesis of (pro)insulin or SGM 110. In normal islets, however, 20 mM-glucose produced a 17-fold increase in (pro)insulin biosynthesis and a 13-fold increase in SGM 110 biosynthesis, compared with only a 2-fold increase in total protein synthesis, as judged by incorporation of [³⁵S]methionine during a 1 h incubation. The effect of glucose on both (pro)insulin and SGM 110 biosynthesis was blocked by the addition of mannoheptulose, but not by the removal of extracellular calcium, both of which conditions inhibit insulin secretion. In contrast tolbutamide, an agent which stimulates insulin secretion, did not enhance the biosynthesis of (pro)insulin or SGM 110. It is concluded that at least one protein component of the insulin secretory granule membrane is synthesized co-ordinately with proinsulin and is subject to similar regulatory mechanisms. Factors which acutely control insulin secretion may also control granule biogenesis, although the two processes are not coupled in an obligatory fashion.

INTRODUCTION

The biosynthesis of proinsulin and its conversion to insulin has received considerable attention (for reviews see Docherty & Steiner, 1982; Steiner *et al.*, 1985), but virtually nothing is known about the synthesis of secretory granule membrane components. For insulin to be properly segregated and stored in the secretory granule it is evident that the hormone and other protein constituents of the secretory granule must be assembled co-ordinately. The biosynthesis of insulin by β -cells in the islets of Langerhans *in vitro* is regulated by ambient glucose concentration, an effect which requires the metabolism of glucose in the cell (Pipeleers *et al.*, 1973; Ashcroft *et al.*, 1976). It is not known though whether the same signals also affect the biosynthesis of other secretory granule proteins. Granule membrane proteins, moreover, constitute a special case in that unlike insulin they are not lost from the cell during exocytosis but may be recycled. There is evidence that in pancreatic β -cells (Orci *et al.*, 1973; Orci, 1974) and in other secretory cells (reviewed by Farquhar, 1985) granule membrane proteins are retrieved from the plasma membrane, passed to the Golgi apparatus and used in the formation of new secretory granules. Such morphological evidence is however indirect and fails to define the intracellular route followed by individual granule membrane proteins and does not allow a quantitative assessment of the

importance of this route versus synthesis *de novo*. The approach we have chosen in studying granule biogenesis has been to exploit the specificity of monoclonal antibodies raised to the granule membrane as granule membrane-specific markers. We report here a study of the biosynthesis of one such protein, SGM 110 (Grimaldi *et al.*, 1987).

METHODS

Materials

Laboratory chemicals of analytical grade were obtained either from BDH Chemicals or Sigma Chemical Co. unless otherwise specified. Radioisotopes were obtained from Amersham International. Monoclonal antibodies, insulinoma cells, subcellular fractions and islets of Langerhans were prepared as previously described (Grimaldi *et al.*, 1987).

Immunoblotting

Proteins were subjected to SDS/PAGE and immunoblotting as previously described (Grimaldi *et al.*, 1987).

Biosynthetic labelling of insulinoma cells and islets

Insulinoma cells [(2–4) × 10⁶] or islets (100–150) were incubated at 37 °C in 400 μ l of modified Krebs bicarbonate buffer (120 mM-NaCl, 5 mM-KCl, 24 mM-

Abbreviations used: PBS, phosphate-buffered saline; PAGE, polyacrylamide-gel electrophoresis; BSA, bovine serum albumin.

* Present address: Galibia SpA, Via Mantellini 22/A, I-100179 Roma, Italy.

† To whom correspondence and reprint requests should be addressed.

NaHCO₃, 1 mM-MgCl₂, 2.5 mM-CaCl₂) containing BSA (1 mg/ml), 20 mM-D-glucose and 100 μ Ci of [³⁵S]-methionine (SJ 204; sp. radioactivity 1000 Ci/mmol) or 400 μ Ci of D-[2,6-³H]-mannose (TRK 675; sp. radioactivity 54 Ci/mmol). The cells were incubated in 1.8 ml capacity sealed tubes (Nunc Cryotubes; Gibco, Paisley, Scotland, U.K.) under an atmosphere of CO₂/O₂ (1:19). For time course experiments the cells were then incubated in medium containing 3 mM-unlabelled methionine or 2 mM-mannose. Cells were washed three times in 10 ml of Krebs/BSA/glucose buffer, resuspended in 1 ml of 25 mM-Na₂B₄O₇, pH 9, containing BSA (1 mg/ml) and 1 mM-phenylmethanesulphonyl fluoride, and subjected to sonication and a single cycle of freeze-thawing. Samples (5 μ l) were mixed with 1 ml of 10% (w/v) trichloroacetic acid and the precipitated protein was recovered by centrifugation for 5 min at 8800 g in a microcentrifuge (MSE Microcentaur). The pelleted material was redissolved in 200 μ l of 1 M-methyl benzethonium hydroxide in methanol; 5 ml of scintillation fluid (0.5% PPO and 0.02% POPOP in toluene) was added and the radioactivity was determined by liquid-scintillation counting (Packard Tri-Carb 3255).

Immunoprecipitation

A portion (1 ml) of the disrupted cell suspension was placed in 1.5 ml capacity microcentrifuge tubes and underlaid with 50 μ l of 60% (w/v) sucrose. Each tube was then placed inside a 15 ml polycarbonate centrifuge tube which contained 13 ml of 10% (w/v) sucrose and centrifuged for 45 min at 75000 g (MSE 6 $\times$ 16.5 rotor, $r_{av.}$ = 11 cm). The supernatant was retained for immunoprecipitation of insulin and the membrane material at the interface was resuspended in 500 μ l of veronal buffer (50 mM-sodium barbitone/85 mM-NaCl containing 5 g of BSA and 0.1 g of NaN₃/l, adjusted to pH 8 with HCl). Samples of the suspension containing (2–5) $\times$ 10⁶ d.p.m. were incubated overnight at 4 °C with 1 μ l of ascitic fluid. Polyclonal rabbit anti-(mouse IgG) (10 μ l of 2 mg/ml) (Miles Scientific) was then added and a further overnight incubation was performed at 4 °C. The mixture was then made to 1 ml with veronal buffer and the tubes were centrifuged as before for 45 min at 75000 g. The supernatant was discarded and the pellet was solubilized by heating for 10 min at 50 °C in medium composed of 80 mM-NaCl, 5 mM-Hepes, 1 mM-EDTA, 1.25% (w/v) NP40, 1.25% (v/v) Zwittergent SB14, 0.1% SDS, 0.1% BSA, 0.05% NaN₃ and 100 μ M-phenylmethanesulphonyl fluoride, adjusted to pH 7.5 with NaOH. Insoluble material was removed by centrifugation for 5 min at 75000 g, and the supernatant was then incubated at room temperature with shaking for 1–2 h with 100 μ l of protein A-Sepharose suspension (Sigma) (equivalent to 10 μ l of swollen gel) in PBS (8.1 mM-Na₂HPO₄, 1.5 mM-KH₂PO₄, 2.7 mM-KCl and 140 mM-NaCl, pH 7.5). The protein A-Sepharose was washed six times with 1 ml of PBS containing BSA (1 mg/ml) and 0.05% Tween 20 (PBS/Tween/BSA), and three times with deionized water. The protein A-Sepharose pellet was eluted twice with 70 μ l of 10 mM-HCl, and the eluates were pooled and prepared for SDS/PAGE (Laemmli, 1970). Electrophoretograms were then prepared for fluorography by the method of Bonner & Laskey (1974). M_r calibration was achieved by using a ¹⁴C-labelled protein M_r standard mixture (BRL, Paisley, U.K.).

Proinsulin, insulin and processing intermediates were

immunoprecipitated using a monoclonal anti-insulin antibody (3B7) coupled to aminocellulose by diazotization (Gray *et al.*, 1987). Cellulose immunoadsorbent (1 mg) was incubated for 1 h at 4 °C with a sample of the soluble protein fraction (see above) containing 2 $\times$ 10⁶ d.p.m. of trichloroacetic acid-precipitable radioactivity. The immunoadsorbent was recovered by centrifugation for 5 min at 8800 g. It was washed twice in a buffer composed of 50 mM-Tris/HCl, pH 7.5, 150 mM-NaCl, 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate and 5 mM-EDTA, four times in PBS/Tween/BSA and three times in deionized water. Protein was eluted with 0.5 ml of 20% (v/v) acetic acid. Samples of the eluate (5 μ l) were removed for the determination of radioactivity, the remainder being lyophilized and then resuspended in 8 M-urea/2.5 mM-Tris/16 mM-glycine, pH 8.6, and electrophoresed in gels polymerized from a solution containing 7.5% (w/v) acrylamide and 0.08% NN'-methylenebisacrylamide in 12.5 mM-Tris base/80 mM-glycine (pH 8.6)/6 M-urea by the addition of 0.07% ammonium persulphate and 0.16% NNN'-tetramethylethylenediamine (Perrie & Perry, 1970). The gel electrode buffer consisted of 12.5 mM-Tris base/80 mM-glycine (pH 8.6). The gel was pre-run for 2 h at 300 V and the cathode buffer was replaced before protein samples were applied. For optimum separation the samples were electrophoresed for 3.5 h at 300 V. The gel was then fixed for 1 h in 25% (v/v) propan-2-ol and subjected to fluorography (Bonner & Laskey, 1974).

RESULTS

Biosynthesis of SGM 110

The immunoprecipitation of biosynthetically labelled granule membrane antigens proved difficult and a number of commonly used methods (Kessler, 1981; Anderson & Blobel, 1983) failed to give satisfactory results. This was attributable to the very low incorporation into the proteins under study, which in turn was probably related to their low abundance in the membrane (estimated to be of the order of 0.01–0.001% of total cell protein). The method which was developed reproducibly immunoprecipitated the biosynthetically labelled protein SGM 110 (Grimaldi *et al.*, 1987) with monoclonal antibody GM 10. Precipitation of the protein SGM 80 described in Grimaldi *et al.* (1987) was less successful, and although a faint specific band was obtained using antibody GM 4 the background of non-specific binding was too high for biosynthesis experiments to be performed satisfactorily for this antigen (K. A. Grimaldi, K. Siddle & J. C. Hutton, unpublished work).

Pulse-chase experiments performed with monoclonal antibody GM 10 showed that radioactivity was initially incorporated into a 97000 Da component which migrated as a narrow band on electrophoresis (Fig. 1a). This disappeared 30–60 min after its synthesis with the concomitant appearance of a higher molecular mass, more diffuse, band of 110000 Da. The latter corresponded in size to the 110000 Da protein seen upon immunoblotting granule membranes with the same antibody (Fig. 2). The 50000 Da peptide seen in immunoblots however was not conspicuous even in cells labelled for 30 min and chased for 20 h (K. A. Grimaldi,

Fig. 1. Biosynthesis and processing of SGM 110

(a) Insulinoma cells were labelled for 30 min with [35 S]methionine and then incubated with unlabelled methionine (3 mM) for 0 (track 1), 30 (track 2), and 120 min (track 3). (b) Insulinoma cells were labelled for 30 min with D-[2,6- 3 H]mannose. The label was chased for 0 (track 1) or 60 min (track 2) with unlabelled mannose (2 mM). Labelled SGM 110 was immunoprecipitated with monoclonal antibody GM 10, then subjected to SDS/PAGE and fluorography.

K. Siddle & J. C. Hutton, unpublished work). The 97000 Da form was also labelled with [3 H]mannose, suggesting that it was cotranslationally glycosylated (Fig. 1b). As in [35 S]methionine-labelled cells, the [3 H]mannose-labelled proteins could be chased into a higher molecular mass form although apparently at a slower rate, since the 97000 Da form was still evident in the latter cells after a 60 min chase incubation. This, however, may be the consequence of a large pool of dolichol oligosaccharide in the cell which permitted further incorporation of [3 H]mannose upon the termination of the pulse-labelling period.

The higher molecular mass form of labelled SGM 110 showed a similar degree of spreading on SDS/PAGE to the protein identified by immunoblotting, suggesting that it represented the major final species in the mature granule. The transformation into the diffuse form was most likely attributable to the addition of terminal glycosyl units which may be expected to occur in the trans Golgi region and which may result in microheterogeneity of molecular forms. Such microheterogeneity has been reported in many membrane proteins, for example the low density lipoprotein receptor which matures from 120000 Da to 160000 Da (Cummings *et al.*, 1983) and the epidermal growth factor receptor which is processed from a 160000 Da precursor to a final form of 170000 Da (Stoscheck *et al.*, 1985).

The failure to observe the 50000 Da component in labelling experiments suggested that it is not a product of a processing reaction in the Golgi apparatus or in the early phase of granule maturation but rather may be generated by later degradative reactions, possibly by lysosomal proteases. The 50000 Da component, as

Fig. 2. Immunoblots of SGM 80 and SGM 110 in insulinoma secretory granule fractions

Membrane proteins from an insulinoma secretory granule fractions (fraction B) (tracks 1 and 4), a fraction containing both granules and lysosomes (fraction A₂) (tracks 2 and 5) and a lysosomal fraction (fraction A₁) (tracks 3 and 6) were separated by SDS/PAGE (50 μ g of protein/track), and transferred to nitrocellulose membrane. The blotted proteins were incubated with monoclonal antibody GM 10 (reacting with SGM 110) (tracks 1–3) or GM 4 (reacting with SGM 80) (tracks 4–6). Binding was detected by autoradiography following incubation with 125 I-labelled sheep anti-(mouse IgG) (100000 d.p.m./protein track).

determined by densitometric scanning of immunoblots prepared with antibody GM10 (Fig. 2) constituted about 20% of the immunoreactive material in a purified secretory granule fraction (fraction B; Docherty & Hutton, 1983) but more than 40% of the material in granule fractions which contained a higher proportion of lysosomal marker proteins (fractions A₁ and A₂). This would be consistent with the 50000 Da component being produced by degradation within a crinophagic vesicle resulting from fusion between a mature secretory granule and a lysosome. It could equally result from fusion between a lysosome and an endocytic vesicle involved in the retrieval of the granule membrane after exocytosis.

The same fractions immunoblotted to reveal the presence of SGM 80 showed that in fraction B the protein migrates as a more diffuse band than in fractions A₁ and A₂. The molecular mass is also lower in the latter two fractions. Such alterations could again have resulted from incorporation of the granule membrane protein into lysosomes within the insulinoma cell. The changes in SGM 80 and SGM 110 immunoblotting patterns did not appear to be an artefact of granule isolation resulting from the exposure of granule proteins to disrupted lysosomal elements, since incuba-

Table 1. SGM 110 and proinsulin biosynthesis in insulinoma cells and islets

Biosynthetically labelled SGM 110 and (pro)insulin were immunoprecipitated from insulinoma cells or islets which had been incubated with [³⁵S]methionine with the indicated modifications to the labelling medium. The labelling of cellular SGM 110 and secreted (pro)insulin were determined by densitometric scanning of fluorographs and are expressed as peak areas on suitably exposed films. For further experimental details see the Methods section. The results tabulated are derived from single experiments as dictated by the mode of analysis. Qualitatively and quantitatively similar data was obtained on at least two other occasions for each experimental condition. N.D., not determined.

Expt. no.	Tissue	Glucose concn. (mM)	Modification	10 ⁻⁶ × Trichloroacetic acid-precipitable radioactivity (d.p.m.)	10 ⁻⁴ × Cellular (pro)insulin (d.p.m.)	Cellular SGM 110 (arbitrary units)	Secreted (pro)insulin (arbitrary units)
1	Insulinoma	3	—	39.6	45.4	8.5	4.5
		20	—	39.0	41.8	8.5	4.5
2	Islets	3	—	3.6	1.5	0.5	0.5
		20	—	8.5	26.9	6.2	4.0
3	Islets	20	—	9.4	19.2	5.5	4.5
		20	+ EGTA (5 mM), no Ca ²⁺	9.3	28.9	8.5	1.2
4	Islets	20	—	10.1	23.6	5.3	4.3
		20	+ Mannoheptulose (20 mM)	6.2	2.4	0.5	0.5
5	Islets	5.6	—	10.7	6.2	3.2	N.D.
		5.6	+ Tolbutamide (0.1 mg/ml)	9.1	4.4	1.9	N.D.

tion of fraction B with fraction A1 at 37 °C for 3 h did not result in any differences in the immunoblotting patterns (K. A. Grimaldi, K. Siddle & J. C. Hutton, unpublished work).

Regulation of SGM 110 biosynthesis: dependence on glucose concentration

With insulinoma cells an increase in the glucose concentration in the labelling medium from 3 mM to 20 mM failed to affect total protein synthesis as judged by [³⁵S]methionine incorporation into trichloroacetic acid-precipitable material (Table 1). The incorporation into (pro)insulin and SGM 110 were similarly unaffected (Figs. 3 and 4 and Table 1). By contrast, in normal pancreatic islets, total protein synthesis was approximately doubled by exposure to 20 mM-glucose compared with 3 mM-glucose (Table 1) and there was a greater than 17-fold increase in the total amount of ³⁵S-labelled (pro)insulin immunoprecipitated. The (pro)insulin biosynthesis relative to total protein was about 8-fold greater at 20 mM (3.2%) than at 3 mM-glucose (0.42%). Under the conditions used, the total (pro)insulin biosynthesis is underestimated since only one of the two rat insulin genes (II) contains methionine (Lomedico *et al.*, 1979).

The effect of glucose on SGM 110 biosynthesis was similar to that on (pro)insulin biosynthesis. The incorporation of radioactivity into SGM 110 in 1 h was at least 13 times greater in islets incubated in 20 mM-glucose than in those incubated in 3 mM-glucose. Relative to trichloroacetic acid-precipitable radioactivity it represented an increase of at least 6.5-fold.

The lack of any effect of glucose on the biosynthesis of granule protein in the insulinoma parallels the lack of sensitivity of the secretory response of this tissue to glucose (Sopwith *et al.*, 1981). Although the tumour contains only one-third to one-fifth of the insulin content of islets, the rate of insulin biosynthesis expressed

Fig. 3. Immunoprecipitation of SGM 110 from islets and insulinoma cells

Islets (tracks 1 and 2) or insulinoma cells (tracks 3 and 4) were incubated for 1 h at 37 °C with [³⁵S]methionine in the presence of either 3 mM-glucose (tracks 1 and 3) or 20 mM-glucose (tracks 2 and 4). Proteins immunoprecipitated from a membrane fraction with monoclonal antibody GM 10 were subjected to SDS/PAGE and fluorography.

relative to total protein synthesis was almost 3-fold greater than basal insulin biosynthesis in islets and about one-third of the maximal rate in islets. It would seem that, rather than being unable to generate a signal for biosynthesis and secretion in response to glucose, the

Fig. 4. Immunoprecipitation of (pro)insulin from islets and insulinoma cells

Islets (tracks 1–4) and insulinoma cells (tracks 5 and 6) were incubated for 1 h at 37 °C with [35 S]methionine in the presence of either 3 mM-glucose (tracks 1, 3 and 5) or 20 mM-glucose (tracks 2, 4 and 6). Insulin was immunoprecipitated both from the cells (tracks 1 and 2, 5 and 6) or from the media surrounding the islets (tracks 3 and 4) using the monoclonal antibody 3B7. The immunoprecipitated proteins were analysed by alkaline urea gel electrophoresis. The migration of proinsulin (Pro), insulin (Ins) and processing intermediates (Int) is indicated. The mobility of the latter is consistent with their being a mixture of des-Arg³¹,Arg³²-proinsulin and des-Lys⁶⁴,Arg⁶⁴-proinsulin.

tumour is already effectively maximally stimulated. In any case the tumour proved an inconvenient model for studying regulation of biosynthesis, and subsequent experiments were therefore performed on isolated islets in spite of the very small amount of material available for the study of such a low-abundance protein as SGM 110.

The proportion of the precursor forms of insulin and SGM 110 precipitated from islets was greater than from insulinoma cells (Fig. 4). This is probably not due to slower processing and maturation, because it has previously been demonstrated that the conversion of proinsulin to insulin occurs at similar rates in both islets and insulinoma tissue (Gold *et al.*, 1984; H. Davidson & J. C. Hutton, unpublished work). It may be better explained by the findings of Permutt & Kipnis (1972) that although there is an immediate 2–3-fold increase in proinsulin biosynthesis there is a delay of approx. 45 min before the principal stimulatory effect of glucose is seen. Therefore, if SGM 110 behaves similarly to proinsulin, in islets labelled for 1 h the majority of the (pro)insulin and SGM 110 precipitated would have been synthesized in the final 15 min of the labelling period and most of the SGM 110 would still be in the precursor form. When islets were labelled for 2 h in the presence of 20 mM-glucose the pattern of SGM 110 immunoprecipitated was similar to that obtained from insulinoma cells labelled for 1 h (K. A. Grimaldi, K. Siddle & J. C. Hutton, unpublished work). In subsequent experiments the incubation time was kept at 1 h because the 97000 Da

component migrates as a narrower band than the higher molecular mass form and is more readily detected by fluorography (2.5 versus 5 weeks exposure time).

Effect of mannoheptulose

In the presence of 20 mM-mannoheptulose plus 20 mM-glucose the incorporation of [35 S]methionine into trichloroacetic acid-precipitable material in islets was reduced to 60% of the control value and the amount of SGM 110 and (pro)insulin immunoprecipitated was reduced to less than 10% of the level in the absence of mannoheptulose (Table 1). Mannoheptulose, which blocks the conversion of glucose to glucose 6-phosphate in islets (Ashcroft & Randle, 1970), thus reversed the effect of high glucose on islet protein biosynthesis. This suggests that the glucose molecule itself is not sufficient to stimulate SGM 110 and (pro)insulin biosynthesis but that metabolism of the sugar is necessary for the generation of the stimulatory signal. Similar observations have been made on glucose-stimulated insulin secretion which is also inhibited in the presence of mannoheptulose (Ashcroft & Randle, 1970).

Effect of extracellular calcium

When islets were incubated in the absence of extracellular calcium there was no change in incorporation into trichloroacetic acid-precipitable material, whereas the amounts of SGM 110 or (pro)insulin immunoprecipitated were increased by about 50% (Table 1). Calcium depletion did however reduce the amount of (pro)insulin immunoprecipitated from the labelling medium. Therefore, although secretion by islet cells required the presence of extracellular calcium, the biosynthesis of the granule proteins did not. It follows that the synthesis of secretory granule membrane is not obligatorily coupled to exocytosis in the cell and that the signal generated by glucose metabolism may be acting primarily on the biosynthetic machinery. The reason for the apparent increase in the amounts of SGM 110 and (pro)insulin synthesized in the absence of calcium is unclear, but it was also found by others investigating insulin biosynthesis by following the incorporation of [3 H]leucine (Steiner *et al.*, 1972; Lin & Haist, 1973). It cannot be explained by the inhibition of secretion *per se* because islets labelled in the presence of extracellular calcium secreted only about 1% of their labelled (pro)insulin during the incubation period (K. A. Grimaldi, K. Siddle & J. C. Hutton, unpublished work) whereas islets labelled in the absence of calcium apparently synthesized 50% more (pro)insulin. Further, this explanation would not account for the similar increase in the amount of labelled SGM 110 which is a membrane protein and would therefore remain in the cell after the exocytotic release of insulin.

Effect of tolbutamide

Tolbutamide is a potent insulin secretagogue which probably exerts its action at the level of the plasma membrane (Gylfe *et al.*, 1984). To investigate the effect of tolbutamide on SGM 110 biosynthesis islets were incubated in 5.6 mM-glucose rather than 3 mM-glucose to ensure a higher-than-basal insulin synthesis (Morris & Korner, 1970) so that inhibitory effects, if present, could be observed. The synthesis of both SGM 110 and (pro)insulin were reduced in the presence of tolbutamide (Table 1). This effect was not specific to granule proteins

as the amount of [^{35}S]methionine incorporated into trichloroacetic acid-precipitable material was reduced to a similar extent.

DISCUSSION

To investigate the biogenesis of insulin secretory granules we have made use of a monoclonal antibody raised to granule membranes, which recognizes a glycoprotein antigen of unknown function, previously designated as SGM 110 (Grimaldi *et al.*, 1987). Studies of the protein composition of the insulin secretory granule show that, although the membrane may contain up to 8% of the total granule protein, this is distributed fairly evenly among up to 100 different proteins (Hutton, 1984). Thus on average a given protein is likely to constitute no more than about 0.1% of the granule protein or 0.02% of total islet protein. The antigen SGM 110 appeared to be of even less than average abundance, judged by the failure to identify it as a distinct band on Coomassie Blue-stained electrophoretograms of isolated insulinoma granules. This accorded with the inability to quantify the incorporation of [^{35}S]methionine into the protein by liquid-scintillation counting techniques which would have been able to detect a protein constituting 0.01% or more of the total trichloroacetic acid-precipitable radioactivity.

The fact that SGM 110 is such a minor granule component put severe limitations on the design of experiments. For example, pulse-chase experiments had to be performed with extended labelling periods and results were at best semiquantitative. Nevertheless, it was possible to address for the first time some of the fundamental questions concerning insulin secretory granule membrane biogenesis.

The first of these concerns the pathway of biosynthesis of newly formed granule membrane proteins. It was probable that SGM 110 was synthesized on bound ribosomes on the endoplasmic reticulum since glycosylation occurred co-translationally as has been documented for a number of secreted and plasma membrane proteins (for review see Meldolesi, 1983). Subsequent passage of SGM 110 through the Golgi stack was indicated by the appearance of further glycosylation occurring some 30–60 min post biosynthesis. At about this time newly synthesized (pro)insulin passes from the trans Golgi (the site of terminal glycosylation) to the coated and then uncoated secretory granule compartment (Orci *et al.*, 1984). Thus it appeared that both the secreted product and the membrane envelope proteins traverse the biosynthetic pathway at approximately the same rate. This may not be simply the product of uniform membrane traffic or bulk flow of proteins through the Golgi stack since it is evident, at least in other cells, that proteins following this same route, such as lysosomal and plasma membrane constituents, traverse the Golgi apparatus at rates differing by greater than an order of magnitude (Kelly, 1985).

A second major question concerns the role of post-translational modification in the segregation of proteins in the secretory granule. SGM 110, in common with many granule membrane proteins, was glycosylated (Abbs & Phillips, 1980). However, unlike proinsulin and another granule matrix protein, betagranin (Hutton *et al.*, 1987), SGM 110 did not appear to undergo proteolytic cleavage during the course of its segregation

into the secretory granule, although clearly the removal of a small peptide of less than 2000 Da from a pro-form of the protein would have passed undetected.

A third question concerns the relationship between granule membrane biogenesis and membrane movements occurring during exocytosis. The observation that tolbutamide-induced secretion failed to activate SGM 110 biosynthesis suggested that granule membrane movement and granule membrane protein biosynthesis are not obligatorily coupled. This was further supported by the finding that removal of Ca^{2+} from the medium, which blocks glucose-induced insulin secretion, did not block glucose-induced SGM 110 biosynthesis.

Since depletion of intracellular Ca^{2+} by its removal extracellularly may also impede intracellular membrane flow, as indicated by morphological studies on insulin granules in intact pancreatic β -cells (Howell *et al.*, 1978), it follows that the control of granule membrane biogenesis lies proximally to the point of segregation of the proteins into the granule. As proinsulin and SGM 110 biosynthesis appeared to be affected in parallel under all conditions studied it is tempting to conclude that their syntheses are regulated at the same level. In both cases the metabolism of the sugar was essential to activation of the biosynthetic process, as indicated by the inhibitory effect of mannoheptulose. It is known for proinsulin biosynthesis that although control is exerted at the level of transcription of the insulin gene and on mRNA stability in the long term (Brunstedt & Chan, 1982; Nielsen *et al.*, 1985; Welsh *et al.*, 1985), in the short term, regulation is achieved by changes in rates of initiation of mRNA translation (Morris & Korner, 1970; Permutt & Kipnis, 1972; Itoh *et al.*, 1978; Itoh & Okamoto, 1980) and possibly release of signal recognition particle arrest (Welsh & Welsh, 1986). Since glucose-stimulated SGM 110 biosynthesis as seen here was activated within 60 min it would seem likely that translational control is operational as in the case of proinsulin.

Preferential stimulus of polypeptide hormone biosynthesis by secretagogues, although commonly found in other endocrine tissues, is generally not effected by control of translational events. One might speculate that structural features of proinsulin mRNA could be important in such regulation. However, the finding that SGM 110 is found in other endocrine tissues suggests that some other factors are important in the selective activation of translation of secretory granule protein mRNAs.

Although SGM 110 did not prove an ideal marker for the study of insulin secretory granule membrane biogenesis, further examination of the regulation of biosynthesis of this protein appears warranted, especially if this can be extended in the future to the analysis of the mRNA(s) and gene(s) encoding this protein. The very fact that the present studies were feasible provides further incentive to the development of monoclonal antibody reagents to more abundant granule membrane protein constituents.

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