*Proc. Natl. Acad. Sci. USA*
Vol. 81, pp. 5007–5011, August 1984
Biochemistry

# Conversion of proglucagon in pancreatic alpha cells: The major endproducts are glucagon and a single peptide, the major proglucagon fragment, that contains two glucagon-like sequences

(hormone biosynthesis/preproglucagon/conversion product/amino acid composition)

CHRISTOPH PATZELT AND EMILE SCHILTZ*

Physiologisch-Chemisches Institut, D-8700 Würzburg, Federal Republic of Germany

*Communicated by Donald F. Steiner, April 9, 1984*

ABSTRACT **It has previously been shown by biosynthetic labeling studies that glucagon is synthesized in mammalian islets via an 18-kDa precursor, proglucagon, that during processing gives rise to glucagon and a secreted peptide of 10 kDa (the major proglucagon fragment, MPGF). We have now developed a simple procedure for the isolation of this peptide from rat pancreatic islets and have characterized it more fully. On the basis of its amino acid composition, MPGF is identified as the COOH-terminal portion of proglucagon that contains two glucagon-related sequences. These sequences do not appear to be liberated from MPGF in alpha cells of the islets of Langerhans but MPGF may be processed further elsewhere in the body or in other cells of the gastrointestinal tract that produce glucagon precursors.**

In previous studies on protein biosynthesis in rat pancreatic islets, an 18-kDa proglucagon was identified by peptide mapping (1). Conversion of this precursor during pulse–chase experiments resulted in the appearance of a 10-kDa peptide, designated the major proglucagon fragment (MPGF), that did not contain glucagon (2). More recently, the amino acid sequences of three mammalian proglucagons have been deduced from the sequence of cloned DNA complementary to preproglucagon mRNA (3–5). These encode 18-kDa proteins whose predicted amino acid sequences confirm the proposed structural relationship between proglucagon and glicentin, a larger form of glucagon from intestinal mucosa (6), indicating that glicentin represents the $NH_2$-terminal portion of the prohormone. The remaining COOH-terminal part of proglucagon is predicted to contain two glucagon-like sequences linked by a short peptide spacer (3–5).

Prohormone conversion products generated in addition to glucagon may be of physiological importance. However, the type of conversion cannot be readily predicted from the primary structure of the prohormone. In particular, the formation and secretion of a stable proglucagon fragment such as the 10-kDa MPGF (2) would be incompatible with the simultaneous liberation from alpha cells of the two glucagon-like peptides predicted in this sequence.

In the present study, the formation and processing of glucagon precursors has been analyzed in view of these structural considerations. A procedure for isolation of MPGF from pancreatic islets is presented. From its amino acid composition, we have identified MPGF as the entire (unprocessed) COOH-terminal portion of the proglucagon molecule including both of the glucagon-like sequences.

## MATERIALS AND METHODS

Isolation of pancreatic islets, labeling of islet proteins, and their electrophoretic separation for fluorography have been described (7). Elution of radioactive proteins from excised gel bits for subsequent two-dimensional analysis of their tryptic peptides and quantitation of electrophoretically resolved proteins by scintillation counting have also been described (1, 8). Isoelectric focusing was carried out on slab gels composed of 5% acrylamide/0.33% *N,N'*-methylenebisacrylamide/8 M urea/2% (vol/vol) Nonidet P-40 and ampholytes of pH ranges 5–7 and 3–10, 1% (wt/vol) each.

**Isolation of Islet mRNA and Cell-Free Translation.** Polyadenylylated RNA was isolated from batches of 2000–4000 rat islets as detailed (9). The average yield of mRNA was $\approx 0.05$ $A_{260}$ unit per 1000 islets. Ten-microliter samples of mRNA, corresponding to 500 islets, were translated in a reticulocyte lysate system (10) in a total volume of 50 $\mu$l for 60 min at 30°C. Where indicated, 1 $\mu$l of microsomal membranes from canine pancreas (11), corresponding to 0.2 $A_{280}$ unit, was included prior to the addition of mRNA.

**Immunoprecipitations.** For immunoprecipitation of glucagon-related peptides, isolated islets were incubated for 2 hr with [$^{35}$S]methionine (1), then sonicated in immunoprecipitation buffer (12) (2 ml for 1000 islets) and heated for 1 min at 100°C. After centrifugation for 1 hr at 40,000 × *g*, 0.2-ml samples of the supernate were incubated with 1 $\mu$l of glucagon antiserum K4023 (Novo Research Institute, Copenhagen, Denmark) for 24 hr at 4°C. To controls, 20 $\mu$g of synthetic glucagon was added. After addition of 50 $\mu$l of anti-rabbit IgG (Miles), incubation was continued for at least 4 hr. Precipitates were washed (13) and lysed in 0.3 ml of 3 M acetic acid, then lyophilized and dissolved in 25 $\mu$l of denaturing solution for electrophoresis (7). For immunoprecipitation of cell-free translational products, samples were heated (1 min, 100°C), diluted with 20 vol of immunoprecipitation buffer and centrifuged (Sorvall HB 4 rotor, 8000 rpm, 20 min). Samples (0.5 ml) of the supernate were precipitated as described above.

**Isolation of MPGF from Pancreatic Islets.** Batches of 2000–4000 islets that had been washed with albumin-free Hanks' buffer were sonicated in 2 ml of 70% ethanol (vol/vol)/0.2 M HCl. After centrifugation (Sorvall HB 4 rotor, 8000 rpm, 15 min, 4°C), the pellet was extracted with an equal volume of HCl/ethanol. The combined extracts were adjusted to pH 5 (10 M NaOH) and kept overnight at −24°C. The flocculant precipitate was collected by centrifugation and extracted with three 2-ml portions of 0.1 M Tris·HCl, pH 10/2 mM phenylmethylsulfonyl fluoride. The combined extracts were fractionated by ammonium sulfate precipitation. Material precipitating between 1.3 and 3.6 M ammonium sulfate was collected and dissolved in 50 mM Tris·HCl, pH 10/1 mM phenylmethylsulfonyl fluoride and any undissolved material was removed by centrifugation. The supernate was subject-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MPGF, major proglucagon fragment; D, duodenal; S, splenic.

*Present address: Institut für Organische Chemie und Biochemie, D-7800 Freiburg, Federal Republic of Germany.

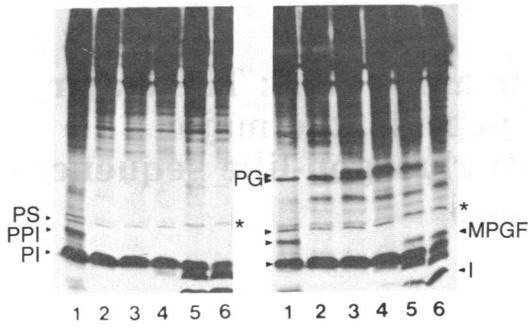


FIG. 1. Pulse–chase experiments with duodenal (*Left*) and splenic (*Right*) islets. Samples of 60 islets were pulse labeled with [$^{35}$S]methionine for 2 min and chased for 0 (lane 1), 3 (lane 2), 8 (lane 3), 28 (lane 4), 58 (lane 5), and 118 (lane 6) min. PPI, preproinsulin; PI, proinsulin; I, insulin chains; PS, prosomatostatin; PG, proglucagon; MPGF, major proglucagon fragment; *, unidentified protein.

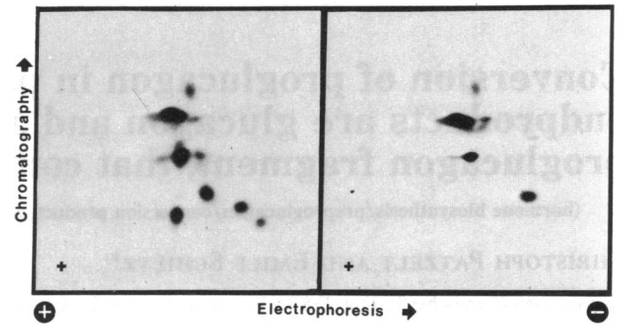


FIG. 2. Two-dimensional analysis of tryptic peptides of proglucagon (*Left*) and MPGF (*Right*), biosynthetically labeled with [$^{3}$H]phenylalanine. Origins were at the lower left corners (+).

ed to a second precipitation at pH 5. The pelleted precipitate was washed once with 50 mM NaOAc (pH 5), dissolved in 2 ml of 20 mM Na phosphate (pH 7), and cleared again of undissolved material. The resulting supernate contained isolated MPGF.

For evaluation of this isolation procedure, two sets of aliquots were removed at the essential steps (see Table 1) and lyophilized. One set of samples was dissolved for electrophoretic analysis (7) and the other was solubilized in 20 mM Tris·HCl, pH 8.0/1% $NaDodSO_4$ for protein determination (14).

**Amino Acid Analysis.** Two nanomoles of isolated MPGF (20 $\mu$g) was hydrolyzed in 6 M HCl for 20 hr at 110°C and analyzed on a Biotronik LC 5000 amino acid analyzer.

## RESULTS

**Synthesis and Processing of Glucagon Precursors *in Vivo* and *in Vitro*.** The demonstration of the components of glucagon biosynthesis in pancreatic islets is facilitated by the existence of two types of islets—those designated "splenic" or S islets that produce glucagon and those called "duodenal" or D islets that are devoid of glucagon. The almost complete regional separation of these two islet populations in the rat pancreas (15) allows for their separate isolation from the respective portions of this organ.

The results of pulse–chase experiments with D and S islets are presented in Fig. 1. The various forms of insulin biosynthesis including preproinsulin, proinsulin, and its conversion products (7) are equally conspicuous in both types of islets. Similarly, prosomatostatin (8) can be clearly discerned on the protein patterns of both D and S islets. By contrast, the forms that have been attributed to glucagon biosynthesis (1) are completely absent from the protein pattern of D islets. Those forms are proglucagon (18 kDa), a posttranslationally modified proglucagon of slightly reduced electrophoretic mobility, and the conversion product of 10 kDa designated MPGF (1, 2). Glucagon itself appears to be covered by heavily labeled insulin chains resulting from stimulated (25 mM glucose) insulin production.

A protein of ≈13 kDa directly above prosomatostatin (Fig. 1, *), which was tentatively identified as an intermediate of proglucagon conversion (1) can no longer be attributed to glucagon biosynthesis because of its formation in both types of islets.

The nature of MPGF as a proteolytic conversion product of proglucagon is confirmed by two-dimensional analysis of the tryptic peptides of biosynthetically labeled proglucagon and MPGF (Fig. 2). Labeling with radioactive phenylalanine has been found to result in particularly clear fingerprints. The structural analysis of MPGF (see below) confirmed the presence of phenylalanine in most of its tryptic peptides (see Fig. 6).

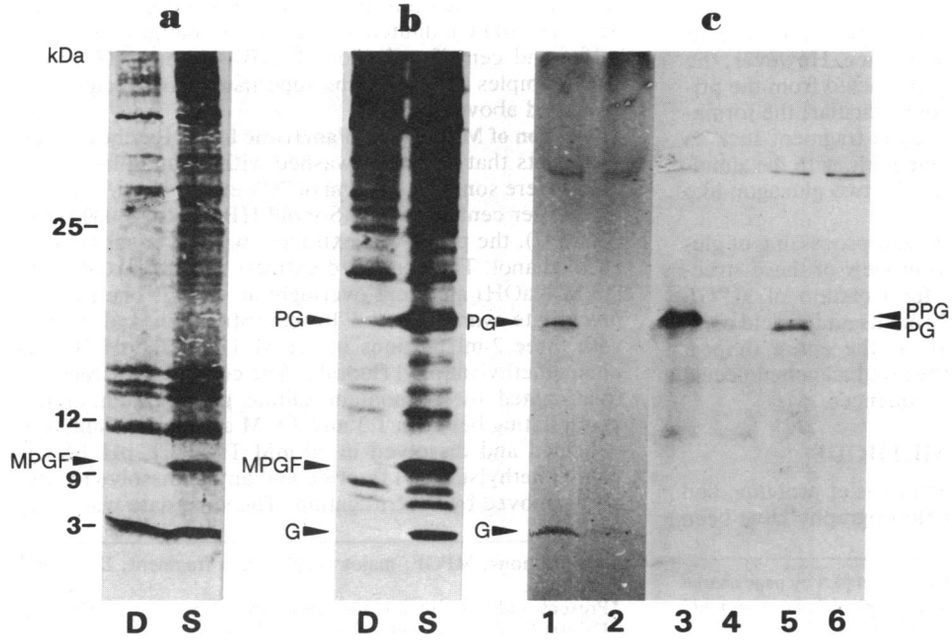


FIG. 3. Comparison of glucagon precursors and related peptides formed *in vivo* and *in vitro*. (*a*) Coomassie blue-stained electrophoretograms of 100 D and S islets, respectively, labeled for 2 hr with [$^{35}$S]methionine. (*b*) Fluorograph of the same gel. (*c*) Lanes: 1, immunoprecipitated extract of labeled S-islets; 3, immunoprecipitated cell-free translation products; 5, immunoprecipitated cell-free translation products processed by microsomal membranes; 2, 4, and 6, control precipitates. PPG, preproglucagon; G, glucagon; other abbreviations are as in Fig. 1.

Table 1. Isolation of MPGF from pancreatic islets

| Fraction | Total protein, μg | $^{35}$S-labeled MPGF,* total cpm | Yield, % | Specific activity, cpm/μg protein |
|---|---|---|---|---|
| Islet lysate | 3920 | 4543 | 100 | 1.2 |
| HCl/ethanol extract | 2640 | 4112 | 91 | 1.6 |
| First pH 5 precipitate (extracted at pH 10) | 1272 | 3471 | 76 | 2.7 |
| Ammonium sulfate precipitate (1.3–3.6 M) (dissolved at pH 10) | 108 | 1005 | 22 | 9.3 |
| Second pH 5 precipitate (dissolved at pH 7) | 15 | 614 | 14 | 40.9 |

A total of 4400 islets was incubated as batches of 200 with [$^{35}$S]methionine for 2 hr (1). Combined material was fractionated as described in *Materials and Methods*.
*Determined from excised and dissolved bits of the gel shown in Fig. 4.

Accumulation of MPGF as a conversion product of proglucagon in pancreatic islets has been shown by its abundance in the pattern of islet proteins on Coomassie blue-stained electrophoresis gels (2). In the fluorograph of islets labeled with [$^{35}$S]methionine for 2 hr (Fig. 3*b*), proglucagon, MPGF, and glucagon can easily be discerned. In this experiment, as in all the following ones, islets were incubated at low glucose concentration (2.5 mM) to suppress insulin biosynthesis. Coomassie blue staining of the fluorographed gel (Fig. 3*a*) showed that the band corresponding to MPGF was the only visible difference between D and S islets. Whereas glucagon again appears to be overlayed by (preformed) insulin chains, proglucagon, like proinsulin, seems not to accumulate sufficiently in the cell to be visible as a stained protein band (Fig. 3*a*).

For immunoprecipitation of glucagon-related peptides from islet extracts or from cell-free translational systems, a glucagon antiserum (K4023) was used that also reacts with larger forms of glucagon such as glicentin (16). In [$^{35}$S]methionine-labeled islet extracts, proglucagon and glucagon were found to be immunoreactive (Fig. 3*c*, lane 1), but not MPGF, in accordance with its previous identification as a glucagon-free conversion product (1). When total islet mRNA was translated in a reticulocyte system, a single protein was immunoprecipitated that can be tentatively identified as preproglucagon (Fig. 3*c*, lane 3). This primary translation product appeared to be only slightly larger than proglucagon in the electrophoresis system used (7), but it was reduced to the size of the prohormone when microsomal membranes were added to the cell-free system (Fig. 3*c*, lane 5).

**Isolation of MPGF from Pancreatic Islets.** To isolate MPGF from pancreatic islets, a multistep precipitation procedure was developed using changes of pH, temperature, and ethanol and ammonium sulfate concentrations (Table 1). The stepwise purification of $^{35}$S-labeled MPGF is illustrated in Fig. 4. Since MPGF (estimated size, 10 kDa) represents a major portion of the prohormone, the concomitant purification of labeled proglucagon is not unexpected (Fig. 4*a*). However, because of the low abundance of the prohormone in pancreatic islets, the final preparation of MPGF can be regarded as being "electrophoretically pure" (Fig. 4*b*, lane 5). On isoelectric focusing gels, isolated labeled MPGF is present as a single band at pH 5 (Fig. 4*c*) whereas labeled proglucagon appears to be resolved into multiple bands of slightly different isoelectric points that are barely visible on the fluorograph (Fig. 4*c*, lane 1).

From the radioactivity in MPGF bands excised from the gel of Fig. 4, a final yield of extracted peptide of 10–20% can be calculated (Table 1). Given that 4400 islets were used as the starting material, the MPGF content was calculated to be 24 ng per islet or about 1/40th of the total islet protein. This figure agrees with the gross appearance of MPGF abundance in Coomassie blue-stained islet proteins (Fig. 3*a*).

**Amino Acid Composition of MPGF and its Structural Assignment to Proglucagon.** Recently, the amino acid sequences of three mammalian proglucagons have been deduced from the structure of preproglucagon cDNA (3–5). In each case the $NH_2$-terminal glucagon-containing portion of the prohormone was composed of the sequence of glicentin whereas the rest of the molecule consisted of two glucagon-related sequences bracketed by basic residues and linked by a spacer sequence (see Fig. 6). This entire COOH-terminal portion of 88 or 89 amino acid residues was close to a molecular weight of 10,000.

To prove the identity of MPGF with this COOH-terminal

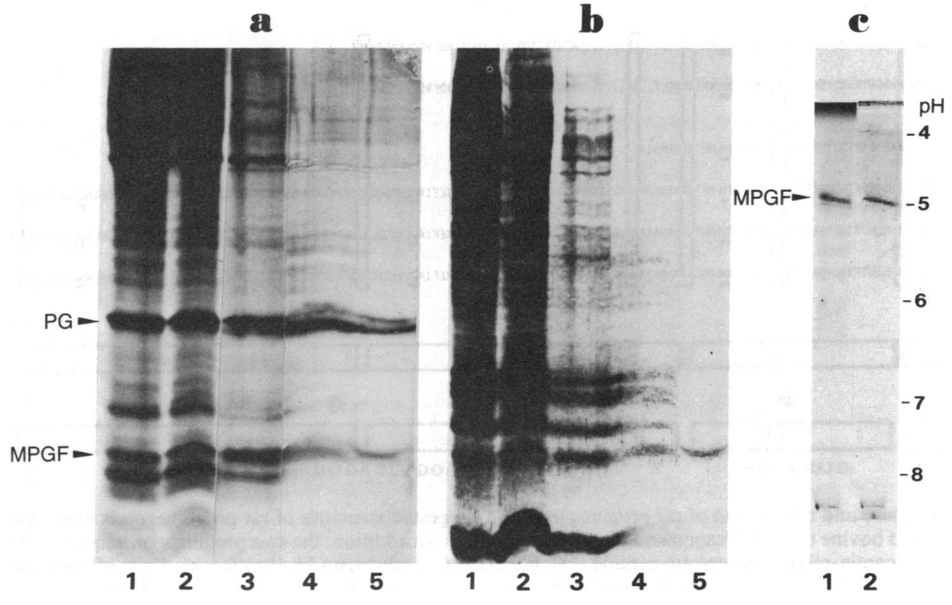


FIG. 4. Isolation of MPGF from pancreatic islets. (*a*) Fluorograph. (*b*) Protein stain. Lane numbers refer to fractions listed in Table 1. (*c*) Isoelectric focusing of MPGF (corresponding to lanes of 5 *a* and *b*). Lane 1, fluorograph; lane 2, protein stain. Abbreviations are as in Fig. 1.

Table 2. Comparison of amino acid compositions of rat MPGF and the COOH-terminal portions of human (4), hamster (3), and bovine (5) proglucagon

| Amino acid | Residues per molecule | | | |
|---|---|---|---|---|
| | Rat* | Man | Hamster | Ox |
| Asx | 8.8 (9) | 11 | 10 | 11 |
| Thr | 4.7 (5) | 5 | 7 | 5 |
| Ser | 6.4 (6) | 5 | 6 | 6 |
| Glx | 12.6 (13) | 12 | 12 | 12 |
| Pro | 1.1 (1) | 1 | 1 | 1 |
| Gly | 7.4 (7) | 6 | 6 | 5 |
| Ala | 7.4 (7) | 8 | 6 | 6 |
| Cys | — | — | — | — |
| Val | 3.8 (4) | 4 | 4 | 5 |
| Met | 1.1 (1) | 1 | 1 | 1 |
| Ile | 4.7 (5) | 6 | 6 | 4 |
| Leu | 6.9 (7) | 6 | 6 | 7 |
| Tyr | 2.0† (2) | 1 | 1 | 2 |
| Phe | 4.6 (5) | 6 | 6 | 6 |
| Lys | 4.8 (5) | 3 | 5 | 4 |
| His | 2.5 (3) | 3 | 3 | 3 |
| Trp | 2‡ (2) | 2 | 2 | 2 |
| Arg | 6.5 (7) | 8 | 7 | 9 |
| Total | 89 | 88 | 89 | 89 |

*Values for rat MPGF are means of analyses of three different protein preparations. Numbers in parentheses represent nearest integers.
†Based on the assumption of two tyrosine residues per molecule.
‡Calculated from [$^3$H]tryptophan-labeled MPGF.

part of the proglucagon molecule, the amino acid composition of MPGF isolated from rat islets was compared with that of the corresponding portions of human (4), hamster (3), and bovine (5) proglucagon (Table 2). Indeed, by its amino acid composition rat MPGF is almost identical to the presumed equivalent sequences of other proglucagons, while a few mismatches may be due to impurities or interspecies differences (Table 2).

Correct determination of the number of tryptophan residues in MPGF appeared to be crucial for its identification because of the consistent and exclusive presence of one tryptophan in each glucagon-related sequence (see Fig. 6). For this purpose, lysates of D and S islets that had been labeled for 2 hr with [$^3$H]tryptophan were electrophoresed on gels designed for optimal resolution of peptides (17) together

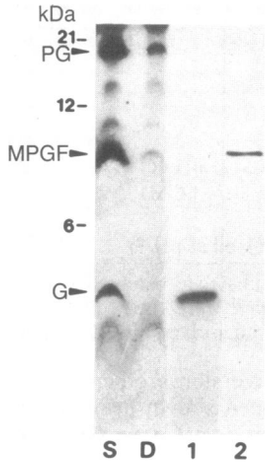


FIG. 5. High-resolution electrophoretic analysis of components of glucagon biosynthesis labeled with [$^3$H]tryptophan. One hundred each S and D islets were labeled and analyzed. Lane 1, $^{125}$I-labeled glucagon, autoradiograph; lane 2, isolated MPGF, protein stain. Abbreviations are as in Figs. 1 and 3.

with $^{125}$I-labeled glucagon and isolated MPGF for reference (Fig. 5). For subsequent fluorography, gels were rinsed for 20 min in 1 M Na salicylate (18) and then dried and exposed. The loss of glucagon by this procedure was negligible as estimated from recovery of $^{125}$I-labeled glucagon.

As shown in Fig. 5, the components of glucagon biosynthesis are the predominant tryptophan-containing proteins at the resolved molecular range. Excision and determination of the radioactivity of MPGF and glucagon bands from the gels of three different experiments gave mean values of 5368 cpm for MPGF and 2451 cpm for glucagon. Since the peptides would be expected to be formed at equimolar rates, these data confirm the presence of two tryptophan residues in MPGF as compared with one in the glucagon sequence, and they also indicate that MPGF is not processed to glucagon-like peptides to any measurable extent.

## DISCUSSION

The present data give a rather clear picture of proglucagon conversion in pancreatic islets. Since only one glucagon precursor has been detected *in vivo* as well as *in vitro*, the assumption of a close structural relationship between rat proglucagon and the evolutionarily well-preserved sequences of other mammalian proglucagons appears to be well justified. Thus, the conversion of the rat prohormone is suggested to result in glucagon, the $NH_2$-terminal peptide that is necessarily released and has already been isolated as glicentin-related pancreatic peptide from porcine pancreas (19), and

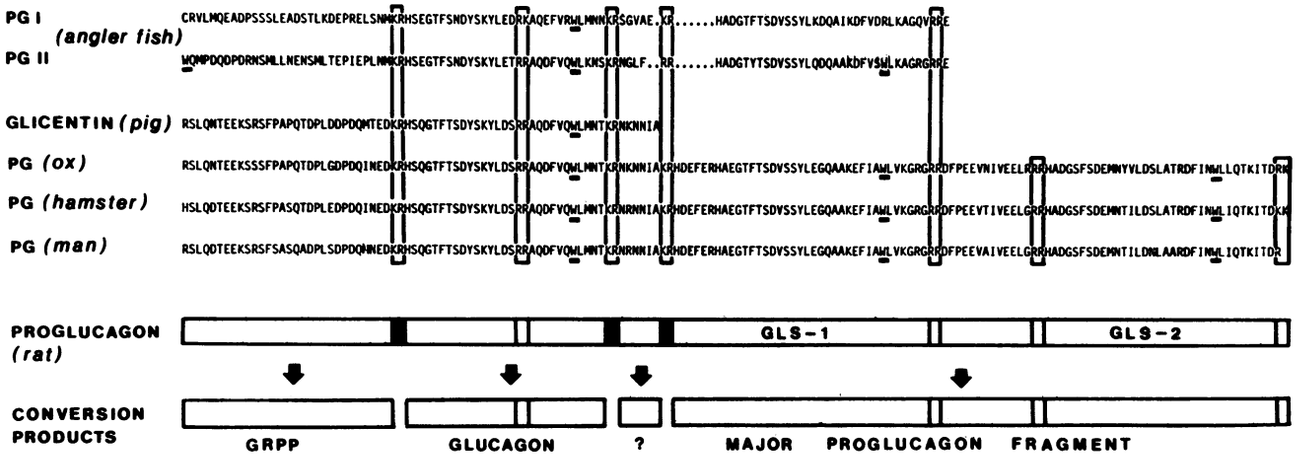


FIG. 6. Schematic presentation of structure and conversion of rat proglucagon. The suggested structure of rat proglucagon is aligned with the sequences of human (4), hamster (3), and bovine (5) proglucagon and porcine glicentin (6). In addition, the two proglucagon sequences from angler fish (20, 21) are presented. GRPP, glicentin-related pancreatic peptide (19); GLS-1 and -2, glucagon-like sequences. Pairs of basic amino acids as potential conversion sites (22) are boxed. Note the position of tryptophan residues (underlined) in glucagon and MPGF.

MPGF as the third conversion product. The fate of the hexapeptide generated in the proteolytic separation of glucagon and MPGF (Fig. 6) is unknown at present.

Immunological evidence has been presented for the presence of glicentin-related pancreatic peptide in secretory granules of glucagon-producing cells (23) and its secretion along with glucagon has been reported (24). Because of an overestimation of its size (16, 24), this peptide was originally thought to be structurally related to MPGF (1, 2). However, the data presented here allow for correct assignment of the arrangement of these sequences in proglucagon. Thus, by its size of 10 kDa, by its lack of glucagon (or glicentin) immunoreactivity and, most importantly, by its amino acid composition, MPGF is identified as the COOH-terminal portion of proglucagon comprising the two glucagon-like sequences (Fig. 6).

A proglucagon fragment consisting of the glucagon moiety with a COOH-terminal extension of eight amino acids has been isolated from pancreas (25) and evidence has been presented for still larger glucagon species as possible intermediates of proglucagon conversion (26). However, these intermediates appear not to accumulate sufficiently to be conspicuous as stained or labeled proteins on electrophoresis gels of fluorographs thereof.

The isolation procedure described here for rat pancreatic islets may be applicable to the enrichment of MPGF from total pancreas of larger animals as a more ample source of this protein. From such crude material, sufficient quantities of highly purified MPGF may be derived for studies of its physiological relevance. Evidence for the secretion of MPGF together with glucagon has been presented (2), and receptor studies will be necessary as the next step in establishing its role as a bioactive peptide. Those studies will also have to take into consideration a possible synergism of MPGF with its biosynthetic and secretory partners, especially glucagon, at the receptor or post-receptor level.

From analysis of the human genome, the presence of only one allelic sequence coding for glucagon has been reported (4). This observation supports the presumed generation of glucagon and glicentin from the same prohormone. For the regulation of a tissue-specific processing of proglucagon, resulting in glucagon in pancreatic islets, and in glicentin in the intestinal mucosa, modifications of the peptide chain of the precursor may be relevant. Such modifications may influence the conformation of the protein thereby changing the accessibility of potential recognition sites (22) (Fig. 6) for the converting enzyme(s).

The evidence for modification of rat proglucagon is twofold. First, the apparent difference in the size of preproglucagon and proglucagon does not account for the size of the signal peptide of 2–3 kDa (3–5). (For comparison, see the difference between preproinsulin and proinsulin in Fig. 1.) This discrepancy is equally evident in the proglucagon found by labeling of intact islets and for the prohormone generated *in vitro* by microsomal processing (Fig. 3*C*). This virtual increase of the size of proglucagon most likely represents an early modification occurring during or directly after translation. Second, an additional modification step resulting in a further virtual increase in size appears after 5–10 min in pulse–chase experiments (Fig. 1).

The prevailing modification of secretory proteins is glycosylation, resulting in *N*-glycosidic linkage of the oligosaccharide chain to an asparagine residue. However, the absence of a typical glycosylation site of the structure Asn-X-Thr (Ser) in all the proglucagons sequenced so far (Fig. 6) makes this type of modification unlikely. The true nature of this phenomenon may ultimately be clarified by the identification of modified amino acids in proglucagon conversion products. Also for this purpose, the elaboration of a procedure for the large scale isolation of MPGF from total pancreas appears to be necessary.

**Note Added in Proof.** After this article was submitted, the sequence of rat preproglucagon was made available to us (G. Heinrich and J. Habener, personal communication), confirming its close structural relationship to other mammalian preproglucagons. By radiosequencing of biosynthetically labeled islet proteins (7), we confirmed the positions of proline, threonine, and serine in sequence cycles 3, 6, and 12, respectively, for the $NH_2$-terminal portion of proglucagon. For MPGF, phenylalanine was found in positions 4 and 12 and alanine was found in position 8, in agreement with our structural assignment of this fragment.

The technical assistance of Mrs. Brigitte Weber, who also assisted in the preparation of the manuscript, and Ms. Birgit Sorg is gratefully acknowledged. This work was supported by the Deutsche Forschungsgemeinschaft.

1. Patzelt, C., Tager, H. S., Carroll, R. J. & Steiner, D. F. (1979) *Nature (London)* **282,** 260–266.
2. Patzelt, C. & Schug, G. (1981) *FEBS Lett.* **129,** 127–130.
3. Bell, G. I., Santerre, R. F. & Mullenbach, G. T. (1983) *Nature (London)* **302,** 716–718.
4. Bell, G. I., Sanchez-Pescador, R., Laybourn, P. J. & Najarian, R. C. (1983) *Nature (London)* **304,** 368–371.
5. Lopez, L. C., Frazier, M. L., Su, C.-J., Kumar, A. & Saunders, G. F. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5485–5489.
6. Thim, L. & Moody, A. J. (1981) *Regul. Pept.* **2,** 139–150.
7. Patzelt, C., Labrecque, A. D., Duguid, J. R., Carroll, R. J., Keim, P. S., Heinrickson, R. L. & Steiner, D. F. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1260–1264.
8. Patzelt, C., Tager, H. S., Carroll, R. J. & Steiner, D. F. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2410–2414.
9. Majzoub, J. A., Kronenberg, H. M., Potts, J. T., Jr., Rich, A. & Habener, J. F. (1979) *J. Biol. Chem.* **254,** 7449–7455.
10. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67,** 247–256.
11. Katz, F. N., Rothman, J. E., Lingappa, V. R., Blobel, G. & Lodish, H. F. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3278–3282.
12. Obata, K., Itoh, N., Okamoto, H., Yanaihara, C., Yanaihara, N. & Suzuki, T. (1981) *FEBS Lett.* **136,** 123–126.
13. Goodman, R. H., Lund, P. K., Jacobs, J. W. & Habener, J. F. (1980) *J. Biol. Chem.* **255,** 6549–6552.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
15. Baetens, D., Malaisse-Lagae, F., Perrelet, A. & Orci, L. (1979) *Science* **206,** 1323–1325.
16. Sundby, F., Jacobsen, H. & Moody, A. J. (1976) *Horm. Metab. Res.* **8,** 366–371.
17. Kyte, J. & Rodriguez, H. (1983) *Anal. Biochem.* **133,** 515–522.
18. Chamberlain, J. P. (1979) *Anal. Biochem.* **98,** 132–135.
19. Thim, L. & Moody, A. J. (1982) *Biochim. Biophys. Acta* **703,** 134–141.
20. Lund, P. K., Goodman, R. H., Dee, P. C. & Habener, J. F. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 345–349.
21. Lund, P. K., Goodman, R. H., Montminy, M. R., Dee, P. C. & Habener, J. F. (1983) *J. Biol. Chem.* **258,** 3280–3284.
22. Steiner, D. F., Quinn, P. S., Chan, S. J., Marsh, J. & Tager, H. S. (1980) *Ann. N.Y. Acad. Sci.* **343,** 1–16.
23. Ravazzola, M. & Orci, L. (1980) *Nature (London)* **284,** 66–67.
24. Moody, A. J., Holst, J. J., Thim, L. & Lindkaer-Jensen, S. (1981) *Nature (London)* **289,** 514–516.
25. Tager, H. S. & Steiner, D. F. (1973) *Proc. Natl. Acad. Sci. USA* **79,** 2321–2325.
26. Tager, H. S. & Markese, J. (1979) *J. Biol. Chem.* **254,** 2229–2233.