THE MAJOR PROGLUCAGON FRAGMENT: AN ABUNDANT ISLET PROTEIN AND SECRETORY PRODUCT

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Received 5 May 1981

1. Introduction

A protein of an estimated molecular mass (M_r) of 18 000 has been identified as proglucagon in biosynthetic studies on isolated rat pancreatic islets [1]. Moreover, a 10 000 M_r peptide was recognized as a major conversion product of this prohormone. The intracellular accumulation of this radioactively labeled fragment, which was found to be devoid of the glucagon sequence, was accompanied by that of newly synthesized glucagon [1].

Here, this major proglucagon fragment is identified as an abundant peptide in the Coomassie blue-stained electrophoretic patterns of rat islet proteins by the following criteria:

(i) Its regional distribution in the rat pancreas corresponds to the difference in glucagon biosynthesis in 'duodenal' and 'splenic' islets, respectively;
(ii) Its electrophoretic and isoelectric characteristics are identical to that of the major conversion product of [^{35}S]methionine-labeled proglucagon.

Moreover, secretion of the newly synthesized major fragment is found to be stimulated by arginine, its release following kinetics similar to that of labeled glucagon. These data strongly support the identification of the major proglucagon fragment as an end-product, besides glucagon itself, of prohormonal proteolytic conversion.

2. Methods

2.1. *Isolation of pancreatic islets and electrophoretic analysis of islet proteins*

Islets were isolated from inbred Wistar rats (200–300 g) by a modified collagenase procedure [2] (collagenase type V, Sigma). Incubation of isolated islets with [^{35}S]methionine, lysis of islets, sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) as well as fluorographic detection of labeled proteins have been described [3]. For two-dimensional analysis 100 islets were suspended in 40 μl lysis buffer [4] followed by repeated freezing and thawing and boiling for 3 min. Supernates of the centrifuged lysates (1000 × *g*, 5 min) were analyzed as in [4].

2.2. *Incubation of islets for secretion studies*

For prolonged incubation batches of 300–400 islets were incubated in 1.0 ml Hank's buffer equilibrated with 95% O_2/5% CO_2, 2.5 mM glucose, 1 mg/ml bovine serum albumin, amino acids and vitamins according to Eagle's minimum essential medium [5] and 10 mM L-arginine where indicated. Islets were stirred constantly by gentle agitation. Media were changed hourly after sedimenting islets at 400 × *g* for 30 s and removal of supernates. Islets were sedimented and lysed at the end of the incubation period.

2.3. *Quantitation of newly synthesized proteins*

For its determination in incubation media the major proglucagon fragment was concentrated by isoelectric precipitation at pH 5.0 after addition of HCl–ethanol extract [6] of 50 islets/medium sample as carrier. These precipitates and islet lysates, respectively, were subjected to SDS–PAGE. From fluorographed gels the labeled major proglucagon fragment was excised (fig.1c, ▷) and gel bits were dissolved and scintilation-counted [3].

For quantitating newly synthesized glucagon 20 μg synthetic glucagon (Serva), known to be

identical to rat glucagon [7], were added as carrier to islet lysates and medium samples, respectively. From media glucagon was then precipitated in 10% trichloroacetic acid. Trichloroacetic acid-precipitates and islet lysates were electrophoresed (SDS–PAGE) and gels were fixed in acetic acid/methanol (9.2%/45.4%) and dried. The lowermost peptide band (i-band, fig.1) containing small peptides like insulin chains and glucagon, was localized by juxtaposition of Coomassie blue-stained lanes and excised. Gel bits were soaked in lysis buffer [4], chopped into small pieces and placed on top of cylindrical gels for isoelectric focusing (IEF). After IEF and fixation in 12% trichloroacetic acid gels were stained with Coomassie blue. Heavily stained glucagon bands were excised to be then dissolved and scintillation-counted.

Losses of glucagon during this procedure were found to be negligible in contrast to its poor preservation in gels treated for fluorography [1].

3. Results and discussion

The demonstration of equimolar amounts of glucagon and the proglucagon fragment in pancreatic islets is so far hampered by the unavailability of a reliable method for quantitating this fragment. Nevertheless, an islet peptide equimolar to glucagon is expected to amount to a few micrograms and, thus, to form a well visible band in the Coomassie blue-stained electrophoretic pattern of proteins of 100 islets.

Indeed, a protein of estimated M_r 10 000 is conspicuous in stained electrophoretograms of islet lysates (fig.1a, ▷). Moreover, this protein is almost absent in the pattern of 'duodenal' islets (isolated from the lower head of the rat pancreas) which have been reported to contain only small amounts of glucagon as compared to 'splenic' islets (isolated from the other regions of the pancreas) [8] (fig.1a). Comparison of Coomassie blue-stained electrophoresis gels with their fluorographs of [^{35}S]methionine-labeled islet proteins allows for the exact alignment of the stained 10 000 M_r protein and the radioactively labeled major conversion product of proglucagon (fig.1b). Again this labeled protein is almost absent in 'duodenal' islets as well as the other easily discernible components of glucagon biosynthesis which are the two bands of proglucagon and a likely intermediate of prohormonal processing of M_r 13 000–

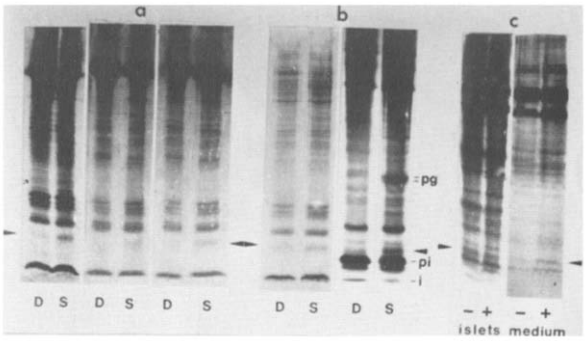

Fig.1. One-dimensional electrophoretic analysis of rat islet proteins: (a) Coomassie blue-stained SDS–PAG electrophoretograms of 100 'duodenal' (D) and 100 'splenic' (S) islets, respectively. Electrophoretograms of 3 individual experiments are shown. (b) Left: Coomassie blue-stained SDS–PAG electrophoretograms of 50 'duodenal' (D) and 50 'splenic' (S) islets, respectively, labeled for 2 h with 0.5 μCi [^{35}S]methionine/islet [1]. Right: Fluorograph of the same gel; pg, proglucagon (M_r 18 000); pi, proinsulin (M_r 9000); i, insulin chains (M_r 3000) (c) Left: Fluorograph of electrophoresed lysates of 100 randomized islets pulse-labeled for 1 h with 0.5 μCi [^{35}S]methionine/islet and chased for another 2 h period either in the absense (–) or in the presence (+) of 10 mM L-arginine. Right: Electrophoresed and fluorographed pH 5.0 precipitates from media of the respective chase incubations. (▷) major proglucagon fragment (M_r 10 000).

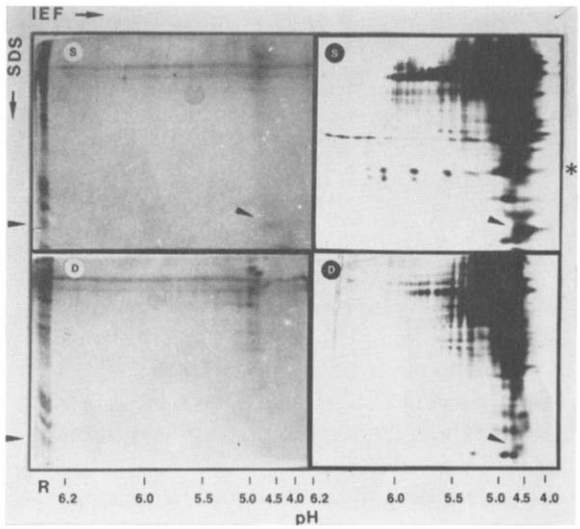

Fig.2. Two-dimensional electrophoretic analysis of proteins of 100 'duodenal' (D) and 100 'splenic' (S) islets, respectively, labeled for 2 h with 0.5 μCi [^{35}S]methionine/islet. For electrophoresis at the second dimension (SDS) lysates of 50 randomized islets were included as reference samples (R). Left: Coomassie blue-stained gels. Right: Fluorographs; (>) major proglucagon fragment; (*) electrophoretic position of proglucagon.

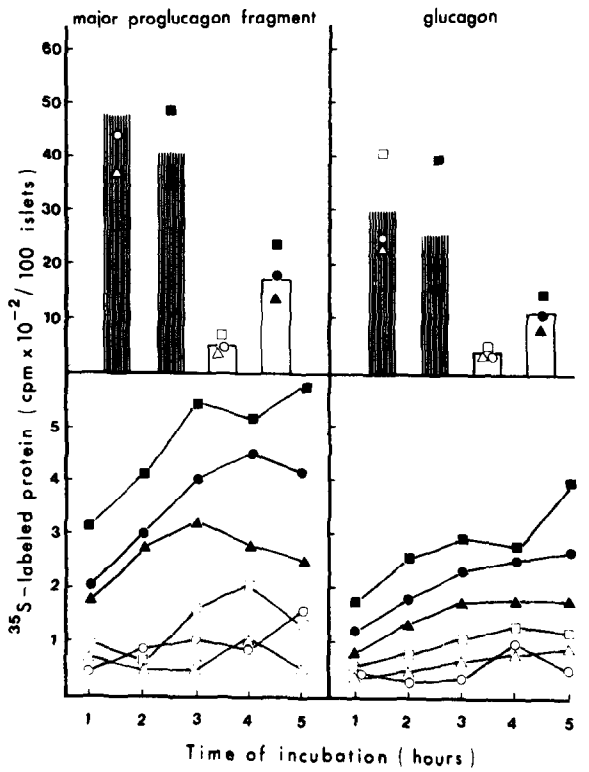

Fig.3. Effect of arginine on secretion of the major proglucagon fragment and of glucagon. Batches of 300–400 isolated islets were pulse-labeled for 1 h with 0.5 μCi [^{35}S]methionine/islet [1]. After washing islets in excess methionine chase incubations and subsequent determination of labeled proteins were performed as in section 2. Symbols refer to 3 individual experiments: open, chase incubations in the absence of additional arginine; closed, chase incubations with 10 mM L-arginine. Top: hatched columns, newly synthesized proteins in islets at the end of the chase period; light columns, labeled proteins released during the 5 h-chase period (sum of the data shown below). Bottom: time course of the release of the labeled proglucagon fragment and of glucagon, respectively.

14 000. Extended documentation on the identification of these and other precursors of islet hormones has been presented in [1,3,9].

The identity of the stained and the radioactively labeled 10 000 M_r protein is substantiated by two-dimensional electrophoretic analysis (fig.2). Both the stained and the radioactive peptide occupy the same position in the two-dimensional area corresponding to an isoelectric point of 4.6 if 'splenic' islet lysates are analyzed, whereas the corresponding spots on plates from 'duodenal' islets are almost unvisible (fig.2). Although other components of glucagon biosynthesis are hard to be discerned on two-dimensional fluorographs an interesting isoelectric heterogeneity of labeled material at the expected position of proglucagon can be observed (fig.2, *) which could be due to the so far unidentified post-translational modification of proglucagon [1].

Conversion products of proglucagon are supposed to be packed into secretory granules to be secreted together with glucagon upon application of an appropriate stimulus. For secretion studies batches of islets were pulse-labeled with [^{35}S]methionine followed by chase periods up to 5 h in the presence of arginine as a secretory stimulus. As shown in fig.1c and 3 the secretion of both glucagon and the major proglucagon fragment is stimulated by 10 mM arginine their release following similar kinetics and reaching a maximum after 3–4 h of incubation (fig.3, bottom). Determination of these labeled proteins in lysates of islets at the end of secretion experiments gave similar ratios of intracellular to released material for both peptides (fig.3, top). Since there is almost twice as much radioactivity found in the 10 000 M_r protein as compared to glucagon it appears that the major fragment contains 2 methionine residues over 1 residue in rat glucagon [7].

The abundance of the major proglucagon fragment in pancreatic islets and its secretion upon stimulation by arginine indicate that this fragment is truely an endproduct of proglucagon conversion. The physiological significance of this fragment is so far completely unknown. A structural relationship between pancreatic proglucagon and glicentin, a larger form (M_r 12 000) of glucagon isolated from intestinal mucosa [10], has been suggested [1,10]. Accordingly, the major proglucagon fragment may partly be homologous to that portion of glycentin unrelated to the glucagon sequence.

From immunohistochemical studies on pancreatic islets the presence of glycentin-related immunoreactivity in secretory granules of glucagon-producing A-cells has been reported [11]. Moreover, such immunoreactive material was found to be secreted together with glucagon from the perfused pancreas [12]. However, this released material differed from glycentin itself by its smaller size, its more acidic isoelectric point of ~4, as well as by its lack of glucagon-immunoreactivity [12]. These findings agree with the present data. It therefore appears that the major proglucagon fragment represents the glycentin-related material released from pancreatic islets.

Acknowledgements

C. P. is a fellow of the Heisenberg-program of the Deutsche Forschungsgemeinschaft. These studies were supported by grant Pa 189/3 from the same institution.

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