Biosynthesis of rat liver pI-6.1 esterase, a carboxylesterase of the cisternal space of the endoplasmic reticulum

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Biosynthesis of the rat liver microsomal esterase with pI 6.1 was investigated in cell-free systems and in cultured hepatocytes, by using a rabbit antiserum. Protein synthesis directed by total rat liver RNA in wheat-germ extract or reticulocyte lysate generated a single immunoprecipitable product, also found with the RNA extracted from bound, but not from free, polysomes. When dog pancreas microsomal fractions were included, reticulocyte lysates gave two processed products, a prominent one slightly larger, and another slightly smaller, than the precursor, both resistant to exogenous proteinases and, hence, segregated within vesicles. The processing was co-translational; it consisted of the removal of a peptide fragment and, for the large component, the addition of a single oligosaccharide chain. Indeed, this component bound to concanavalin A–Sepharose and gave the small one ($\sim 2000 M_r$ loss) by cleavage with endo- β -N-acetylglucosaminidase H (endo-H). A single labelled peptide was precipitated from hepatocytes incubated with [35 S]methionine. Its apparent M_r was decreased by ~ 2000 after treatment with endo-H; it was then identical with that of an unglycosylated form produced in hepatocytes poisoned with tunicamycin. Even in that case, immunoreactive peptides were not detected in the culture medium. Whether synthesized in reticulocyte lysate or in hepatocytes, the glycosylated forms migrated in SDS/polyacrylamide-gel electrophoresis as the purified enzyme labelled with [3 H]di-isopropyl fluorophosphate. Thus, although pI-6.1 esterase is not secreted, its biosynthesis is, as yet, indistinguishable from that of secretory proteins. Its oligosaccharide moiety is apparently not the structural element that retains it in the endoplasmic reticulum.

INTRODUCTION

Previous studies on the biogenesis of the endoplasmic reticulum (ER) have been mainly focused on integral proteins of the membrane, including amphipathic proteins which expose a functional domain at the cytoplasmic surface. Cytochrome b_5 and NADH–cytochrome b_5 reductase, which are proteins anchored to the phospholipid bilayer by their C-terminal end, are assembled on free polysomes and inserted post-translationally into the ER membrane (Rachubinski *et al.*, 1980; Borgese & Gaetani, 1980, 1983). NADPH–cytochrome P-450 reductase, another amphipathic protein of the cytoplasmic aspect, is anchored via its N-terminal end, synthesized on bound polysomes and co-translationally inserted without proteolytic cleavage (Okada *et al.*, 1982). Cytochrome P-450 and epoxide hydrolase are similarly synthesized at their mature size on bound polysomes and co-translationally inserted (Okada *et al.*, 1982; Bar-Nun *et al.*, 1980; Gonzalez & Kasper, 1980). The two trans-membrane glycoproteins ribophorin I and II are made on bound polysomes as a precursor that is inserted, proteolytically processed and glycosylated during elongation (Rosenfeld *et al.*, 1984). Much less was known about the biosynthesis of enzymes acting at the luminal side of the ER membrane. This prompted us to investigate the biosynthesis of esterases (carboxylesterases, EC 3.1.1.1).

Previous sub-fractionation studies by Beaufay *et al.*

(1974b) and Amar-Costesec *et al.* (1974) revealed that the microsomal esterases are associated with the ER. They are located at the luminal side (Robbi & Beaufay, 1983), possibly in a form loosely bound to the membrane. All migrate in SDS/polyacrylamide gels as peptides of about 60 kDa. They are monomers, except the pI-6.1 enzyme, which is a trimer (Mentlein *et al.*, 1980; Robbi & Beaufay, 1983). We have reported previously that the esterases focusing at pH 5.0 are synthesized in a manner highly reminiscent of that of most secretory proteins (Robbi & Beaufay, 1986), except for the relatively large difference in M_r (~ 6000) resulting from the proteolytic processing of the precursor polypeptide. To investigate whether these results could be extended to other esterases, we also studied the biosynthesis of the pI-6.1 enzyme, which is a major protein of the microsomal fraction, much more abundant than each of the other microsomal esterases. This enzyme, identified as ES10A (Mentlein *et al.*, 1987), is genetically distinct from the other microsomal esterases (Simon *et al.*, 1985). Although its precise physiological function is still uncertain, it is active on several natural and artificial substrates, including monoacylglycerols of medium chain length, clofibrate, procaine and butanilcaine (Mentlein *et al.*, 1984a; Mentlein & Heymann, 1984). Hence, this esterase is most probably involved in detoxication of xenobiotics and presumably also in the metabolism of natural substrates.

In the present paper we show that the biosynthesis of

the pI-6.1 esterase involves the assembly, on bound polysomes, of a prepolyptide that is co-translationally translocated into microsomal vesicles, and converted into a mature, proteolytically processed and glycosylated molecule. The *N*-linked oligosaccharide chain is not involved in the retention of the enzyme in the ER. A preliminary report of this work has been published in abstract form (Robbi & Beaufay, 1985).

EXPERIMENTAL

Materials

Adult male Wistar rats, non-inbred strain Han:Wist (180–220 g) were purchased from Proefdierencentrum, Heverlee, Belgium; untoasted wheat germ was kindly provided by General Mills, Vallejo, CA, U.S.A.; carrier-free Na¹²⁵I was from The Radiochemical Centre, Amersham, Bucks., U.K. Other reagents were as described by Robbi & Beaufay (1986).

Purification of the pI-6.1 esterase

The esterases were isolated from rat liver microsomes (microsomal fractions) as described previously, up to the preparative isoelectric-focusing step (Robbi & Beaufay, 1983). The fractions focusing around pH 6.1 were then applied to a DEAE Protein-Pak column (Waters DEAE 5 PW; 7.5 mm × 75 mm) equilibrated with 5 mM-Tris/HCl, pH 7.5. The column was washed for 15 min with this buffer, before elution with a 40 min linear gradient of 0–0.3 M-NaCl in the buffer, at a flow rate of 1 ml/min in a Waters h.p.l.c. apparatus. The fractions containing pI-6.1 esterase activity were further purified by gel-filtration on a Superose 12 column (1.6 cm × 70 cm) equilibrated with 20 mM-Tris/HCl (pH 7.4)/0.15 M-NaCl, at a flow rate of 12 ml/h at 4 °C. Throughout the purification, esterase activity was monitored with *o*-nitrophenyl acetate as substrate (Beaufay *et al.*, 1974a), and the various esterase isoenzymes were revealed by PAGE followed by histochemical staining of the gel with α -naphthyl acetate and Fast Blue RR (Ljungquist & Augustinsson, 1971).

Antibodies

The purified enzyme preparation was used to generate antibodies in a 1.5 kg female rabbit (Termonde White strain). The antigen (100 μ g of protein in 0.5 ml of 10 mM-potassium phosphate buffer (pH 7.4)/0.15 M-NaCl, emulsified with an equal volume of complete Freund's adjuvant) was first injected in the popliteal lymph nodes of both hind legs (Sigel *et al.*, 1983) and subcutaneously at several spots along the back. Then 1 month later, a booster injection (80 μ g of protein in Freund's complete adjuvant) was given subcutaneously at several sites in the back, followed the next day by an intravenous injection (50 μ g of protein in the phosphate-buffered saline); 10 days later, blood was removed from the ear vein. The serum obtained from this initial bleeding was used throughout this work. Unless otherwise stated, experiments were performed with the antiserum passed over a column of rat serum proteins linked to Sepharose 4B equilibrated in 10 mM-potassium phosphate buffer (pH 7.4)/0.15 M-NaCl.

Immunological procedures

Crossed immunoelectrophoresis was performed in an LKB Multiphor flat-bed system at 4 °C. Running buffer

was 25 mM-diethylbarbituric acid/75 mM-Tris/0.5 mM-calcium lactate/2 mM-Na₃N₃, pH 8.6. The sample proteins, adjusted to pH 8.6, were first resolved in a 1% agarose gel at 300 V for 100 min, and then driven electrophoretically at right angles, for 16 h at 50 V, into a second 1% agarose gel containing the antiserum (80 μ l in 12 ml of gel solution). After the second run, the gel was blotted between filter papers, washed in 0.1 M-NaCl at room temperature for 3 h, dried, and stained for esterase activity, or with Coomassie Blue. Immunoblotting was as described by Towbin *et al.* (1979).

Biosynthetic labelling in cultured hepatocytes

Confluent monolayers of hepatocytes were prepared as described by Limet *et al.* (1980) from male Wistar rats (180–220 g). The cells (3.3×10^6 cells/dish) were incubated successively, in the presence or in the absence of tunicamycin (2 μ g/ml), for 1 h in RPMI-1640 medium, for 1 h in methionine-free RPMI-1640 medium and finally for 4–18 h in 3 ml of methionine-free RPMI-1640 medium which also contained 10% (v/v) dialysed horse serum and 30 μ Ci of [³⁵S]methionine (≥ 1000 Ci/mmol). The culture media were removed by suction and centrifuged for 10 min at 2000 rev./min (Beckman JS-4.2 rotor) and then for 30 min at 39000 rev./min (rotor 40). The supernatants were supplemented with 200 KIU of Trasylol/ml, 0.25 mM-phenylmethanesulphonyl fluoride and 0.2% Triton X-100 and kept at 4 °C. The cells were rinsed twice with ice-cold 10 mM-phosphate buffer (pH 7.4)/0.15 M-NaCl and once with 0.25 M-sucrose containing 3 mM-NaHCO₃, pH 7.4, and 1 mM-EGTA (medium A), scraped off the Petri dish with 1 ml of medium A and fractionated as described by Darte & Beaufay (1983). Esterases were extracted from the microsomes by resuspension (Dounce homogenizer, ten passes of tight-fitting pestle) in a solution containing 0.2% Triton X-100, 10 mM-phosphate buffer, pH 7.4, 200 KIU of Trasylol/ml and 0.25 mM-phenylmethanesulphonyl fluoride (0.3 ml/dish) and centrifugation (1 h at 38000 rev./min; Beckman Ti 60 rotor). Components related to pI-6.1 esterase were then immunoprecipitated from these different microsomal extracts and culture media.

Other techniques

Preparation of cell-free systems for protein synthesis, extraction and translation of rat liver RNA *in vitro*, immunoprecipitation of polypeptides synthesized *in vitro* or *in vivo*, PAGE with or without SDS and fluorography were all performed as described by Robbi & Beaufay (1986). Silver-staining of gels was as described by Merrill (1981).

RESULTS AND DISCUSSION

A purified pI-6.1 esterase preparation, homogeneous after SDS/PAGE and silver-staining (Fig. 1a), was used to generate antibodies in a rabbit. After it had been passed over a column of rat serum proteins bound to Sepharose 4B, the antiserum was analysed by crossed immunoelectrophoresis against the material extracted from liver microsomes and focusing near pH 6.1. A single strong immunoprecipitation line was observed on staining for esterase activity with α -naphthyl acetate (Fig. 1b). The pattern was unchanged when the gel was subsequently stained for protein with Coomassie Blue (results not shown).

Fig. 1. Analysis of the purified pI-6.1 esterase (a) and of the corresponding rabbit antiserum (b)

In (a) the polypeptides were resolved by SDS/PAGE and revealed by silver staining: lane 1, M_r markers; lane 2, chromatographically purified pI-6.1 esterase (1.5 μ g). Panel (b) shows crossed immunoelectrophoresis of the antiserum against microsomal proteins. The well in the lower left corner was loaded with the crude material extracted from liver microsomes with Triton X-100 (0.2%) and isoelectrically focused around pH 6.1. Proteins were resolved in the first dimension by electrophoresis in agarose gels (Tris/barbiturate buffer, pH 8.6; see the Experimental section) and subsequently driven electrophoretically in the second dimension through agarose gels which contained the rabbit antiserum preadsorbed on rat serum proteins linked to Sepharose 4B (see the Experimental section). The gel was stained for esterase activity with α -naphthyl acetate as substrate and Fast Blue RR. The same pattern was revealed after re-staining with Coomassie Brilliant Blue R250.

Immunoblots further substantiate the specificity of the antiserum. Duplicate samples of the proteins extracted from microsomes with Triton X-100 were subjected to PAGE. Half of the gel was stained for esterase activity (Fig. 2a, lane 1). The components of the other sample were blotted on nitrocellulose. The autoradiogram of the immunostained blot (lane 2) showed a single band at the position of the pI-6.1 esterase on the enzymically stained gel (lane 1). Fig. 2(b) is an immunostained blot of an SDS/polyacrylamide gel loaded with rat liver microsomes which were quickly boiled in sample buffer for SDS/PAGE (lane 1), and with the purified pI-6.1 esterase preparation (lane 2). A single, apparently identical, polypeptide is revealed in each case.

Our antisera against the pI-5.0 esterases (Robbi & Beaufay, 1983) and against the pI-6.5 esterases (M. Robbi & H. Beaufay, unpublished work) do not react with the pI-6.1 enzyme, and none reacts with the pI-5.5 esterases (results not shown). It thus appears, in agreement with Mentlein *et al.* (1984b), that these liver esterases are largely unrelated in their immune properties. The antigenic specificity of the pI-6.1 esterase regarding other liver esterases also agrees with the genetic data, since this enzyme is encoded by a gene different from those that code for the other major rat liver carboxyl-

Fig. 2. Analysis of the antiserum by immunoblotting

A crude Triton X-100 (0.2%) extract from rat liver microsomes (100 μ g of microsomal protein) was subjected to non-denaturing PAGE (panel a). In lane 1, the gel was stained for esterase activity (see Fig. 1); in lane 2 the components were transferred to nitrocellulose, which was subsequently incubated with the rabbit antiserum (diluted 1:50) and with ¹²⁵I-labelled Protein A for autoradiography. Liver microsomal proteins were also resolved by SDS/PAGE on a 9%-acrylamide gel (panel b) before transfer to nitrocellulose and immunochemical staining. Lane 1, total microsomal protein (100 μ g); lane 2, purified esterase preparation (2.5 μ g).

esterases (Simon *et al.*, 1985). In addition, immunoreplicas indicate that the antigen did not suffer any perceptible proteolytic degradation during its purification.

After translation of the unfractionated rat liver RNA in a wheat-germ extract in the presence of [³⁵S]-methionine (Fig. 3), a single labelled polypeptide was precipitated by the antiserum (lane 1). The precipitation of this component was competed out by addition of the purified pI-6.1 esterase (lane 2). Its synthesis was also revealed in the wheat-germ extract programmed by RNA extracted from bound polysomes (lane 4), but not when RNA extracted from free polysomes was used (lane 3). This esterase-related product was not precipitated by the pre-immune serum (lanes 5–7). Precipitation of prealbumin and catalase from translations with the same RNA preparations by specific antisera showed the presence of one or another product, depending on whether the RNA included in the translation was derived from bound or from free polysomes (Robbi & Beaufay, 1986). Thus both RNA preparations were active, and contained distinct mRNA species.

Total rat liver RNA was also translated in a nuclease-treated reticulocyte lysate, with or without addition of rough microsomes obtained from dog pancreas and stripped by treatment with EDTA (10 mM; 50 A_{280} units of rough microsomes/ml) (Fig. 4). In the absence of microsomes, a single prominent product was identified by the antiserum (lane 1). Its electrophoretic mobility

Fig. 3. Synthesis of the pI-6.1 esterase in a wheat-germ extract

Rat liver RNA extracted from total (T), free (F) and bound (B) polysomes were translated in wheat-germ extract in the presence of [35 S]methionine (1 mCi/ml; 1200 Ci/mmol). Translation products were precipitated by the antiserum (lanes 1–4). They were also made to react with pre-immune serum (lanes 5–7). In lane 2 the products were diluted with 50 μ g of purified pI-6.1 esterase. The immunoprecipitates were analysed by SDS/PAGE and fluorography (exposure for 24 h at -80°C to Kodak X-Omat AR-5).

was not perceptibly different from that of the component recovered from the translations made in a wheat-germ extract (Fig. 3, lane 1). In the presence of microsomes, two new forms became prominent in the immunoprecipitate. Their apparent M_r values were slightly larger and smaller, respectively, than that of the primary product (lane 3). These processed polypeptides were segregated in the lumen of the microsomal vesicles. Indeed, whereas the precursor was completely degraded upon post-translational incubation with trypsin and chymotrypsin (lane 2), the processed forms were largely resistant to the proteolytic enzymes (lane 4), unless the microsomal vesicles were disrupted by addition of Triton X-100 (lane 5). Post-translational incubation with dog pancreas microsomes did not alter the size of the preformed precursor (lane 7), nor its susceptibility to proteinases (lane 8), indicating that the processing and segregation were co-translational processes.

The authentic enzyme labelled *in vitro* with [^3H]di-isopropyl fluorophosphate co-migrates with the major segregated and processed form produced in the reticulocyte lysate supplemented with dog pancreas microsomes (cf. lanes 4 and 6). The apparent M_r , derived from the mobility in SDS/PAGE, is 59000 for the precursor polypeptide, 58000 and 60000 for the processed forms, and 60000 for the subunit of the authentic enzyme labelled with [^3H]di-isopropyl fluorophosphate. The M_r -58000 processed form was not observed in all experiments.

Fig. 4. Translocation and processing of the precursor form of pI-6.1 esterase by dog pancreas microsomes

The RNA extracted from total rat liver was translated in reticulocyte lysate by incubation for 2 h at 26°C , in the presence or absence of rough microsomes derived from dog pancreas and treated by EDTA. Esterase-related products were immunoprecipitated, analysed by SDS/PAGE and revealed by fluorography. Lanes 1 and 2, translation in the absence of microsomes; lanes 3–5, translation in the presence of microsomes. The material in lanes 2 and 4 was incubated with trypsin and chymotrypsin before immunoprecipitation; the material in lane 5 was similarly proteolysed in the presence of 1% Triton X-100. The well in lane 6 was loaded with pI-6.1 esterase labelled with [^3H]di-isopropyl fluorophosphate. Lanes 7 and 8 were as lanes 1 and 2, respectively, except that the products were post-translationally incubated with microsomes for 2 h at 26°C . Each sample was derived from 100 μl of the translation medium.

The microsomal pI-6.1 esterase is a glycoprotein of the high-mannose type (Robbi & Beaufay, 1983). It is therefore likely that the processing observed when the liver RNAs were translated in the presence of microsomal vesicles consists, at least in part, of the addition of one or several oligosaccharide chains. Therefore the capacity of the various translation products to bind concanavalin A was examined (Fig. 5a). As expected, the precursor synthesized in the absence of microsomes showed no affinity for the lectin (cf. lanes 1 and 3). In contrast, the slower form synthesized in the presence of microsomes was efficiently removed (cf. lanes 2, 4 and 7), except when the lectin was pre-loaded with α -methyl mannoside (lanes 5 and 8).

The effect of incubation with endo-H, an enzyme which cleaves the oligosaccharides of the high-mannose-type glycoproteins between the two *N*-acetylglucosamine residues (Tarentino & Maley, 1974), is shown in Fig.

Fig. 5. Evidence for the glycosylation of the processed translation product

Translations were carried out, as described in Fig. 4, in the absence (*a*, lanes 1, 3 and 6; *b*, lanes 1 and 2) or in the presence of dog pancreas microsomes (other lanes). In the latter case the products were incubated with trypsin and chymotrypsin, to remove the non-segregated polypeptides. In the experiment shown in (*a*), the esterase-related polypeptides were immunoprecipitated for analysis by SDS/PAGE and fluorography, either directly (lanes 1 and 2), or after reaction with concanavalin A (lanes 3–5) under the following conditions. The samples were diluted 7.5-fold with the equilibration medium for concanavalin A (10 mM-Tris/HCl, pH 7.5, 0.15 M-NaCl, 1 mM-CaCl₂, 1 mM-MnCl₂, 1 % Triton X-100, 5 mM-methionine, 0.1 % serum albumin) containing no (lanes 3 and 4), or 0.2 M- α -methyl mannoside (α -MMan) (lane 5), and agitated for 3 h at room temperature with 75 μ l of packed concanavalin A-Sepharose beads. The beads were separated by centrifugation, and the esterase-related products recovered by immunoprecipitation from the supernatants (lanes 3–5), and from the pellets after elution in 2 % SDS at 100 °C (lanes 6–8). In the experiment shown in (*b*), the total immunoprecipitates were incubated for 16 h at 37 °C with (lanes 2 and 4) or without (lanes 1 and 3) endo-H before SDS/PAGE and fluorography.

5(*b*). The position of the unglycosylated polypeptide precursor synthesized in the absence of membranes was not changed after endo-H treatment (lanes 1 and 2), as expected; that of the segregated and glycosylated form synthesized in the presence of microsomes was shifted to a lower M_r (lanes 3 and 4). The apparent decrease in M_r (~ 2000) resulting from the endo-H treatment of this component is consistent with the presence of a single *N*-linked oligosaccharide chain. It corresponds to the difference in M_r between the two processed products (Fig. 4, lane 4). Thus the fast esterase-related product probably represents a segregated, unglycosylated, form.

Fig. 6. Biosynthetic labelling of pI-6.1 esterase in cultured hepatocytes

Hepatocytes cultivated in monolayers were incubated in methionine-free RPMI 1640 medium in the presence or in the absence of tunicamycin and subsequently in the presence of [³⁵S]methionine for 4 h (see the Experimental section). The culture medium was supplemented with proteinase inhibitors (200 KIU of Trasylol/ml and 0.25 mM-phenylmethanesulphonyl fluoride) and Triton X-100 (0.2%) and kept at 4 °C. The cells were homogenized and fractionated to give the microsomal fraction, which was extracted with Triton X-100 (0.2%). All samples were then simultaneously precipitated with the antiserum. Lanes: 1, purified rat liver pI-6.1 esterase labelled with [³H]di-isopropyl fluorophosphate; 2, immunoprecipitate of microsomal extract; 3, as in lane 2, but microsomal extract was diluted with a competing amount of purified esterase (20 μ g); lanes 4 and 5, as in lanes 2 and 3 respectively, except that microsomal extracts were derived from cells cultivated in the presence of tunicamycin (TM); lanes 6 and 7, immunoprecipitates from culture media of the cells cultivated without and with tunicamycin respectively; lanes 8 and 9, as in lane 2, but immunoprecipitated products were incubated for 16 h at 37 °C in the absence (lane 8) or in the presence (lane 9) of endo-H.

As it migrates also slightly faster than the precursor synthesized in the absence of microsomes, it is presumably a proteolytically processed form of the precursor. Obviously this presumption holds for the glycosylated product.

Further evidence that the pI-6.1 esterase is a glycoprotein came from labelling studies *in vivo*. Monolayers of hepatocytes were labelled for 4 h with [³⁵S]methionine in the absence or in the presence of tunicamycin, as described in the Experimental section. The pI-6.1-esterase-related products were immunoprecipitated from the microsomal extracts and from the culture media, and analysed by SDS/PAGE and fluorography (Fig. 6). The microsomal extract from control cells shows a single immunoprecipitable product (lanes 2 and 8), which co-

migrates with the M_r -60000 subunit of the mature enzyme isolated from rat liver microsomes and labelled with [3 H]di-isopropyl fluorophosphate (lane 1), and is competed out by the authentic enzyme (lane 3). This biosynthetically labelled product is converted into a component migrating slightly faster on incubation with endo-H (cf. lanes 8 and 9). Since the product remains sensitive to endo-H even after an 18 h labelling period (results not shown), it seems that the mature enzyme does not move through the Golgi complex. Indeed, most asparagine-linked oligosaccharide chains that move in or through the Golgi complex become fully resistant to endo-H (Rothman, 1981). A fast-migrating, presumably unglycosylated, pI-6.1 esterase was synthesized in the cells poisoned with tunicamycin (cf. lanes 4 and 5 with lanes 2 and 3). The M_r of the polypeptide synthesized in poisoned cells (lane 4) and that of the glycopeptide deglycosylated by endo-H treatment (lane 9) appear identical. The difference in M_r between these forms and the glycosylated product (~ 2000) again indicates that a single core-oligosaccharide is present in the glycoprotein. At variance with the behaviour expected for a secretory product, components related to this enzyme were not detected in the culture medium (lane 6); nor were they detected when the cells were poisoned with tunicamycin (lane 7). It seems therefore that addition of the oligosaccharide to the polypeptide chain is not a prerequisite for the retention of this enzyme in the ER.

Hepatocytes synthesize many serum proteins, and Mentlein & Heymann (1984) have reported that their antiserum against the liver pI-6.1 esterase cross-reacts with an esterase of the rat serum. We made the antiserum react with rat serum proteins bound to Sepharose 4B, and this step did not remove the antibodies directed against the pI-6.1 enzyme. Thus we consider that there is at present no evidence supporting the concept that the liver enzyme is a secretory product. The finding that it cannot be detected in the culture medium of hepatocytes, even after 4 h, also argues against that concept. This argument, however, is not compelling, for some proteins are secreted very slowly (Strous & Berger, 1982), the movement from the ER to the Golgi complex being often the limiting step (Choi & Hynes, 1979; Lodish *et al.*, 1983).

While this manuscript was in preparation, Munro & Pelham (1987) reported that the C-terminal tetrapeptide Lys-Asp-Glu-Leu, shared by three proteins located in the lumen of the ER (glucose-regulated protein 78 and 94 and protein disulphide-isomerase), is necessary to retain glucose-regulated protein 78 in the ER and sufficient to convert lysozyme into a derivative that does not exit from the ER. This is apparently not the case for the pI-6.1 enzyme, the C-terminal sequence of which is Ala-Val-Leu (Mentlein *et al.*, 1980). Thus the C-terminal sequence Lys-Asp-Glu-Leu may not be a general retention signal present in all intraluminal proteins.

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