Occurrence of an HIV-1 gp160 endoproteolytic activity in low-density vesicles and evidence for a distinct density distribution from endogenously expressed furin and PC7/LPC convertases

Sandrine Wouters[a,*], Etienne Decroly[a], Michel Vandenbranden[a], Daniela Shober[b], Renate Fuchs[b], Valérie Morel[c], Michèle Leruth[c], Nabil G. Seidah[d], Pierre J. Courtoy[c,1], Jean-Marie Ruysschaert[a,1]

[a] *Laboratoire de Chimie Physique des Macromolécules aux Interfaces (LCPMI), CP206/2, Université Libre de Bruxelles, Boulevard du Triomphe, B-1050 Brussels, Belgium*
[b] *Dept. of General and Experimental Pathology, University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria*
[c] *Unité de Biologie Cellulaire, Université Catholique de Louvain Medical School and Christian de Duve Institute of Cellular Pathology, 75, Avenue Hippocrate, B-1200 Brussels, Belgium*
[d] *Clinical Research Institute of Montreal, 110 Pine Ave West, Montreal Que. H2W 1R7, Canada*

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Abstract Human immunodeficiency virus (HIV) glycoprotein (gp) 160 processing by host cell proteinases is an essential step for viral fusion and infectivity. We have identified a rat liver subcellular fraction which specifically processes gp160 into gp120 and gp41. Using equilibration of microsomes in sucrose gradients, the gp160 cleavage activity was associated with particles equilibrating at low densities, well-separated from the endoplasmic reticulum, *cis*-Golgi network, Golgi stacks, lysosomes and plasma membrane. Its density distribution was compatible with light secretory vesicles derived from the *trans*-Golgi network (TGN) or to endosomes, but association with endosomes was not supported by free flow electrophoresis. Although furin and pro-protein convertase (PC) 7/LPC have been proposed as the major gp160 processing convertases, the rat liver microsomal gp160 processing activity was essentially resolved from furin and only partially overlapped PC7/LPC. These data suggest that proteinase(s) other than furin and PC7/LPC, presumably located in TGN-derived vesicles, may participate in the gp160 processing into gp120 and gp41.

Key words: Glycoprotein 160; Cleavage; Convertase; Low-density vesicle

1. Introduction

Critical steps in human immunodeficiency virus (HIV) infection include binding and fusion with CD4-positive cells. Fusion between the virus and host cell membranes involves both viral envelope glycoproteins gp120 and gp41 [1]. These HIV-1 envelope glycoproteins are synthesized as an inactive precursor gp160 that is cleaved at the conserved sequence R-E-K-R↓A-V during its transport through the host cell secretory pathway. Correct endoproteolytic maturation of gp160 is essential for the fusion process, hence for viral infectivity. The cleavage capacity has been tentatively assigned to the *cis*/medial cisternae of the Golgi complex [2,3] or to secretory vesicles [4,5]. Clear-cut genetic evidence of the participation of any endoprotease to gp160 processing in vivo is lacking.

The family of kexin/subtilisin-like serine proteinases is implicated in the endoproteolytic maturation of protein precursors at sequences (R-X-K/R-R↓), similar to that found in the classical cleavage site of gp160, located at the carboxy-terminal of Arg_{511}. Members of this family, also known as pro-protein convertases (PCs), include furin, PC1/3, PC2, paired basic amino acids converting enzyme (PACE) 4, PC4, PC5/6 and PC7/LPC [6,7]. Two such convertases, furin and PC7/LPC, have been suggested as the best candidates for gp160 processing in infected lymphocytes [8].

Furin was first shown to be able to cleave gp160 into gp120/gp41 using the vaccinia virus overexpression system [9], but recent studies have implicated other members of the mammalian PC family in this process. Specific cleavage of gp160 has been demonstrated by in vitro assays with furin, PC1/3, PACE4 and PC5/6 and in co-expression studies for furin, PACE4, PC5/6 and PC7/LPC [8,10–12]. However, involvement of a convertase in the maturation process of a given substrate requires its co-localization with the substrate in the subcellular compartment where cleavage normally takes place. Since overexpression used in the afore-mentioned studies may saturate retention/retrieval mechanisms and thereby alter intracellular protein targeting [13], a definitive proof is still lacking that these convertases normally co-localize with gp160 where it is cleaved. Therefore, alternative approaches are warranted.

In addition, the variability of tissue-specific expression of convertases, in conjunction with HIV's ability to infect several non-lymphocytic cells (dendritic cells, chromaffin cells, hepatocytes and glial cells: [14]), suggests that the virus may be cleaved by various PCs, depending on the infected cell type. Alternatively, the isolation from lymphocytes of viral envelope glycoprotein maturase (VEM), a non-subtilisin-like protease able to cleave gp160 in vitro [15], suggested other enzymes for gp160 processing. However, VEM has not yet been

*Corresponding author. Fax: (32) (2) 650 53 82.
E-mail: swouters@ulb.ac

[1] The two last authors contributed with equal responsibility to this work.

Abbreviations: HIV, human immunodeficiency virus; gp, glycoprotein; PACE, paired basic amino acids converting enzyme; PC, pro-protein convertase; VV, vaccinia virus; TGN, *trans*-Golgi network; RER, rough endoplasmic reticulum; FFE, free flow electrophoresis; α_2M, α_2-macroglobulin; VEM, viral envelope glycoprotein maturase

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cloned and its role in the gp160 maturation process in infected cells is still uncertain.

The obvious difficulty is that infected cells may contain redundant activities and that no cell line lacking multiple members of processing secretory endoproteases is available. In the absence of an unambiguous system to identify gp160 converting enzymes, we decided to apply subcellular fractionation to a reference tissue, in order to determine the intracellular distribution of specific gp160 cleaving activity, in comparison with endogenously expressed furin and PC7/LPC. Rat liver was selected because furin and PC7/LPC are naturally expressed at a basal level in liver cells and because fractionation procedures have been extensively characterized in this experimental system, in contrast to lymphocytes [16–18].

A low-density subcellular compartment containing a specific gp160 processing activity was resolved from the *trans*-Golgi network (TGN) and from endosomes and probably corresponds to the secretion vesicles dispatched from the TGN. The gp160 processing activity isolated was also essentially resolved from the subcellular compartments enriched in furin and only partially overlapped PC7/LPC. These data suggest that, at least in the rat liver, furin and PC7/LPC do not represent the major endoproteases that cleave gp160.

2. Materials and methods

2.1. Sucrose density fractionation

Microsomes (P fraction), prepared as described [18], were brought to a density of 1.27 g/ml by the slow addition of concentrated sucrose and layered below either discontinuous or linear sucrose gradients (from 1.05 to 1.26 g/ml in density) for flotation isopycnic centrifugation (16 h at 49 000 rpm (VTi 50 rotor, Beckman), i.e. $192\times10^6\times g$). Four fractions were collected from the discontinuous sucrose gradients: the 8.2/33% and 33/36% sucrose interfaces (referred to as fraction 8/33 and fraction 33/36) and the 36 and 43% sucrose layers (fraction 36 and fraction 43). To identify integral membrane proteins, fraction 8/33 (1 mg/ml) was prepared in 200 μl 10 mM Tris-HCl, pH 7.4, 150 mM NaCl and 1% Triton X-114 at 0°C. Separation of the proteins was performed following the procedure of Bordier [19]. 14 Fractions were collected from the linear sucrose gradients, weighed and analyzed for density, enzyme activities and antigen content by quantitative Western blotting. Distributions of marker enzymes and those of furin and PC7/LPC have recently been reported [18].

2.2. Endoproteolytic maturation of gp160

Cleavage reactions were performed in 100 μl of 30 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 1 mM $CaCl_2$, pH 7.4, in the presence of 10 μl ^{35}S-labelled gp160 (10 000 cpm), purified as reported [10], and diluted sucrose gradient fractions (typically 0.7 mg protein/ml of the 8/33% sucrose interface) at 25°C. After overnight digestion, samples were run on an 8% SDS-polyacrylamide gel. Following autoradiography, the intensities of the bands were quantitated by densitometry of the X-ray film. The percentage of specifically cleaved protein was calculated as described [10]. These endoproteolytic activities in the liver sucrose gradients were finally normalized according to Leighton et al. [16].

2.3. Microsequencing of [^{3}H]Leu-labelled gp41

[^{3}H]leucine-radiolabelled gp160 was incubated with fraction 8/33 and the products were separated by 8% polyacrylamide SDS-PAGE (30×14 cm). The gel was blotted on a PVDF membrane, autoradiographed and the dissected gp41-containing band (noted by an asterisk) was layered over a protein filter (Beckman). Microsequencing was performed on a LF3400 protein sequencer (Beckman). The radioactivity recovered in each cycle was estimated by liquid scintillation counting (Tri-Carb model 1600CA).

2.4. Free flow electrophoresis (FFE)

FFE was performed as described [20] except that the sample was subjected to 0.3% trypsin treatment (0.3 mg trypsin/mg of protein). Pooled fractions were analyzed for gp160 cleavage as described above and by Western blotting [18] using a rabbit antiserum anti-TGN38 (TGN marker), provided by G. Banting, University of Bristol [21], and a mouse hybridoma 6F4C5 against a p54 protein (medial Golgi marker), provided by A. Tartakoff, Cleveland University [22].

3. Results

3.1. Specific cleavage of gp160 by a low-density subcellular fraction

Endoproteolytic maturation of newly synthesized HIV gp160, which is essential for viral infectivity, is mediated within the secretory pathway by host cell proteases. In order to localize subcellular compartments containing potential gp160 processing activity, rat liver microsomes were isolated by differential centrifugation and further resolved in sucrose gradients. First, discontinuous gradients were used and four fractions, collected at the 8/33% and 33/36% sucrose interfaces, as well the 36 and 43% sucrose layers, were incubated with [^{35}S]gp160 purified from vaccinia virus (VV):env-infected CV-1 cells.

Fluorography of the SDS-PAGE gel (Fig. 1) revealed that the light fractions (8/33 and 33/36), which are enriched in Golgi membranes [16], similarly cleaved gp160 into proteins migrating respectively at 120 and 41 kDa. The bands were identified as gp120 and gp41 by Western blotting (not shown). The absence of other gp160 cleavage products, even at five times higher enzyme/substrate ratios (not shown), demonstrated the specificity of the activity isolated in the 8/33 subcellular fraction. In contrast, the high-density 36 and 43 fractions, enriched in rough endoplasmic reticulum (RER) and lysosomes [16], cleaved gp160 non-specifically, without detectable gp120/gp41 intermediate production even after shorter incubation times.

Upon closer inspection, gp41 generated in vitro by the 8/33 fraction was found to migrate at a slightly higher molecular

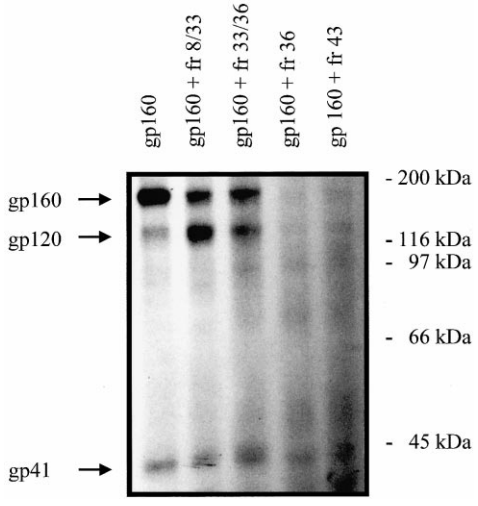

Fig. 1. Cleavage of the purified HIV-1 envelope glycoprotein precursor by rat liver subcellular fractions. Fluorography of a SDS-polyacrylamide gel (8% under reducing conditions). Lane 1, [^{35}S]gp160; lanes 2–5, [^{35}S]gp160 incubated with rat liver microsomes fraction 8/33 (lane 2), 33/36 (lane 3), 36 (lane 4) and 43 (lane 5). Digestions were performed in 100 μl of 30 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 1 mM $CaCl_2$, pH 7.4. Positions of molecular weight markers are indicated at the right. These experiments were reproduced >10 times with identical results.

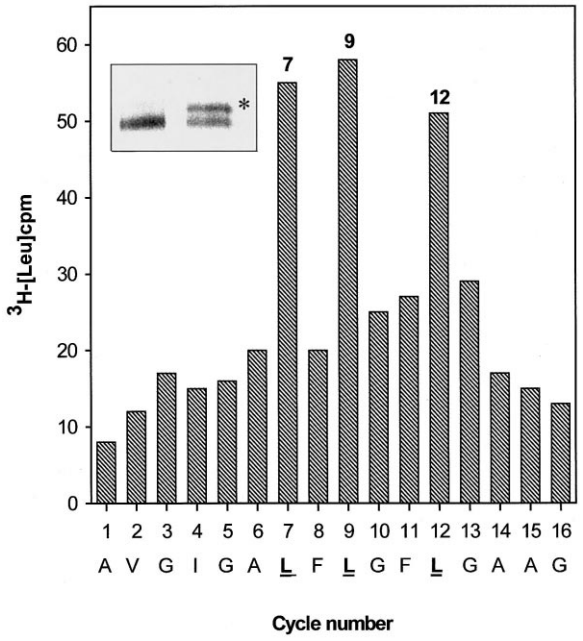

Fig. 2. Microsequence identification of the gp160 cleavage site by fraction 8/33. [^{3}H]leucine-gp41 resulting from digestion of gp160 by fraction 8/33 (upper band noted by an asterisk in the insert) was separated by SDS-PAGE (8) and sequenced. The deduced leucine positions are emphasized along the primary sequence following the gp120/gp41 cleavage site between Arg_{511} and Ala_{512} (here numbered at position 1).

weight position than gp41 which was produced by VV:env-infected CV-1 cells and contaminated the gp160 substrate preparation. This slight difference in electrophoretic mobility, barely detectable on the small gels (Fig. 1), was estimated at ~1–2 kDa on longer gels (insert in Fig. 2). A similar difference, also observed when gp41 was produced by convertases in vitro [10,11], could reflect glycosylation differences between the intracellular mature gp41 and that derived from the in vitro digestion of high-mannose gp160 by the 8/33 fraction. Alternatively, the molecular weight difference could result from cleavage at a second potential site ($KAKR_{503}\downarrow R$), located eight amino acids upstream of the natural site $REKR_{511}\downarrow A$ [23]. To clarify this issue, the NH_2-terminal sequence of gp41 produced in vitro by the 8/33 fraction was determined.

3.2. Authenticity of cleavage, as shown by NH_2-terminal sequencing of gp41 generated in vitro by the 8/33 fraction

The 8/33 fraction digestion products of [^{3}H]leucine-labelled gp160 were separated on a 30 cm long SDS-PAGE and the upper band was transferred onto a PVDF membrane and microsequenced. The NH_2-terminal sequence revealed the presence of leucine residues at positions 7, 9 and 12 (Fig. 2), confirming that cleavage occurred between Arg_{511} and Ala_{512} of gp160 ($KAKR_{503}RVVQREKR_{511}\downarrow AVGIGALFLGFLGAAG$) at the relevant position. These data prompted us to further characterize the cleavage activity of the 8/33 fraction and to better define its subcellular distribution.

3.3. Occurrence of furin and PC7/LPC in the low-density rat liver subcellular fraction

Since PCs were proposed as the major gp160 processing enzymes, we examined by immunoblot analyses which of these were present in the rat liver 8/33 fraction. Furin and PC7/LPC, migrating respectively at ~98 and 92 kDa, were clearly demonstrated (data not shown, see [18] for a comparable demonstration on low-density fractions from linear sucrose gradients), but neither PC1/3 nor PC2 were detected (results not shown), as expected from the absence of detectable PC1/3 and PC2 mRNA in liver tissue [6]. Due to the lack of anti-rat PACE4 and PC5/6-specific antibodies, we were unable to

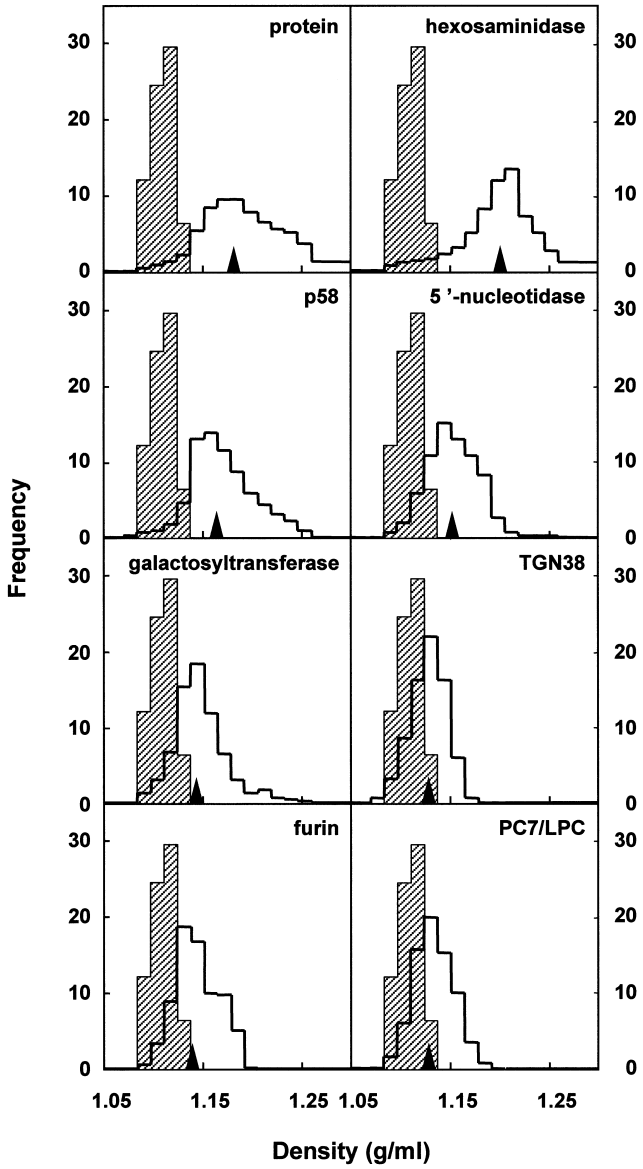

Fig. 3. Density distribution of the gp160 processing activity, furin and PC7/LPC after isopycnic equilibration of microsomes in sucrose gradients. Liver was homogenized and a total microsomal fraction (P) was equilibrated by flotation in a linear sucrose gradient (1.05–1.26 g/ml in density). 14 Fractions were collected and their cleaving activity was tested on [^{35}S]gp160. The distribution of gp160 processing activity is represented by the shaded area superimposed on profiles of the subcellular markers (*N*-acetyl-β-hexosaminidase: lysosomes, p58: *cis*-Golgi network, 5′-nucleotidase: plasma membrane, galactosyltransferase: *trans*-Golgi cisterna, TGN38: TGN) and of the convertases furin and PC7/LPC. This experiment has been reproduced once with identical results.

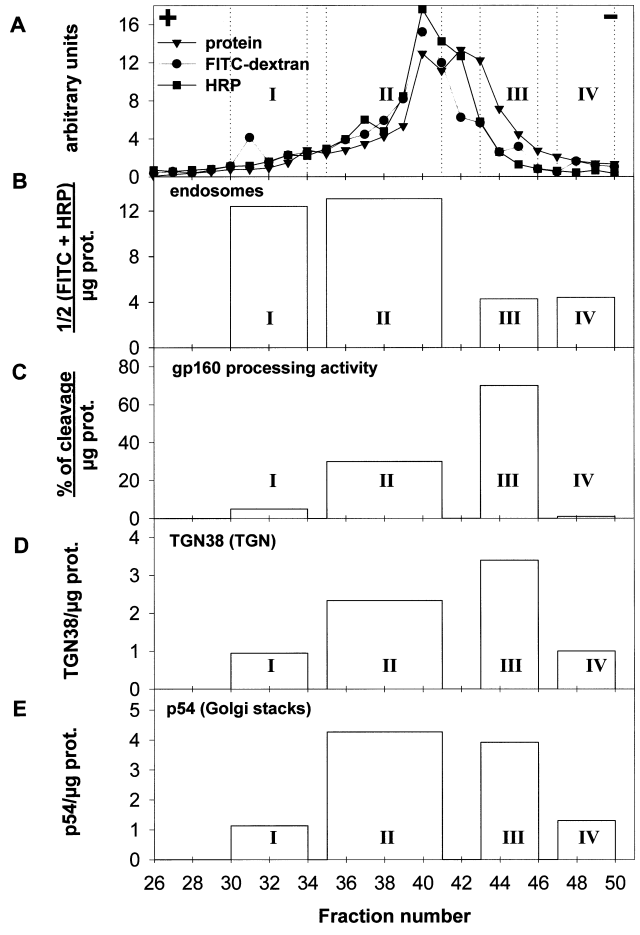

Fig. 4. FFE of low-density microsomes. Early and late endosomes were labelled with HRP and FITC-dextran by in situ perfusion for 1 min or using a 4 min pulse and 10 min chase at 37°C, respectively. Low-density microsomes were then injected into the FFE chamber at the cathodic site (opposite to outlet # 66), separated by FFE and the distributions of protein (triangles), HRP (squares) and FITC-dextran (circles) were determined (A). Positions of the anode and the cathode are indicated at the top. Data are expressed as percentage of the total amount recovered after FFE. Fractions were grouped in four pools (I, II, III and IV), their endosomal content (mean of HRP- and FITC-dextran-labelled endosomes) was calculated (B) and the enzymatic activity of each of these was tested on [^{35}S]gp160 (C). Immunoblot analyses were performed on the four pools using antibodies against the TGN38 protein (D) and p54 protein (E). This experiment has been reproduced once with identical results.

search for these proteins in the 8/33 fraction, although PACE4 and PC5/6 mRNA are abundantly expressed in the liver [6]. Thus, fraction 8/33 contained at least two members of the subtilisin-like convertases, furin and PC7/LPC, proposed as the main candidates for the natural processing of gp160 [8]. We therefore compared some intrinsic characteristics of gp160 processing activity with those of furin and PC7/LPC as well as their subcellular distribution.

3.4. Partial characterization of gp160 processing activity in the 8/33 low-density fraction

First, the 8/33 fraction was solubilized in Triton X-114 at 0°C, then separated into aqueous and micellar phases at 20°C. gp160 processing activity was mainly (80%) partitioned in the micellar phase, suggesting its association with integral membranes or amphiphilic enzymes. This result is compatible with a role of PCs.

Second, the gp160 cleavage activity by the 8/33 low-density fraction was tested for pH-dependence and was found to exhibit a broad pH range from 5.5 to 9 (data not shown). This is again compatible with PCs and strongly argues against the involvement of endosomal or lysosomal proteases such as cathepsin B, which shows optimal activity at pH 4–5 [24].

Third, since processing of gp160 in living cells is blocked by intracellular calcium depletion [12,25] and since activity of PCs depends on calcium [11], the calcium requirement of gp160 processing activity by the 8/33 low-density fraction was determined. Cleavage was inhibited by EDTA at concentrations well above 2 mM and restored after the addition of excess $CaCl_2$, demonstrating that the activity is calcium-dependent and does not belong to the metallo-proteinases family (data not shown).

3.5. Localization of gp160 processing activity in a compartment lighter than TGN vesicles

To better define its subcellular localization, cleavage was monitored in rat liver microsomes that were equilibrated in continuous sucrose gradients and recently characterized [18]. The distribution pattern of the gp160 processing activity was analyzed by densitometry and compared with established subcellular markers: 5′-nucleotidase (plasma membrane), *N*-acetyl-β-hexosaminidase (lysosomes), p58 (*cis*-Golgi network), galactosyltransferase (*trans*-Golgi cisternae) and TGN38. As previously reported [18], in the absence of ethanol treatment, furin and PC7/LPC essentially co-distributed with TGN38.

The gp160 processing activity was recovered around a median density of 1.113 g/ml (Fig. 3). Its distribution was clearly distinct from the lysosomal (1.203 g/ml) and *cis*-Golgi network (1.166 g/ml) markers, marginally overlapped the plasma membrane (1.152 g/ml) and Golgi stacks (1.144 g/ml) distributions and only partially overlapped the TGN (1.133 g/ml), furin (1.141 g/ml) and PC7/LPC (1.133 g/ml) distributions. The low abundance of furin and PC7/LPC in the lightest fractions, that were the most enriched in gp160 processing activity, suggested that other enzymes, possibly activated by convertases, were directly responsible for the cleavage of gp160 into gp120 and gp41 in liver cells. In addition, these observations excluded a primary location of gp160 processing activity in lysosomes, the RER, the plasma membrane and the Golgi stacks. Instead, they indicated that the activity was associated with a low-density compartment overlapping, but lighter than the TGN, such as TGN-derived vesicles or endosomes. To distinguish between these two possibilities, we resorted to FFE.

3.6. Resolution of gp160 processing activity from endosomes by FFE

FFE has previously been used to isolate endosomal populations from cultured cells and tissues [20]. Separation is based on the difference in the surface charge of particles (proteins, membrane-bound organelles) subjected to electrophoresis. Early and late endosomes were labelled by in situ perfusion of rat liver with fluid phase endocytosis markers, using respectively horseradish peroxidase (HRP) for 1 min and FITC-dextran for 4 min of pulse and 10 min of chase. After perfusion, light microsomes were isolated and separated by FFE (Fig. 4A). HRP- and FITC-dextran-labelled endosomes essentially

co-migrated as a major intermediate peak. In contrast, protein distribution was bimodal, one peak co-migrating with endosomes and the other peak extending towards the cathode.

To localize the gp160 endoproteolytic activity, the FFE fractions were concentrated in four pools and each was tested on [^{35}S]gp160. The specific cleavage activity was the highest in pool III, which was largely depleted of endosomes, and lower in pool II, which was more enriched in endosomes (cf. Fig. 4B and C), pointing to a compartment distinct from the endosomes. In contrast, the distribution profile of the gp160 processing activity after FFE partially overlapped those of the Golgi stacks (marker p54, Fig. 4D) and the TGN (marker TGN38, Fig. 4E). Taken together, continuous density gradient and FFE analyses suggested that the gp160 processing activity localized in vesicles dispatched from the TGN such as secretory vesicles.

4. Discussion

In the present work, we have identified in the liver an endoproteolytic activity of the appropriate specificity towards gp160 and have compared its properties and subcellular distribution with those of convertases that cleave gp160 in vitro or when overexpressed in cultured cells. The unusually high concentration of EDTA required to inhibit cleavage and the distinct distribution of this activity from furin and PC7/LPC argue against these two PCs being the only actors responsible for gp160 processing activity in our system.

Even though biosynthesis of HIV-1 envelope glycoprotein gp160 and its endoproteolytic processing into gp41 and gp120 have been extensively studied, the exact subcellular localization of the cleavage step is still uncertain. The best candidates are the Golgi complex, the TGN or the secretory vesicles [2–5]. The present subcellular fractionation study identifies a specific endoproteolytic gp160 processing activity that is not associated with lysosomes, endoplasmic reticulum and Golgi stacks, but rather with very low-density vesicles. Since these vesicles could correspond either to TGN-derived vesicles [18] or to endosomes [17], FFE was used and the ensuing results argued against a primary location of gp160 processing activity in early and late endosomes. Furthermore, in contrast to the gp160 processing activity that partitioned in the Triton X-114 micelle phase and was maximal at a broad neutral pH, known endosomal/lysosomal proteases are soluble enzymes and act at low pH. Therefore, we suggest that the gp160 processing activity localizes in TGN-derived vesicles. This hypothesis is compatible with temperature reversion experiments showing that, when HIV-infected Jurkat cells were incubated at 20°C, gp160 accumulated in the TGN and was not cleaved. When the temperature was shifted to 37°C, secretion resumed and gp160 was processed, suggesting that the endoproteolytic processing of gp160 takes place after exit from the TGN [5].

The nature of the cleaving enzyme(s) remains elusive. While liver expresses endogenous furin and PC7/LPC, both reported as the main gp160 cleaving enzymes [8], particles containing the gp160 processing activity were largely depleted of furin and did not strictly co-distribute with PC7/LPC. In analytical subcellular fractionation, due to the polydisperse nature of biological particles, evidence of overlapping between an activity and a particular enzyme cannot by itself demonstrate physical linkage, whereas dissociation is good evidence of distinct activities and compartments. In addition, a single activity should strictly correlate with the distribution of the presumptive actor. The lower right panel of Fig. 3 clearly shows that the peaks of gp160 endoproteolytic activity and PC7/LPC are distinct and that about half of PC7/LPC is associated with fractions 5–7, where no gp160 endoproteolytic activity can be detected. The lack of proportionality between PC7/LPC and gp160 endoproteolytic activity in the gradient fractions indicates that an activity distinct from PC7/LPC has been identified that is crucial for gp160 maturation in this experimental system.

Since furin displays a similar subcellular localization among various cell lines [26], we suggest that HIV-1 gp160 processing could be mainly effected by endoproteases other than furin and PC7/LPC. This suggestion is entirely consistent with three other lines of experimental evidence. First, two furin-defective cell lines, RPE.40 and LoVo cells, are able to correctly process gp160 [27,28]. Second, gp160 processing in COS-1 cells is not inhibited by the co-expression of FUR-rα_2-macroglobulin (M), an α_2M mutant known to drastically inhibit endogenous substrate processing by furin, suggesting that furin and gp160 do not share the same secretory pathway [29]. Third, gp160 is a poor substrate for furin, which is about 1000 times less sensitive to cleavage by the purified enzyme than anthrax toxin PA [28,30].

Could other convertases do the job? Northern blot analyses have previously revealed that PC5/6 and PACE4 are also expressed in rat liver and efficiently cleave gp160 in vitro and in cultured cells [11,12]. Although the membrane-bound forms of PC5/6 (PC5/6-B) [31] and PACE4 (PACE4-E) [32] are concentrated in the TGN, they could cycle to the cell surface and may also play a role in gp160 processing. However, in the absence of good antibodies against these enzymes which would work on Western blots, their possible contribution to the processing of gp160 could not be addressed.

Finally, the activation of a rate-limiting non-subtilisin enzyme by convertases can be envisaged. Indeed, two reports have implied that convertases may activate another, as yet undefined, gp160 processing enzyme [8,28]. PC7/LPC has been proposed to interact with other cellular proteins or to activate another enzyme, since it is able to cleave gp160 only by co-expression, but not in vitro [8]. PACE4 was also shown to participate in the activation of a protease in the furin-deficient RPE.40 cells, which efficiently convert gp160 into gp120/gp41 [28]. VEM, the non-subtilisin protease purified from a lymphocytic Golgi fraction and able to cleave gp160 into its two bona fide subunits, remains an interesting candidate [15]. Finally, it cannot be excluded that several enzymes are able to cleave gp160, as the exploitation of redundant systems is a well-conceivable viral strategy for this critical step for HIV activation.

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References

[1] Berger, E.A., Murphy, P.M. and Farber, J.M. (1999) Annu. Rev. Immunol. 17, 657–700.
[2] Pal, R., Hoke, G.M. and Sarngadharan, M.G. (1989) Proc. Natl. Acad. Sci. USA 86, 3384–3388.
[3] Stein, B.S. and Engleman, E.G. (1990) J. Biol. Chem. 265, 2640–2649.
[4] Willey, R.L., Bonifacino, J.S., Potts, B.J., Martin, M.A. and Klausner, R.D. (1988) Proc. Natl. Acad. Sci. USA 85, 9580–9584.
[5] Kantanen, M.L., Leinikki, P. and Kuismanen, E. (1995) Arch. Virol. 140, 1441–1449.
[6] Seidah, N.G., Chrétien, M. and Day, R. (1994) Biochimie 76, 197–209.
[7] Seidah, N.G., Hamelin, J., Mamarbachi, M., Dong, W., Tardos, H., Mbikay, M., Chrétien, M. and Day, R. (1996) Proc. Natl. Acad. Sci. USA 93, 3388–3393.
[8] Decroly, E., Benjannet, S., Savaria, D. and Seidah, N.G. (1997) FEBS Lett. 405, 68–72.
[9] Hallenberger, S., Bosch, V., Angliker, H., Shaw, E., Klenk, H.-D. and Garten, W. (1992) Nature 360, 358–361.
[10] Decroly, E., Vandenbranden, M., Ruysschaert, J.-M., Cogniaux, J., Jacob, G.S., Horward, S.C., Marshall, G., Kompelli, A., Basak, A., Jean, F., Lazure, C., Benjannet, S., Chrétien, M., Day, R. and Seidah, N.G. (1994) J. Biol. Chem. 269, 12240–12247.
[11] Decroly, E., Wouters, S., Di Bello, C., Lazure, C., Ruysschaert, J.-M. and Seidah, N.G. (1996) J. Biol. Chem. 271, 30442–30450.
[12] Vollenweider, F., Benjannet, S., Decroly, E., Savaria, D., Lazure, C., Thomas, G., Chrétien, M. and Seidah, N.G. (1996) Biochem. J. 314, 521–532.
[13] Marks, M.S., Woodruff, L., Ohno, H. and Bonifacino, J.S. (1996) J. Cell Biol. 135, 341–354.
[14] Levy, J.A. (1993) Microbiol. Rev. 57, 183–289.
[15] Kido, H., Kamoshita, K., Fukutomi, A. and Katunuma, N. (1993) J. Biol. Chem. 268, 13406–13413.
[16] Leighton, F., Poole, B., Beaufay, H., Baudhuin, P., Coffey, J.W., Fowler, S. and de Duve, C. (1968) J. Cell Biol. 37, 482–513.
[17] Courtoy, P.J. (1993) in: Subcellular biochemistry (Bergeron, J.J.M. and Harris, J.R., Eds.), Endocytic Components: Identification and Characterization, Vol. 39, pp. 29–68, Plenum Press, New York.
[18] Wouters, S., Leruth, M., Decroly, E., Vandenbranden, M., Creemers, J.W.M., van de Loo, J.-W.H.P., Ruysschaert, J.-M. and Courtoy, P.J. (1998) Biochem. J. 336, 311–316.
[19] Bordier, C. (1981) J. Biol. Chem. 256, 1604–1607.
[20] Fuchs, R., Male, P. and Mellman, I. (1989) J. Biol. Chem. 264, 2212–2220.
[21] Luzio, J.P., Brake, B., Banting, G., Howell, K.E., Braghetta, P. and Stanley, K.K. (1990) Biochem. J. 270, 97–102.
[22] Chicheportiche, Y., Vassalli, P. and Tartakoff, A.M. (1984) J. Cell Biol. 99, 2200–2210.
[23] Fenouillet, E. and Gluckman, J.-C. (1992) Virology 187, 825–828.
[24] Berg, T., Gjoen, T. and Bakke, O. (1995) Biochem. J. 307, 313–326.
[25] Moulard, M., Montagnier, L. and Bahraoui, E. (1994) FEBS Lett. 338, 281–284.
[26] Shapiro, J., Sciaky, N., Lee, J., Bosshart, H., Angeletti, R.H. and Bonifacino, J.S. (1997) J. Histochem. Cytochem. 45, 3–12.
[27] Ohnishi, Y., Shioda, T., Nakayama, K., Iwata, S., Gotoh, B., Hamaguchi, M. and Nagai, Y. (1994) J. Virol. 68, 4075–4079.
[28] Inocencio, N.M., Sucic, J.F., Moehring, J.M., Spence, M.J. and Moehring, T.J. (1997) J. Biol. Chem. 272, 1344–1348.
[29] Van Rompaey, L., Ayoubi, T., van de Ven, W.J.M. and Marynen, P. (1997) Biochem. J. 326, 507–514.
[30] Inocencio, N.M., Moehring, J.M. and Moehring, T.J. (1994) J. Biol. Chem. 269, 31831–31835.
[31] De Bie, I., Marcinkiewicz, M., Malide, D., Lazure, C., Nakayama, K., Bendayan, M. and Seidah, N.G. (1996) J. Cell Biol. 135, 1261–1275.
[32] Mori, K., Kii, S., Tsuji, A., Nagahama, M., Imamaki, A., Hayashi, K., Akamatsu, T., Nagamune, H. and Matsuda, Y. (1997) J. Biochem. (Tokyo) 121, 941–948.