Localization of α1,3-fucosyltransferase VI in Weibel–Palade bodies of human endothelial cells

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Surface glycosylation of endothelial cells is relevant to various processes including coagulation, inflammation, metastasis, and lymphocyte homing. One of the essential sugars involved in these processes is fucose linked α1→3 to *N*-acetylglucosamine. A family of α1,3-fucosyltransferases (FucTs) called FucT-III, IV, V, VI, VII, and IX is able to catalyze such fucosylations. Reverse transcription–PCR analysis revealed that human umbilical vein endothelial cells express all of the FucTs except FucT-IX. The predominant activity, as inferred by acceptor specificity of enzyme activity in cell lysates, is compatible with the presence of FucT-VI. By using an antibody to recombinant soluble FucT-VI, the enzyme colocalized with β4-galactosyltransferase-1 to the Golgi apparatus. By using a polyclonal antiserum raised against a 17-aa peptide of the variable (stem) region of the FucT-VI, immunocytochemical staining of FucT-VI was restricted to Weibel–Palade bodies, as determined by colocalization with P-selectin and von Willebrand factor. SDS/PAGE immunoblotting and amino acid sequencing of internal peptides confirmed the identity of the antigen isolated by the peptide-specific antibody as FucT-VI. Storage of a fucosyltransferase in Weibel–Palade bodies suggests a function independent of Golgi-associated glycosylation.

The endothelial storage granules called Weibel–Palade (WP) bodies were first described by Weibel and Palade as distinct ultrastructural entities specific for endothelial cells (1, 2). WP bodies belong to the regulated secretory pathway and are able to exteriorize their ingredients on appropriate stimulation (see refs. 3–5 and refs. cited therein). WP ingredients are involved in a variety of phenomena of vascular biology, such as regulation of vascular tonus [endothelin-1 and its converting enzyme (6)], blood clotting [von Willebrand factor (vWF) and tissue plasminogen activator (7, 8)], and inflammation [P-selectin, CD63 (9–11) and histamine (12)].

An early step in inflammation is expression of E- and P-selectin on the endothelial cell surface inducing rolling of leukocytes. Whereas this phenomenon has been investigated in great detail (for review, see ref. 13), the structure and biosynthesis of endothelial selectin ligands, which may contribute to this process by virtue of L-selectin binding, have, in comparison, received less attention.

An important component of most selectin ligands is peripheral fucose that is attached by an α1,3-glycosidic linkage to an oligosaccharide epitope known as sialyl-Lewis^x (for review, see ref. 14). Fucosylation may take place by one or several of six different α1,3-fucosyltransferases (FucTs) (15, 16). Because one of the possibilities for interference with inflammatory events would consist of inhibiting interaction of selectins with their ligands, it was of interest to investigate fucosylation processes in human endothelial cells in more detail.

FucT-VI, the focus of this study, is highly homologous to the Lewis type FucT-III and to FucT-V (17). Thus far, FucT-VI was found to be expressed mainly in the liver, which also seems to be the major source of the soluble plasma type of this enzyme (18). Liver FucT-VI was purified by Johnson *et al.* (19) and found to be essential for peripheral fucosylation of acute phase proteins (20). Its dominant presence in hepatocyte-derived HepG2 cells was shown by Borsig *et al.* (21). Here we describe the presence of FucT-VI as a Golgi-associated enzyme in human endothelial cells and, unexpectedly, its storage in WP bodies.

Materials and Methods

Endothelial Cells. Endothelial cells from human umbilical cord veins were harvested by collagenase digestion and seeded on human fibronectin-coated Falcon culture flasks or glass coverslips for immunofluorescence. Cultures were grown either in medium (EGM, Clonetics, San Diego) supplemented with growth factor and 10% FCS or in Medium 199 enriched with sodium heparin (90 μg/ml; Novo Industries, Bagsvaerd, Denmark), endothelial cell growth supplement (15 μg/ml; Collaborative Research), and 20% human serum (22). At the time of the experiment, the cells were in their second or third passage. Stimulations were performed with 10 ng/ml tumor necrosis factor and IL-1 for 24 h.

Material. Pansorbin was purchased from Calbiochem with a binding capacity of 2 mg IgG/ml suspension. Protein A agarose (binding capacity 30 mg IgG/ml) was obtained from Fluka and antipain, aprotinin, leupeptin, pepstatinA, PMSF, and white egg trypsin inhibitor from Sigma. GDP fucose and GDP-[U-^{14}C] fucose were from Oxford Glycosystems (Rosedale, NY) and Amersham, respectively. Soluble recombinant FucT-VI expressed in Chinese hamster ovary cells used for control was kindly provided by CIBA–Geigy (23).

Antibodies. The polyclonal rabbit antisera PEP6B (against the peptide) and OLI (against soluble recombinant FucT-VI) were described previously (23). Both antibodies were affinity purified with an Affi-Gel 10 column (Bio-Rad) coupled to peptide PEP6B or FucT-VI, respectively (23). The OLI antibody shows crossreactivity to FucT-III and V but not to FucT-IV or VII (21, 23). The affinity-purified PEP6B antibody was highly specific (23). An alignment of the different FucTs with the location of the PEP6B antibody-binding site is given in supplemental sequence

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Abbreviations: FucT, α1,3-fucosyltransferase; GalT-1, galactosyltransferase-1; LacNAc, *N*-acetyllactosamine; LNB-I, lacto-*N*-biose I; sLacNAc, sialyl-*N*-acetyllactosamine; WP, Weibel–Palade; HUVECs, human umbilical vein endothelial cells; RT-PCR, reverse transcription–PCR; vWF, von Willebrand factor.

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alignments (www.unizh.ch/physiol/). The mouse mAbs KG7/30 against vWF and AC1.2 against P-selectin (anti-CD62) were purchased from BMA Biomedicals and Becton Dickinson, respectively. The mouse mAb to galactosyltransferase-1(GalT-1) mAb2/36/118 was described previously (24). The secondary antibodies, horseradish peroxidase-conjugated goat anti-rabbit and goat anti-mouse, used for immunoblotting were from Sigma and Santa Cruz Biotechnology, respectively. FITC-conjugated goat anti-mouse and Texas red-conjugated goat anti-rabbit antibodies were from Dako and Cappel (ICN), respectively.

Activity Measurements. Human umbilical vein endothelial cells (HUVECs) were lysed in PBS containing 1% Triton X-100, 1 μg/ml each of antipain, aprotinin, and pepstatinA, 0.5 μg/ml leupeptin, and 0.2 mM PMSF. The activity was assayed in a reaction mixture of 50 μl containing 25 mM sodium cacodylate (pH 6.2), 10 mM $MnCl_2$, 10 mM L-Fucose, 5 mM ATP, 101 μM GDP-Fucose ($\approx$5,000 cpm/nmol), 30–60 μg of protein, and 5 mM of acceptor substrate [*N*-acetyllactosamine (LacNAc), lacto-*N*-biose I (LNB-I), or sialyl-*N*-acetyllactosamine (sLacNAc)] or 1 mM of respective substrates linked to $(CH_2)_8COOCH_3$ aglycone (kindly provided by CIBA–Geigy). Controls without acceptor were assayed in parallel. After incubation at 37°C for 2–4 h, the reaction mixture was diluted with cold water and applied to a Dowex 1X8–400 column. Flowthroughs and water eluates were collected. In case of octyl spacer substrates, the reaction was stopped with 500 μl of cold water, and the reaction mixture was separated on Sep-Pak cartridges (Millipore; ref. 25). Eluted products were counted in 10 ml of Instagel (Packard, IL) with a liquid scintillation counter (rackbeta 1219, LKB).

Immunodetection of FucT-VI. Immunoblotting was carried out according to Towbin *et al.* (26). Samples separated on 4–15% polyacrylamide gradient gel by SDS/PAGE were transferred to 0.45 μm PVDF membranes by using 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid buffer, pH 11, with 0.5 mM DTT and 10% methanol. Proteins of interest were detected by incubation with respective first antibodies followed by incubation with horseradish peroxidase-conjugated second antibodies. Bands were visualized by applying the enhanced chemiluminescence developing kit according to instructions of the manufacturer (Amersham).

Cell Fractionation and Enrichment of WP Bodies. Endothelial cells of 24 dishes (15 cm diameter) were fractionated according to the procedure of Vischer and Wagner (11). For immunoblot analysis, Percoll fractions obtained were precipitated with 70 μl/ml 150% (wt/vol) trichloroacetic acid and 7 μl/ml 2% deoxycholate for 1 h on ice. Obtained pellets were washed with acetone-HCl (1 drop HCl/100 ml acetone), dried, and resuspended in Laemmli sample buffer (27). WP bodies were enriched in fractions nos. 8 to 12. These fractions were pooled and applied to a Nycodenz (Sigma) gradient as described in ref. 11.

Immunoprecipitation for Immunoblotting and Protein Sequencing. A total of 32 culture flasks (75 cm^2) of HUVECs in their third passage were grown to confluency and harvested by trypsin–EDTA treatment (2 ml/flask). One milliliter of FCS per flask was added to stop trypsination. All following steps were carried out at 4°C. Six flasks at a time were processed. Cells were collected in 50-ml tubes filled with PBS and sedimented. Pellets were combined and washed three times with cold PBS and resuspended in 2 ml cold lysis buffer containing 20 mM Tris, 1% Triton-100, 1 μg/ml each of antipain, aprotinin, and pepstatinA, 0.5 μg/ml leupeptin, 0.2 mM PMSF, and 3 mg/ml white egg trypsin inhibitor. Lysate was incubated for 30 min on a rocking plate and centrifuged for 15 min at 14,000 $\times$ *g*. Supernatant was collected and transferred to a new tube. Two different procedures for the precipitation were applied. (*i*) The lysate was preabsorbed with Pansorbin (500 μl suspension) for 60 min. After centrifugation for 15 min, supernatants were transferred to a new tube and Pansorbin coupled to the PEP6B antibody (Pansorbin pellet of 500 μl original suspension resuspended in 200 μl of antibody solution was incubated for 2 h at room temperature and washed three times with PBS) was added. (*ii*) Lysate supernatant was preabsorbed with 200 μl protein A agarose for 60 min and centrifuged. Supernatant was transferred to a new tube and 200 μl PEP6B antibody was added. After incubation overnight at 4°C, the antibody–antigen complex was absorbed to 300 μl protein A agarose. The mixture was incubated for 2 h. Precipitates *i* and *ii* were collected by centrifugation at 14,000 $\times$ *g*, and pellets were washed once with lysis buffer and three times with PBS. Pellets were resuspended in nonreducing SDS buffer according to Laemmli (27) and incubated at room temperature for 30 min. Samples were cleared by centrifugation for 5 min, and supernatants were recovered, heated for 3 min, and applied to a 10% nonreducing SDS/PAGE gel. Proteins were transferred (see above) to two superposed PVDF membranes, a 0.1 μm Immobilon^{SQ} PVDF membrane (first membrane, in direct contact with the gel), and a 0.4 μm Immobilon^{Q} PVDF membrane (second membrane, used for immunostaining with PEP6B). Bands on the first membrane, which corresponded to immunoreactive bands on the second membrane, were cut out and treated for 40 h with 70% formic acid at 37°C to overcome the N-terminal blockage, as described by Sonderegger *et al.* (28). Membrane cuts were dried under nitrogen: Edman degradation was performed without further purification steps. Both immunoprecipitation procedures described above yielded the same result.

Immunofluorescence Microscopy. HUVECs grown on coverslips to 80–90% confluency were fixed at room temperature with 3% formaldehyde in Hanks' balanced salt solution (GIBCO/BRL) for 10 min and processed for immunofluorescence as described (21). Cells were analyzed on a Leica laser-scanning microscope (Wetzlar, Germany). Absorption of the affinity-purified PEP6B antibody with the peptide used for immunization abolished the signal obtained (data not shown).

RT-PCR Reaction. Total RNA was isolated from HUVECs by using the RNA-Kit from Fluka. Reverse transcription was performed in 50 μl or 20 μl according to the protocol of Stratagene or Sigma, respectively, by using Oligo-dT. The amplification was performed in 50 μl reaction mix containing 20–25 mM $MgCl_2$, pH 8–9.5, 3 μl of RT-product, 5 units *Taq* DNA polymerase, 0.2 mM dNTP, and 100 μM of respective primers. The specific primer pairs used for FucT-V and FucT-VI (29) and for FucT-III, IV, and VII (21) have been previously described. The primers for FucT-IX were 5′-CATTGAAATCCATACCTACGGGCAAG-3′ and 5′-AAATCTCCACCAAAATATACACGTTACC-3′ and for β-actin were 5′-CCCAGCACAATGAAGATCAAGAT-3′ and 5′-TCCTCGGCCACATTGTGAACTT-3′. For all samples, hot-start PCR was performed as follows: All samples were denatured at 95°C for 3 min, and *Taq*-polymerase was added when samples had reached 80°C. The conditions for the amplification were: for FucT-VII and IV (37 cycles), 60°C for 45 s, 72°C for 50 s, and 95°C for 45 s; for FucT-V and FucT-VI (35 cycles), 70°C for 35 s, 72°C for 45 s, and 95°C for 35 s; for FucT-III (30 cycles), 65°C for 35 s, 72°C for 45 s, and 95°C for 35 s; and for FucT-IX (35 cycles), 54°C for 35 s, 72°C for 50 s, and 95°C for 35 s. β-actin was amplified with 35 cycles (60°C for 35 s, 72°C for 40 s, and 95°C for 35 s). Amplification of genomic DNA was performed with 70 ng DNA for control. Samples were separated on a 3% NuSieve agarose gel (Fluka)

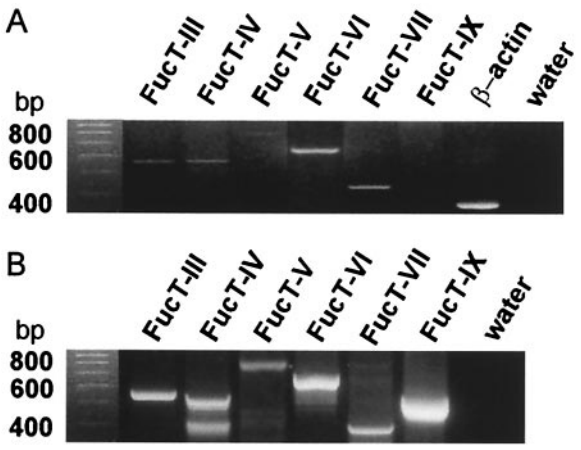

Fig. 1. Detection of FucTs by RT-PCR. RT-PCR products separated on 3% NuSieve agarose gel and stained with ethidium bromide are shown (*A*). Positive-control reactions for the FucT-PCR were performed on genomic DNA and are represented in *B*. RNA quality was checked with the amplification of β-actin (*A*). The H_2O controls are shown on the rightmost lane. A 100-bp ladder was used as standard (first lane).

containing 40 mM Tris-acetate and 2 mM EDTA, pH 8.0, and visualized with ethidium bromide.

Results

Expression of FucTs in Human Endothelial Cells. To identify those α3FucTs that contribute to surface fucosylation of endothelial cells, HUVECs were screened by RT-PCR for the different members of the α3FucT family. Expression of FucT-III, FucT-IV, FucT-V, FucT-VI, and FucT-VII was found, whereas FucT-IX was not detected (Fig. 1).

The presence of FucT-VI in human endothelial cells was ascertained by determination of FucT activity in lysates of HUVECs. To distinguish the different α3FucT activities, three specific acceptors, e.g., LacNAc, LNB-I, and sLacNAc with and without octyl spacer, were used. As summarized in refs. 15 and 30, FucT-IV and FucT-IX are highly specific for LacNAc and FucT-VII for sLacNAc but not LacNAc. FucT-V and -VI both use LacNAc and sLacNAc, whereas LNB-I can be fucosylated only by FucT-III. Stimulation with vasoagonists tumor necrosis factor and IL-1 (22) increased transfer to LacNAc by 2.2- and to sLacNAc by 2.5-fold, whereas little increase (1.3-fold) was detected with LNB-I as an acceptor (Table 1). Because LNB-I is only a substrate for FucT-III, this result suggests that FucT-III is present but not induced. Parallel activity changes of both LacNAc and sLacNAc indicate the presence of FucT-V or FucT-VI on stimulation. However, by using specific antibodies to FucT-V (31), no evidence for its protein expression has been obtained by immunofluorescence (not shown). In summary, these data on the α3FucT activity in HUVECs can be ascribed to FucT-VI and FucT-III.

Table 1. FucT activity in HUVECs

Acceptor	Activity, pmol/min/mg total protein: Resting	Stimulated	Induction factor
LacNAc	7.2 (100%)	16.2 (100%)	2.2
LNB-I	15.3 (210%)	22.4 (138%)	1.4
sLacNAc	0.9 (12%)	2.3 (14%)	2.5
LacNAc-OR[a]	12.2 (100%)	27 (100%)	2.2
LNB-I-OR	10 (82%)	13.1 (48%)	1.3
sLacNAc-OR	1.5 (12%)	2 (7%)	1.4

Immunolocalization of FucT-VI in HUVECs. The presence of FucT-VI was further investigated by using a polyclonal antiserum against a 17-aa peptide of the variable (stem) region, termed PEP6B (see *Material and Methods*; also see supplemental sequence alignment, www.unizh.ch/physiol/). Affinity-purified PEP6B antibody was able to recognize FucT-VI specifically *in situ* and on immunoblots without crossreactivity to the cognate FucT-V and FucT-III (21, 23). An antibody raised against a recombinant soluble form of FucT-VI (OLI) derived from Chinese hamster ovary cells (23) was used as a control.

By dual immunofluorescence staining for FucT-VI with the OLI antibody (crossreacting with FucT-III and V), FucT-VI/III was colocalized with GalT-1 in a compact and juxtanuclear structure typical of the Golgi apparatus (32) (Fig. 2 *A–F*). A strikingly different staining for FucT-VI was observed by using the peptide antibody PEP6B (Fig. 2 *G–M*). Although only some overlap of FucT-VI with GalT-1 was observed (Fig. 2*M*), a dominant presence of FucT-VI in a body-shaped structure characteristic of WP bodies was found. This was verified by dual staining of FucT-VI and the WP body markers vWF (7) or P-selectin (9, 10), respectively. FucT-VI showed a high degree of overlap with both vWF (Fig. 2 *N–P*) and endothelial P-selectin (Fig. 2 *Q–S*). Identical stainings were obtained without fixation and in the presence of 0.1% Triton X-100 as permeabilizing agent (not shown).

Immunoisolation and Identification of Endothelial FucT-VI. To ascertain authenticity of the antigen recognized by PEP6B as FucT-VI, WP bodies were enriched from HUVECs fractionated on a Percoll density gradient and analyzed by SDS/PAGE followed by immunoblot analysis. PEP6B antibodies recognized a major band of about 49 kDa (Fig. 3*A*). A band of the same molecular weight was identified in pooled and concentrated fractions of WP bodies by using the OLI antibodies (Fig. 3*A*), supporting the evidence that FucT-VI is the antigen recognized by PEP6B in WP bodies. A similar band was identified by immunoblotting using the OLI antiserum after immunoprecipitation of HUVEC lysate with PEP6B antibodies (Fig. 3*A*).

Analysis of single fractions collected from a density gradient revealed that FucT-VI recognized by PEP6B was mainly found in the denser fractions and cosedimented with vWF (Fig. 3*B*). The specific Golgi marker GalT-1 was restricted to the Golgi fraction (Fig. 3*B*).

Protein Sequencing. Final proof for the identity of the WP body-associated antigen recognized by the peptide antibody PEP6B was obtained by sequence analysis of the antigen immunoprecipitated by using the PEP6B antibodies (Fig. 3*A*). Internal peptides were obtained by chemical cleavage of the lower molecular band around 49 kDa and the higher molecular band at 95 kDa, respectively. Edman degradation resulted in an almost identical pattern for both bands, implying that they represented mono and dimeric forms of the same protein. The amino acid patterns (Fig. 4*A*) were compared with the full-length FucT-VI protein sequence. Some combinations matched with several stretches of the FucT-VI sequence (Fig. 4*B*). Thus, we conclude that the antigen recognized by PEP6B antibodies in WP bodies is FucT-VI.

Discussion

This work presents evidence for an unusual and surprising localization of a Golgi-associated glycosyltransferase within a storage organelle. FucT-VI, which may participate in the synthesis of selectin ligands, has been found colocalized with P-selectin and vWF in WP bodies. This serendipitous finding was

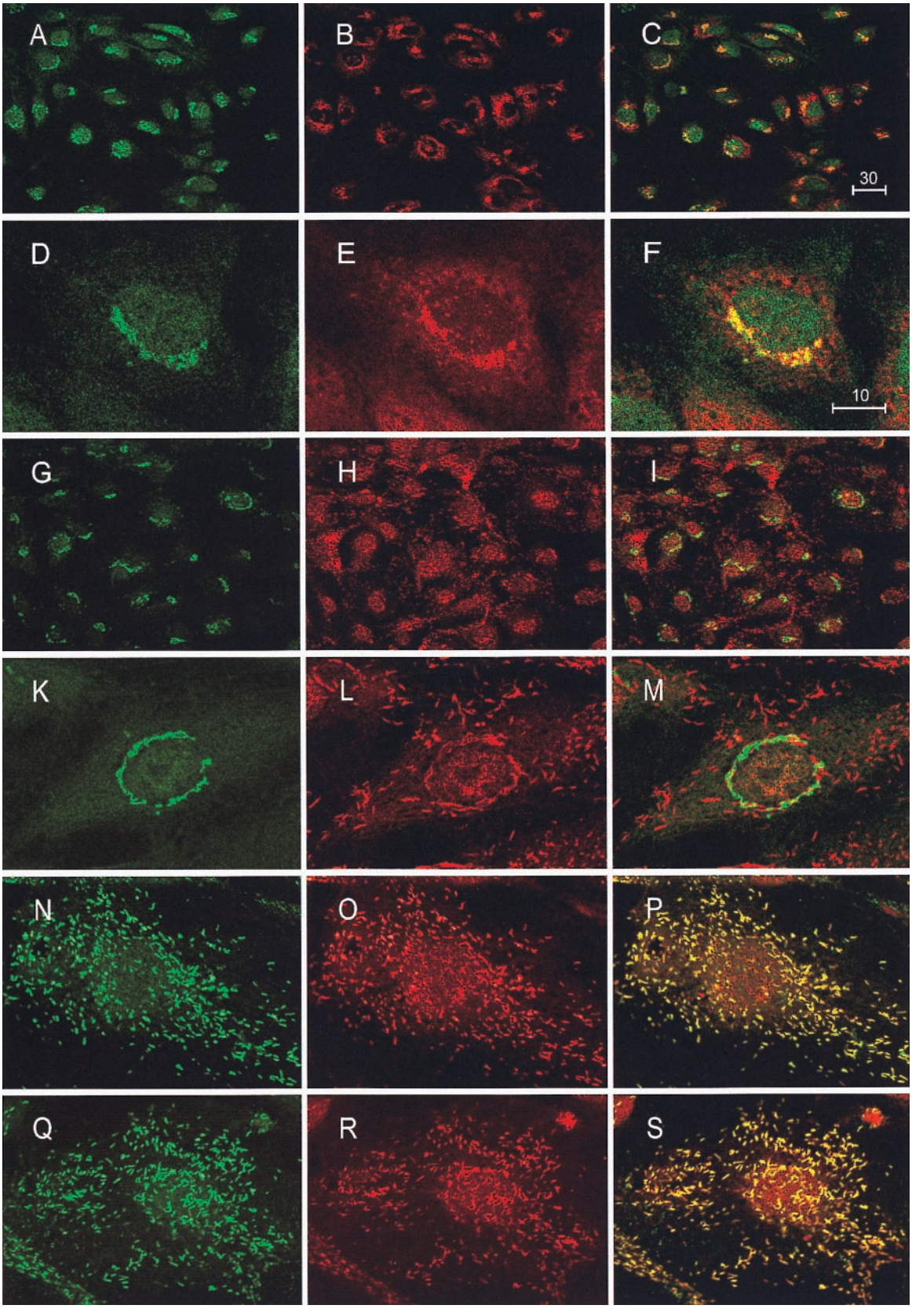

Fig. 2. Immunolocalization of FucT-VI in HUVECs. Dual confocal immunofluorescence on HUVECs was performed as described in *Materials and Methods*. A double staining for GalT-1 (*A* and *D*) and FucT-VI/III (*B* and *E*) with the OLI antibody is shown in an overview in the first row (*A–C*) and on a single cell level in the second row (*D–F*). The overlays of the respective pictures are given in the last column. Colocalization resulted in a yellow color. Bars = 30 μm and 10 μm, respectively. GalT-1/PEP6B (*G, H, K*, and *L*) dual staining is represented in rows 3 (overview) and 4 (single cell). Single-cell sections of double stainings with P-selectin (*N*)/PEP6B and vWF (*Q*)/PEP6B are shown in row 5 (*N–P*) and 6 (*Q–S*).

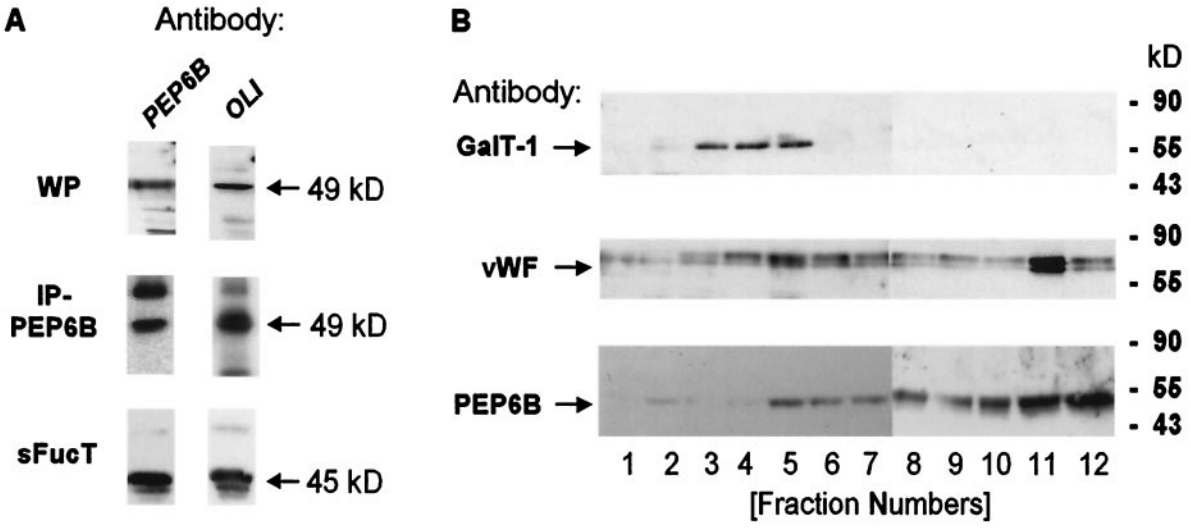

Fig. 3. Immunodetection of FucT-VI in human endothelial cells. HUVECs were analyzed by SDS/PAGE followed by immunoblotting for the presence of FucT-VI (*A*). In the WP bodies enriched fraction (WP), both antibodies PEP6B (lane 1) and OLI (lane 2) recognized a major band migrating at 49 kDa (*A*, row 1). A band of the same molecular weight was obtained after immunoprecipitation with PEP6B from HUVEC lysate given in row 2 (IP-PEP6B). Immunostaining of recombinant soluble FucT-VI (sFucT) is shown as a control (bottom row). In *B*, the localization of FucT-VI by PEP6B in Percoll density gradient fractions (see *Materials and Methods*) is represented. Fractions were tested for the presence of GalT-1 (row 1), vWF (row 2), and FucT-VI with the PEP6B antibody (row 3). The molecular standards are indicated (*Right*).

because of the peptide-specific antibody PEP6B, which recognized its cognate epitope in a form whose confirmation is different from that associated with the Golgi apparatus. This epitope may also be accessible during an early stage of biosynthesis of FucT-VI. This was concluded from the following observations: First, a slight decrease in molecular weight obtained after peptide-*N*-glycanase F treatment of immunoprecipitated FucT-VI of endothelial cells metabolically labeled for 3 h, from a double band at 44 kDa and 46 kDa to a dominant band of 43 kDa (data not shown), which corresponds to the full-length non-*N*-glycosylated form of FucT-VI (23), suggests that an early biosynthetic form of FucT-VI is recognized by the peptide antibody PEP6B (23). Second, PEP6B detects recombinant FucT-VI *in situ* as long as the enzyme is associated with the endoplasmic reticulum: Golgi staining by using these antibodies was observed only after pretreating HUVECs (data not shown) or Chinese hamster ovary cells (23) with guanidinium. Third, FucT-VI blotted on a membrane, hence partially denatured, also reacts with PEP6B (Fig. 3). Conversely, the OLI antiserum raised against recombinant soluble and functional FucT-VI detects the enzyme in the Golgi apparatus where it overlaps with GalT-1 in endothelial cells (see Fig. 2) or HepG2 cells (21). Importantly, both antisera detect purified enzyme and the same antigens in lysates and enriched WP bodies when blotted on a membrane (Fig. 3; ref. 23). This finding implies that there are conformational differences between native FucT-VI associated with the Golgi apparatus vs. WP bodies.

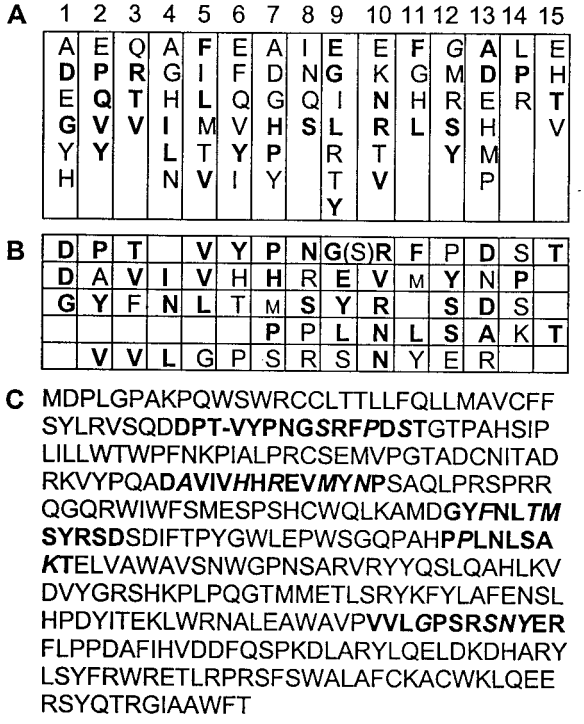

Fig. 4. Edman degradation of internal peptide fragments of FucT-VI. The 49-kDa band blotted on PVDF membrane was sequenced after partial cleavage with formic acid *in vitro* to overcome the N-terminal blockade. The obtained amino acid patterns of each sequencing cycle [number given (*Top*)] are shown in *A*. The amino acid stretches present in FucT-VI are given in *B*. *C* shows localization of the correlating peptides within the full-length amino acid sequence of FucT-VI. In bold are given those amino acids that match exactly with FucT-VI; in italic, the amino acids not clearly identified.

Post-Golgi localization of terminal glycosyltransferases has been a controversial issue for many years, especially with respect to GalT-1 (for review, see ref. 33). In fact, poorly differentiated cell lines in culture usually do not exhibit localizations of glycosyltransferases other than in the Golgi apparatus. The situation in primary cell cultures, such as HUVECs, or in tissues may be more complex. In the case of ST3Gal III, perfusion-fixed tissue sections showed a clear post-Golgi staining in rat kidney epithelial cells in addition to the canonical Golgi localization (34). Other examples are the polysialyltransferases PST and STX, which have been localized to the cell surface and even the extracellular space (35).

Although the function of Golgi-associated glycosyltransferases is clearly defined in elongation and termination of glycan chains, little is known about their possible post-Golgi functions except for those on the cell surface. Our data do not support an

enzymatic function in WP bodies, because activity in corresponding subcellular fractions was little over background (not shown). At present, the functional significance of the data reported here remains to be defined. An attractive working hypothesis relates to lectin-like functions in which sugar-binding properties are conserved while catalytic functions are removed. Evidence for this possibility has been provided by Ma and Colley (36), who showed that α2,6sialyltransferase (ST6Gal 1) can form a homodimer by a disulfide bond that is catalytically inactive but that retains the galactose-binding property. Lectin-like interactions with ligands require stoichiometric rather than catalytic amounts of protein; thus, accumulation in storage granules may be required for such a function.

Another interesting problem relates to the sorting process to WP bodies, which is still unclear. No common targeting signals of the WP proteins have so far been identified. Recently, evidence has been provided to show similarities between endocrine and endothelial cells in protein sorting to storage granules: the cDNA encoding proinsulin was transfected into HUVECs, which sorted this recombinant molecule to WP bodies (8). Fucosylated WP-body proteins are possible candidates for being transported by interaction with FucT-VI, such as tissue plasminogen activator and urokinase (37). Sorting of proteins based on lectin-like interactions has been described for zymogens that are transported from the Golgi to zymogen granules in pancreatic cells (38) and for specific proteins from the endoplasmic reticulum to the Golgi (39).

Besides the functionally active full-length form of FucT-VI, two shorter splice variants have been described as occurring in liver and kidney (29). Interestingly, these variants are catalytically inactive. Current efforts are aimed at investigating whether the species of FucT-VI accumulated in WP body corresponds to a splice variant. In trying to delineate the physiological significance of WP body-associated FucT-VI, the fact that a polymorphism leading to catalytic inactivity occurs in 9% of the Indonesian population (18) needs to be kept in mind. All affected individuals express a completely or partially inactivated enzyme, which still could keep some functional properties that may be conserved in the form retained in WP bodies.

In summary, we describe accumulation of a FucT-VI variant in WP bodies of endothelial cells. Its accumulation in storage granules suggests a function not directly related to its catalytic activity.

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