Role of the Low Density Lipoprotein-related Protein Receptor in Mediation of Factor VIII Catabolism*

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Evgueni L. Saenko‡, Alexey V. Yakhyaev, Irina Mikhailenko, Dudley K. Strickland, and Andrei G. Sarafanov

From the Holland Laboratory, American Red Cross, Rockville, Maryland 20855

In the present study, we found that catabolism of coagulation factor VIII (fVIII) is mediated by the low density lipoprotein receptor-related protein (LRP), a liver multiligand endocytic receptor. In a solid phase assay, fVIII was shown to bind to LRP ($K_d$ 116 nM). The specificity was confirmed by a complete inhibition of fVIII/LRP binding by 39-kDa receptor-associated protein (RAP), an antagonist of all LRP ligands. The region of fVIII involved in its binding to LRP was localized within the A2 domain residues 484–509, on the ability of the isolated A2 domain and the synthetic A2 domain peptide 484–509 to prevent fVIII interaction with LRP. Since vWf did not inhibit fVIII binding to LRP, we proposed that LRP receptor may internalize fVIII from its complex with vWf. Consistent with this hypothesis, mouse embryonic fibroblasts that express LRP, but not fibroblasts genetically deficient in LRP, were able to catabolize 125I-fVIII complexed with vWf, which was not internalized by the cells. These processes could be inhibited by RAP and A2 subunit of fVIII, indicating that cellular internalization and degradation were mediated by interaction of the A2 domain of fVIII with LRP. In vivo studies of 125I-fVIII-vWf complex clearance in mice demonstrated that RAP completely inhibited the fast phase of the biphasic 125I-fVIII clearance that is responsible for removal of 60% of fVIII from circulation. Inhibition of the RAP-sensitive phase prolonged the half-life of 125I-fVIII in circulation by 3.3-fold, indicating that LRP receptor plays an important role in fVIII clearance.

The plasma glycoprotein factor VIII (fVIII) functions as a cofactor for factor IXa in the factor X activation enzyme complex in the intrinsic pathway of blood coagulation, and its level is decreased or the protein is nonfunctional in patients with hemophilia A. The fVIII protein consists of a homologous A and C domains and a unique B domain which are arranged in the order A1-A2-B-A3-C1-C2 (1). It is processed to a series of Mo$^{2+}$-linked heterodimers produced by cleavage at the B-A3 junction (2), generating a light chain (LCh) which consists of an acidic region and A3, C1, and C2 domains and a heavy chain (HCh) which consists of the A1, A2, and B domains (Fig. 1).

Transplantational studies both in animals and humans demonstrated that liver hepatocytes are the major fVIII-producing cells (3, 4). Immediately after release into circulation, fVIII binds with a high affinity ($K_d < 0.5$ nM (5, 6)) to its carrier protein vWF to form a tight, noncovalent complex. The binding to vWF is required for maintenance of a normal fVIII level in circulation, since vWF stabilizes association of the LCh and HCh (7). This prevents fVIII from binding to phospholipid membranes (8), activation by factor Xa (9), and protein C-catalyzed inactivation (10). vWF comprises a series of high molecular mass, disulfide-bonded multimers with molecular mass values as high as 2$\times$10$^7$ Da (11) and circulates in plasma at 10 $\mu$g/ml or 50 nM assuming a molecular mass of 270 kDa for vWF monomers (12). Since the concentration of fVIII in plasma is approximately 1 nM (13), one fVIII molecule is bound per 50 vWF monomers in plasma (14).

Activation of fVIII by thrombin leads to dissociation of activated fVIII (fVIIIa) from vWF and to at least 100-fold increase of the cofactor activity. The fVIIIa is a A1/A2/A3-C1-C2 heterotrimer (15) which does not aggregate and is capable of aggregating in electrostatic forces (15). Spontaneous dissociation of the A2 subunit from the heterotrimer results in non-proteolytic inactivation of fVIIIa (15).

Infusion of fVIII-vWF complex, purified plasma, or recombinant fVIII into patients with severe hemophilia A who do not have fVIII (16, 17) or in normal individuals (18) results in a similar fVIII disappearance with a half-life of 12–14 h. Although the complex formation between fVIII and vWF is crucial for the normal half-life and level of fVIII in circulation, the mechanisms associated with turnover of fVIII-vWF complex are not well defined. We proposed that the fVIII-vWF complex is eliminated from plasma via a clearance receptor and tested the possibility whether this receptor is a low density lipoprotein-related protein receptor (LRP). LRP-mediated cellular endocytosis was shown to be a mechanism of removal of a number of structurally unrelated ligands including several proteins involved in coagulation or fibrinolysis. These ligands are complexes of thrombin with antithrombin III, heparin cofactor II (19), protease nexin I (20), urokinase-type and tissue-type plasminogen activators, respectively, complexes with plasminogen activator inhibitor (21, 22), thrombospordin (23), tissue factor pathway inhibitor (24), and factor Xa (25, 26).

LRP, a large cell-surface glycoprotein identical to $\alpha_2$-macroglobulin receptor (27), is a member of the low density lipoprotein receptor family which also includes the LDL receptor, very low density lipoprotein receptor, vitellogenin receptor, and gly
coprotein 330 receptor. LRP receptor consists of the noncovalently linked 515-kDa α-chain (28) containing binding sites for LRP ligands, and the 85-kDa transmembrane β-chain. The cluster of 31 cysteine-rich class A repeats participates in binding of different ligands (29). The presence of multiple repeats may be responsible for wide ligand diversity of LRP and its ability to serve as a multiligand clearance receptor. In contrast to the acidic ligand-binding region in LRP, its ligands expose regions rich in positively charged amino acid residues (30).

LRP is a major endocytic receptor in the liver (31) but it also expressed in many cell types and tissues including placenta, lung, and brain (32). A 39-kDa receptor-associated protein (RAP) binds to LRP with high affinity \( K_d = 4 \text{ nm} \) (27) and inhibits binding and LRP-mediated internalization and degradation of all ligands (30, 33), therefore serving as a useful tool for testing whether LRP is involved in endocytosis of a given ligand.

In the present study we demonstrated that FVIII specifically binds to LRP, and that LRP mediates the internalization and subsequent degradation of FVIII in cultured fibroblasts and plays a significant role in the clearance of FVIII in vivo. We also found that interaction of the A2 domain of FVIII with LRP is responsible for mediating catabolism of FVIII and localized the A2 domain region that is directly involved in the binding.

**EXPERIMENTAL PROCEDURES**

**Monoclonal Antibodies**—The monoclonal anti-A2 antibodies (mAbs) T5 (epitope within the residues 701–740) and 8860 were kindly provided by Dr. Carol Fulcher (Scripps Clinic and Research Foundation, La Jolla, CA) and by Baxter/Hyland Healthcare Inc. (Glendale, CA). MAb 413 (epitope within A2 domain residues 484–509) was prepared as described previously (36).

**Proteins**—LRP was isolated from human placenta as described (37). Human recombinant RAP was expressed in bacteria and purified as described previously (36). The monoclonal anti-A2 antibodies (mAbs) T5 (epitope within A2 domain residues 484–509) was prepared as described previously (36).

**Preparation of FVIII**—Human recombinant FVIII was purified using ion exchange chromatography of thrombin-activated FVIII on a Resource S column (Amersham Pharma Biotech) (39).

**Solid-phase Binding Assays**—Homologous and heterologous ligand displacement assays were performed as described previously (33). Microtiter wells were coated with purified LRP or BSA (3 μg/ml) in 50 mM Tris, 0.15 mM NaCl, pH 8.0, for 16 h at 4 °C and then blocked with 3% BSA in TBS. Coated wells were incubated with 125I-FVIII in 20 mM Tris-buffered saline, pH 7.4, containing 5 mM CaCl₂, 0.05% Tween 20 in the presence or absence of unlabeled competitors (vWF, FVIII, or RAP) for 1 h at 37 °C. In the experiment using vWF, 125I-FVIII was preincubated in the above buffer in the presence of varying concentrations of vWF for 30 min at 37 °C. This was followed by determination of radioactivity bound to the wells. Affinity constants were derived from homologous and heterologous displacement data using the computer program LIGAND (41).

**Cell-mediated Ligand Internalization and Degradation Assays**—A normal mouse embryonic fibroblast line (MEF) and a mouse embryonic fibroblast cell line that is genetically deficient in LRP biosynthesis (PNA 13) were obtained from Dr. Joachim Herz (University of Texas Southwestern Medical Center, Dallas, TX) and maintained as described (42). Cells were seeded at 1 × 10⁵ cells/well and allowed to grow Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (Life Technologies, Inc.) for 24 h at 37 °C, 5% CO₂. Cellular internalization and degradation assays were conducted as described previously (43). Internalization and degradation of the 125I-labeled FVIII and A2 was measured after incubation for varying time intervals at 37 °C in 0.5 ml of Dulbecco’s modified Eagle’s medium containing 2% BSA. Surface binding of radiolabeled ligand was defined as the amount of radioactivity releasable by treatment of the cells by trypsin (50 μg/ml) and proteinase K (50 μg/ml) (Sigma) in a buffer containing 5 mM EDTA (44). This treatment was previously shown to release radioligands bound to cell surface (43), therefore a ligand which remained associated with the cells after this treatment was considered as internalized. Degradation was defined as radioactivity in the medium that is soluble in 10% trichloroacetic acid. The value of degradation was corrected for noncellular mediated degradation by subtracting the amount of degradation products generated in parallel wells lacking cells.

**Cleavage of 125I-FVIII/vWF Complex from Mouse Plasma**—Prior to the experiment, 125I-FVIII, vWF, and RAP were dialyzed into 20 mM Hepes, 0.15 mM NaCl, pH 7.2, containing 5 mM CaCl₂. The complex of 125I-labeled FVIII with vWF was formed by incubation of 125I-FVIII (15 nM) and vWF (750 nM) in the presence or absence of RAP (50–250 μM) for 30 min at 25°C. Sixteen BALB/c mice (12–14 weeks old, weight 20–24 g) were injected with 100 μl of the above solutions through the tail vein. Blood samples of 35–40 μl were withdrawn from each mouse via retroorbital puncture into 15 μl of 0.1 M sodium citrate buffer, pH 7.3, at the selected time intervals (1, 5, 10, 30, 60, 120, 240, 360, and 480 min). The samples were centrifuged and the radioactivity of plasma aliquots was measured and corrected for volumes of the blood sample withdrawn. The percentage of ligand remaining in the circulation was calculated considering the radioactivity of the aliquot taken at 1 min after injection as 100%. The time course of 125I-FVIII/vWF clearance for each RAP concentration in the injected sample (0, 50, 150, and 250 μM) was examined in four mice and averaged. At the end of experiment, animals were sacrificed, liver lobules and kidneys were excised and
RESULTS

Factor VIII Binds to LRP and Its Binding Is Prevented by RAP—The ability of fVIII to bind to LRP in vitro was examined in the homologous displacement binding assay. In the assay, binding of \(^{125}\text{I-fVIII} (1 \text{ nM})\) to purified LRP, but not to BSA-coated wells, was inhibited (>90%) by an excess of unlabeled fVIII (Fig. 2A). The quantitative data regarding fVIII interaction with LRP were derived from the homologous displacement of \(^{125}\text{I-fVIII}\) by unlabeled fVIII, which was adequately described by a model containing a single class of fVIII-binding sites with \(K_d\) of 116 nM. To elucidate whether fVIII in a complex with vWF is also able to bind to LRP, we tested the effect of vWF on \(^{125}\text{I-fVIII}\) binding to immobilized LRP. In this experiment, \(^{125}\text{I-fVIII}\) was preincubated with vWF as described under "Experimental Procedures" to allow complex formation prior to its addition to LRP-coated wells. As shown in Fig. 2A, the binding of fVIII to LRP was not inhibited by addition of up to 1000 nM vWF, which is 20-fold higher than its concentration in plasma (50 nM) (14). This indicates that the complex formation with vWF does not affect fVIII ability to bind to LRP.

RAP, an antagonist of LRP-ligand binding, completely inhibited the binding of \(^{125}\text{I-fVIII}\) to LRP-coated microtiter with \(K_i\) of 2.5 nM (Fig. 2B), the value is similar to the previously determined affinity (4 nM) of RAP for LRP (27). Together, these results demonstrate specific fVIII binding to LRP.

The Amino Acid Residues 484–509 within the fVIII A2 Domain Are Responsible for fVIII Binding to Purified LRP—In order to localize the fVIII region(s) involved in interaction with LRP, the binding between \(^{125}\text{I-fVIII}\) and immobilized LRP was competed by unlabeled fVIII fragments. As shown in Fig. 3, HCh and A2 domains of fVIII, but not LCh (AR-A3-C1-C2) or A1/A3-C1-C2 dimer, displaced \(^{125}\text{I-fVIII}\) from LRP in the heterologous ligand displacement from a single class of binding sites using the program LIGAND.

Fig. 2. Binding of \(^{125}\text{I-fVIII}\) to purified LRP by ligand competition assay. \(^{125}\text{I-fVIII} (1 \text{ nM})\) was incubated for 1 h at 37 °C in wells coated with LRP (●) or BSA (○) in the presence of increasing concentrations of competing ligands: unlabeled fVIII (●, ○) or vWF (△) (panel A) and RAP (●, ○) (panel B). Following incubation, the wells were washed and \(^{125}\text{I-fVIII}\) binding was determined. Binding of \(^{125}\text{I-fVIII}\) in the presence of unlabeled fVIII, vWF, or RAP is expressed as the percentage of \(^{125}\text{I-fVIII}\) binding, when no competing ligand was added. Each point represents the mean value of triplicates and the error bars display the standard deviation. The curves show the best fit of the data to the model describing heterologous ligand displacement from a single class of binding sites using the program LIGAND.

Fig. 3. Effect of fragments of fVIII on its binding to LRP. \(^{125}\text{I-fVIII} (1 \text{ nM})\) and increasing concentrations of unlabeled HCh (●), A2 (△), LCh (○), or A1/A3-C1-C2 (△) were incubated with LRP as described in the legend to Fig. 2. Each point represents the mean value and the standard deviation of the triplicate. The data were fitted as in Fig. 2 to a model describing heterologous ligand displacement from a single class of binding sites with \(K_i\) values of 120 and 132 nM for HCh and A2, respectively.
binding to LRP in a dose-dependent fashion, indicating that the region 484–509 of the A2 domain does contain residues critical for FVIII binding to LRP. In the control experiment, no binding of 125I-FVIII to BSA-coated wells was observed in the presence of peptide 484–509 (Fig. 4B).

**Internalization and Degradation of 125I-FVIII Complex with vWf by Cultured Fibroblasts Is Mediated by LRP**—Since the above data demonstrated a specific interaction between FVIII and LRP, and vWf did not interfere with this interaction, we hypothesized that LRP may also be capable of mediating the cellular internalization of 125I-FVIII from its complex with vWf. To examine this hypothesis, the cellular uptake and degradation experiments were conducted on MEF which express LRP and on PEA 13 fibroblasts that are genetically deficient in LRP (42). The FVIII-vWf complex was prepared by mixing FVIII and vWf at their plasma concentrations of 1 and 50 nM, respectively. As shown in Fig. 5, A and B, MEF cells, but not PEA 13 cells lacking LRP, were capable of internalizing and degrading 125I-FVIII in the presence of vWf. In addition, internalization and degradation of 125I-FVIII by MEF but not by PEA 13 fibroblasts was inhibited by RAP, an antagonist of ligand binding to LRP. The ability of RAP to block the uptake and degradation of FVIII/vWf in MEF cells and inability of PEA 13 cells to efficiently mediate the uptake and degradation indicates that LRP is a mediator of FVIII/vWf catabolism. To further characterize the degradation pathway of FVIII in MEF cells, we tested the effect of chloroquine (an agent that blocks lysosomal degradation) on FVIII degradation. As shown from Fig. 5B, the degradation of 125I-FVIII is completely inhibited by chloroquine.

To elucidate whether cellular mediated endocytosis of FVIII in the absence of vWf is also mediated by LRP, we compared internalization and degradation of 125I-FVIII-vWf complex and isolated 125I-FVIII (Fig. 6). As seen from Fig. 6, A and B, both internalization and degradation of isolated 125I-FVIII by MEF fibroblasts is approximately 2-fold higher than that in the presence of vWf. RAP inhibited internalization and degradation of 125I-FVIII to a lesser degree than that of 125I-FVIII-vWf complex. In addition, LRP-deficient PEA 13 fibroblasts were able to internalize and degrade isolated 125I-FVIII. This indicates that the LRP-mediated pathway is not the sole mechanism of internalization and degradation of FVIII not complexed with vWf.

To determine whether vWf bound to FVIII is also internalized and degraded by MEF cells, we measured internalization and degradation of 125I-labeled vWf complexed with FVIII. As shown in Fig. 6, A and B, the amounts of internalized and degraded 125I-vWf by both MEF and PEA13 cells were less than 5% of the corresponding amounts of 125I-FVIII catabolized from its complex with vWf under the same experimental conditions. This indicates that vWf does not follow FVIII in the LRP-mediated pathway and possibly dissociates from FVIII at the early stage of endocytosis, prior to entry of the complex into endosomal compartments.

**The A2 Subunit of FVIII Inhibit Endocytosis and Degradation of 125I-FVIII/vWf by LRP-expressing Cells**—Since we demonstrated that the A2 subunit of FVIII is responsible for interaction of FVIII with purified LRP in vitro, we next examined whether A2 is also involved in LRP-mediated internalization and degradation of FVIII-vWf complex by LRP-expressing cells. Fig. 7, A and B, demonstrate that 1000-fold excess of the A2 subunit over 125I-FVIII-vWf complex effectively inhibited internalization (by >70% after 4 h) and degradation (by >60% after 4 h) of this complex. In contrast, the A1/A3-C1-C2 heterodimer, which did not inhibit FVIII interaction with purified LRP in the above experiments, did not have any effect on 125I-FVIII endocytosis and degradation by MEF cells (Fig. 7).

**Effect of RAP on the Plasma Clearance of 125I-FVIII**—To determine whether LRP is capable of catabolizing FVIII from its complex with vWf in vivo, the effect of RAP on the clearance rate of 125I-FVIII-vWf complex was tested in mouse model. In the experiment, each mouse was injected with approximately 1 μg of 125I-FVIII (45) complexed with vWf in the presence or absence of RAP. The amount of administered FVIII was similar to that previously used to study FVIII clearance in mice (48, 49). As shown in Fig. 8, RAP increased the half-life of 125I-FVIII in mouse plasma. The time courses of FVIII clearance in the presence of 150 or 250 μM RAP in the injected sample, suggested that RAP concentration of 150 μM is saturating and its further increase does not appreciably affect the FVIII clearance. In the presence of the saturating concentration of RAP the half-life of 125I-FVIII was prolonged by approximately by 3.3-fold. In addition, in the absence of RAP, most radioactivity was found in the liver but not in kidney, results that are consistent with LRP presence in a high abundance in hepatic tissues (31).

To determine whether 125I-FVIII remains bound to vWf after injection into mice, some aliquots of mice plasma taken at various time intervals (5–480 min) were also subjected to fast protein liquid chromatography as above. It was found that for the time intervals of up to 480 min, 125I-FVIII (>90% of the total eluted radiactivity) was eluted as a single peak in the void.
volume of the column as would be expected for the FVIII-vWf complex, demonstrating that $^{125}$I-FVIII remained bound to vWf in circulation in the course of clearance studies.

In the absence of RAP, clearance of FVIII was biphasic, requiring a double exponential model (Equation 1, "Experimental Procedures") to adequately fit the data. The results are consistent with the existence of fast ($k_1 = 0.0196$ min$^{-1}$) and slow phases ($k_2 = 0.00329$ min$^{-1}$) of FVIII removal from the circulation. By contrast, in the presence of a saturating concentration of RAP (250 µM), the clearance could be well described by
a single exponential equation with \( k = 0.00334 \, \text{min}^{-1} \), a value close to that of the slow phase in the absence of RAP. Since the slow phase of clearance was not RAP-sensitive, we then fitted all four curves to Equation 1 using three fitting parameters: \( C_1, C_2, \) and \( k_1 \), and a constant value for \( k_2 \) as described under “Experimental Procedures.” At indicated time points, blood samples were taken and counted for radioactivity. The percentage of labeled remaining in circulation was calculated considering radioactivity of the aliquot taken at 1 min after injection as 100%. \( ^{125}\text{I}-\text{fVIII} \) clearance for each RAP concentration was examined in four mice, and the data plotted represent the average value ± S.D. The solid lines show the best fit of the experimental data to Equation 1 (“Experimental Procedures”) describing bi-phasic exponential clearance of fVIII. Residual plots showing deviations of the experimental data from the fitted curves are presented for the experiments performed in the absence of RAP (●) and in the presence of 250 \( \mu\text{M} \) RAP (□).

**Table I**

| RAP  | \( C_1 \) | \( C_2 \) | \( k_1 \) | \( k_2 \) |
|------|----------|----------|----------|----------|
| \( \mu\text{M} \) | % | \( \text{min}^{-1} \) | % | \( \text{min}^{-1} \) |
| 0     | 56.6 ± 3.6 | 43.4 ± 2.7 | 0.0196 ± 0.0026 | 0.00329 ± 0.0009 |
| 50    | 28.8 ± 5.6 | 71.2 ± 5.8 | 0.0106 ± 0.0054 | 0.00329 ± 0.0009 |
| 150   | 11.2 ± 4.8 | 88.8 ± 6.3 | 0.001433 ± 0.0005 | 0.00329 ± 0.0009 |
| 250   | 5.9 ± 2.6 | 94.1 ± 4.8 | 0.0008 ± 0.0005 | 0.00329 ± 0.0009 |

Based on the observation that vWF did not inhibit fVIII binding to purified LRP, we propose that RAP-mediated internalization of fVIII from its complex with vWF may be a physiological mechanism of fVIII catabolism. Indeed, fibroblast cells that express LRP, but not fibroblasts genetically deficient in LRP, were able to internalize and degrade \( ^{125}\text{I}-\text{fVIII} \) from its complex with vWF. These processes were also inhibited by RAP, indicating that cellular internalization and degradation were mediated by interaction of fVIII with LRP.

The physiological relevance of observations utilizing the LRP-expressing cell model system was supported by in vivo clearance studies of \( ^{125}\text{I}-\text{fVIII}/\text{vWf} \) complex in mice which demonstrated that RAP prolonged the half-life of \( ^{125}\text{I}-\text{fVIII} \) in the circulation by 3.3-fold, indicating that a RAP-sensitive receptor, most likely LRP, contributes to the plasma clearance of fVIII. We found that fVIII clearance is a biphasic process consisting of fast and slow components. It is essential to recognize that only the fast phase of the clearance is LRP-mediated since RAP inhibited only this phase but not the slow one. Thus, the slow phase of fVIII clearance is not LRP-mediated and the mechanism responsible for this pathway remains to be identified.

Biphasic fVIII clearance in mice, observed in the present study, is in agreement with results of a previous study in which fVIII clearance in mice was described by the sum of two exponentials (45). The biphasic nature of fVIII clearance was also demonstrated for dogs (50) and humans (17). Based on these parallels, we predict that the fast phase of fVIII clearance in humans is also LRP-mediated. One might then anticipate that inhibition of this pathway would increase the half-life of infused fVIII by several fold. This would be especially important for the prophylactic treatment of hemophilia A patients who require frequent fVIII infusions to maintain its minimal level in circulation. This might significantly reduce the cost of the care of hemophilia A patients by reducing the frequency of infusions.

One approach to suppress LRP-mediated clearance of fVIII would be to generate a fVIII mutant in which the LRP-binding site is disrupted. We localized the 25-amino acid region 484–509 within the A2 domain was inferred from finding that a monoclonal antibody with an epitope within residues 484–509 completely inhibited fVIII interaction with LRP. At the same time, inhibition of fVIII/LRP binding by the synthetic peptide corresponding to residues 484–509 also indicated that this
region of the A2 domain is likely to be directly involved in VIII binding to LRP.

The region 484–509 contains 6 positively charged residues, 3 lys at positions 493, 496, and 499 and 3 arg at positions 484, 489, and 490. Basic residues in lipoprotein lipase (47), urokinase-type/plasminogen activator inhibitor 1 complex (50), and \(\alpha_2\)-macroglobulin (52) were previously shown to be critical for electrostatic interaction with LRP. Alanine substitution of the basic amino acid residues involved in binding to LRP in the above ligands leads to a substantial reduction of affinity for ligand binding to LRP and partial (51) or complete (52) inhibition of internalization and degradation of the mutants. Therefore, mutation of charged residues within the 484–509 region of fVIII may be a feasible approach for generation of a recombinant fVIII with a lower rate of LRP-mediated endocytosis and a longer half-life in circulation.

We found that internalization and degradation of isolated fVIII by LRP-presenting MEF cells was more effective than the corresponding processes for fVIII bound to vWF. Faster catabolism of fVIII in the absence of vWF is consistent with the demonstrated shorter half-life of fVIII in patients with severe von Willebrand disease lacking plasma vWF in comparison with those for LCh by 25-fold (57), which raises a possibility that the corresponding processes for fVIII bound to vWF. Faster catabolism of fVIII may be a feasible approach for generation of a recombinant fVIII region of the A2 domain is likely to be directly involved in binding to LRP.

In the recent study related to fVIII interaction with LRP, Lenting et al. (57) made several observations consistent with our study. (i) fVIII binds to purified LRP with the affinity similar (within 2-fold) to that determined in our study. (ii) RAP completely inhibits fVIII binding to purified LRP. (iii) LRP-expressing cells degrade fVIII and this process is only partially inhibited by RAP. In addition, it was shown using the biosensor technique, that (iv) immobilized HCh interacts with the recombiant domain II of LRP, containing 8 out of 31 cysteine-rich similar (within 2-fold) to that determined in our study. (v) fVIII and secondary vWF-mediated release of endogenous fVIII in von Willebrand disease lacking plasma vWF in comparison with those for LCh by 25-fold (57), which raises a possibility that the corresponding processes for fVIII bound to vWF. Faster catabolism of fVIII may be a feasible approach for generation of a recombinant fVIII with a lower rate of LRP-mediated endocytosis and a longer half-life in circulation.

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