Matrix glycoprotein differentially changes in liver regeneration and cirrhosis: Glycosylation alters ligand binding in matrix remodeling

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Abstract

Background

Vitronectins (VN) are multifunctional adhesive glycoproteins that are present in plasma and the extracellular matrix of most tissues. We previously reported that the collagen-binding activity of VN is enhanced by a change in glycosylation *in vitro* and during liver regeneration after partial hepatectomy *in vivo* [Uchibori-Iwaki *et al.*, *Glycobiology* (2000) **10**, 865-874]. Plasma concentrations of VN declined in rats during liver regeneration 24 h after partial hepatectomy, while carbohydrate concentrations of VN decreased to 2/3 of that in sham-operated rats. Carbohydrate composition and lectin reactivity indicated that the *N*-glycan structures and sialylation significantly changed without affecting the peptide portion after partial hepatectomy. VN from partially hepatectomized rats were found to exhibit markedly enhanced binding to type I collagen. The enzymatic deglycosylation of VN demonstrated that collagen binding increased by 1.2 times after de*N*-glycosylation of VN, while it increased by more than 2.9 times after desialylation. To elucidate the biological significance of glycan modulation, changes of VN in cirrhosis were studied and compared with those during liver regeneration.

Methods

VNs purified from patients' and normal plasma were examined for plasma concentration and collagen-binding activity by ELISA, reactivity against lectins by dot blotting, and carbohydrate composition.

Results

Plasma concentrations of VN declined in chronic liver diseases in the order of hepatitis>cirrhosis>hepatocellular carcinoma with cirrhosis. Lectin reactivity and carbohydrate analyses of purified VN from cirrhotic plasma (LC-VN) indicated that sialylation was elevated. LC-VN exhibited decreased binding to type I collagen. Collagen-binding studies of plasma before and after urea-treatment indicated that the active form of VN increased in cirrhotic plasma.

Conclusions

The attenuated collagen-binding activity of LC-VN is attributable to a change of glycosylation. The increase of active VN in cirrhotic plasma may contribute to the matrix incorporation of VN. These findings suggest that the collagen binding activity of VN is modulated by the alteration of peptide glycosylation during liver regeneration after partial hepatectomy and during the pathological processes, which may contribute to the tissue remodeling processes.

Introduction

Tissue homeostasis depends on spatially and temporally controlled expression of multifunctional adhesive glycoproteins and their receptors. Vitronectin (VN) is a multi-functional adhesive glycoprotein that originates mainly in hepatocytes and circulates in the blood at high concentrations (0.2 mg/ml in humans). VN is also found in the extracellular matrix of most tissues. Tissue VN is present as an active multimeric form that interacts with various matrix ligands, such as integrins, type I plasminogen activator inhibitors, and urokinase receptors (reviewed in [1]) (Fig. 1). By providing a link between plasmin-regulated matrix proteolysis and integrin-mediated cell migration, activated VN plays a central role in matrix remodeling. VN can also bind to various types of collagen through its conformational transition from the native inactive form to an active form [2]. Collagen binding may incorporate VN into the extracellular matrix by providing an entry into the tissue and distributing VN to regulate the matrix reorganization. The collagen-binding activity was affected by the presence or absence of *N*-glycan covalently linked to VN [3]. Previously we determined the structures of *N*-glycans on human and porcine VNs [4, 5] (Fig. 2). Recently, we found that the change in glycosylation after partial hepatectomy enhanced the collagen-binding activity of VN [6]. On the other hand, the collagen-binding VN in plasma in chronic liver diseases was reported to increase and correlate with certain fibrous markers [7]. In this study, to elucidate the glycan modulation, changes in VN during liver cirrhosis were studied and compared with the changes during liver regeneration.

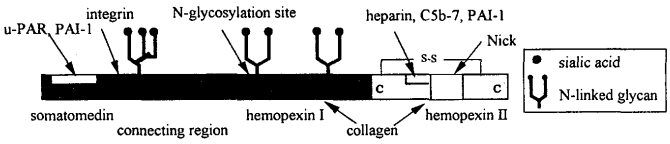

Fig. 1. Glycosylation and domain structure model of human VN.

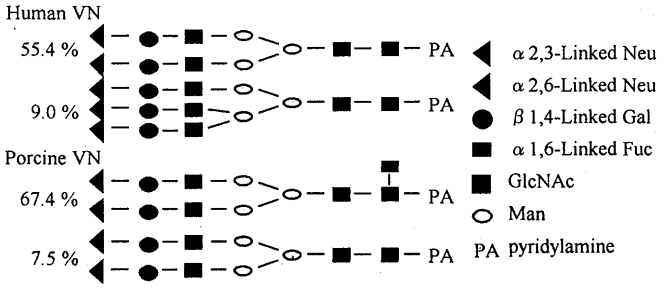

Fig. 2. Major oligosaccharide structures of human and porcine VNs.

Materials and Methods

Materials

Sheep anti-human VN IgG was purchased from the Binding Site Ltd. (Birmingham, England), and horseradish peroxidase (HRP)-conjugated rabbit anti-sheep IgG and various lectins were commercially obtained and labeled with biotin in our laboratory. Type I collagen from porcine skin and other reagents were special grade from Wako Pure Chemicals (Osaka, Japan).

Purification of VN from human plasma

Human VNs were purified from plasma of 8 patients (3 with hepatitis, 3 with cirrhosis, 2 with hepatocellular carcinoma with cirrhosis) and 2 healthy controls as previously described [6].

Carbohydrate analyses

Neutral, amino sugars and sialic acids were analyzed as previously reported [6]. The amounts of purified VN were estimated by the absorbance at 280 nm using the absorption coefficient of 1.38 at 1 mg/ml.

Protein determination

Total protein was measured by modified Bradford method and the VN concentration in plasma was measured by an enzyme-linked immunosorbent assay, ELISA, with an anti-VN monoclonal antibody.

Reactivity of VNs with biotin-lectins

Samples (200 μl) were dot-blotted onto PVDF membrane (Millipore) and reacted with biotinyl lectins in 0.13 M NaCl in 10 mM phosphate buffer (pH 7.5, PBS). After reacting with avidin biotin complex-HRP, the membrane was developed with 4-chloro-1-naphthol/H_2O_2 according to the method previously reported [6].

Assays for binding of VN to immobilized type I collagen

Collagen-binding activities of purified normal (N-) or LC-VNs, or VNs in plasma before and after urea treatment were assayed by ELISA according to the method reported previously [3]. For activation by denaturation, urea was added to plasma, and it was diluted to 10 volumes with PBS containing 5 mM EDTA and 1 mM phenylmethylsulfonyl fluoride to a final concentration of 8 M urea. The solution was incubated for 2 hours at 37°C and diluted with 4 volumes of PBS for ELISA.

Results

Plasma concentration and purification of VNs from normal and cirrhotic subjects

In immunoassays, absorbances at 490 nm were considered to be proportional to the amount of VNs because the immunoreactivities of LC- and N-VNs toward the antibodies used in the assay were unchanged (data not shown). As shown in Fig. 3, the VN concentration in cirrhotic plasma decreased to about 2/3 of that in normal plasma in parallel with the decrease of total protein concentration in plasma. Yields of purified VN markedly decreased in liver cirrhosis, *i.e.*, the amount of purified N-VN from 1 ml of plasma was about 0.13 mg, but that of LC-VN was about 0.04 mg. Both LC-VN and N-VN gave double bands corresponding to about 65 and 75 kDa on SDS-PAGE.

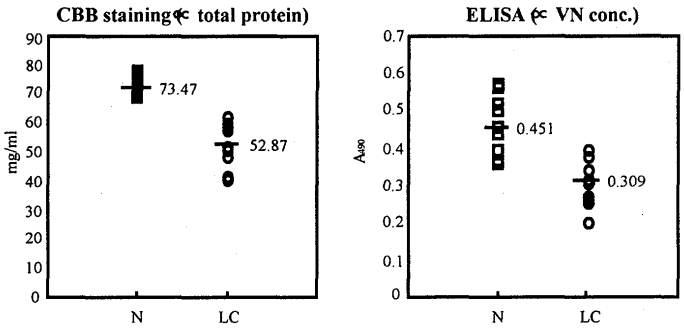

Fig. 3. VN and total protein concentrations in plasma from 10 normal (square) and 10 cirrhotic (circle) subjects as described under Materials and Methods.

Interaction of VNs with biotin-lectins

VNs were reactive with *N*-glycan-specific lectins, concanavalin A, and *Ricinus communis agglutinin*. Enhanced reactivity with *Datura stramonium*, *Aleuria aurantia*, and *Sambucus nigra* indicated increases of tri- or tetraantennary lactosamine branching, fucosylation to the innermost or outermost GlcNAc residue, and sialyl α 2-6Gal sequences in LC-VN (Table 1). Carbohydrate analyses indicated that both VNs contained GlcNAc, Gal, Man and Fuc, suggesting that the presence of *N*-linked glycans. Sialic acid had increased in LC-VN by 1.6-fold that of N-VN concomitantly

with the increase of Gal residues.

lectin	N	LC	specificity
ConA	++	++	*N*-linked biantennary or□ oligomannose type
RCA	++	++	nonreducing terminal Gal
DSA	+	++	tri- or tetra-antennary or□ (Gal β 1-4GlcNAc)n
LEL	−	−	(Gal β 1-4GlcNAc)n
AAL	+	++	Fuc
SNA	+	++	sialyl α 2-6Gal
SNA*	±	±	

SNA* : reactivity after desialylation

Table 1. The lectin reactivity of VNs illustrates the change of glycans during cirrhosis. Reactivity was measured with biotinyl lectins on the membrane as described in Materials and Methods and expressed as staining intensities.

Type I collagen-binding activity of purified VNs and in plasma before and after denaturation

The collagen-binding activity was measured by ELISA. VNs in untreated plasma exist mainly in native cryptic form, and they are activated by denaturing treatment [1]. As shown in Fig. 4 and reported previously by Yamada *et al.* [7], VN in untreated cirrhotic plasma bound to collagen better than VN in normal plasma did, whereas urea treatment increased the reactivity of VN in normal plasma much more than in cirrhotic plasma. Urea treatment increased the reactivity about 6-fold in normal plasma but 2-fold in cirrhotic plasma. In contrast, the collagen-binding activity of LC-VN was 63% of N-VN (data not shown). Together with the low VN concentration in cirrhotic plasma (Fig. 3), the results suggest that the percentage of the active form of VN is considerably higher in cirrhotic plasma than normal plasma.

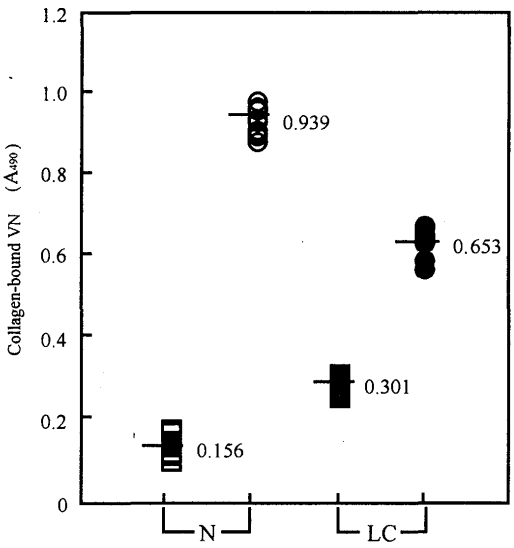

Fig. 4. Activation of collagen binding of native VNs in plasma by treatment with urea. Collagen-binding activities of VN in normal 10 (open) and cirrhotic 10 (closed) plasma before (square) or after (circle) urea treatment were assayed by ELISA as described in Materials and Methods.

Discussion

This study showed that VN in cirrhotic subjects differs from VN in normal individuals in its plasma concentration, glycosylation and collagen-binding activity (schematically represented in Fig. 5). The attenuated collagen-binding activity of LC-VN is attributable to a change of glycosylation, *i.e.*, increased sialylation of LC-VN compared to N-VN, because decreased sialylation enhances the collagen binding of VN produced during liver regeneration [6] (Fig. 5). In cirrhotic plasma, a considerably higher percentage of VN was found to be in the active form than in normal plasma. This may account for the low purification yield of LC-VN, because active VN in plasma binds to the heparin column under a non-denatured condition and is removed by the first heparin affinity chromatography. The increase of active VN in cirrhotic plasma may contribute to the matrix incorporation of VN and subsequent repair or remodeling processes.

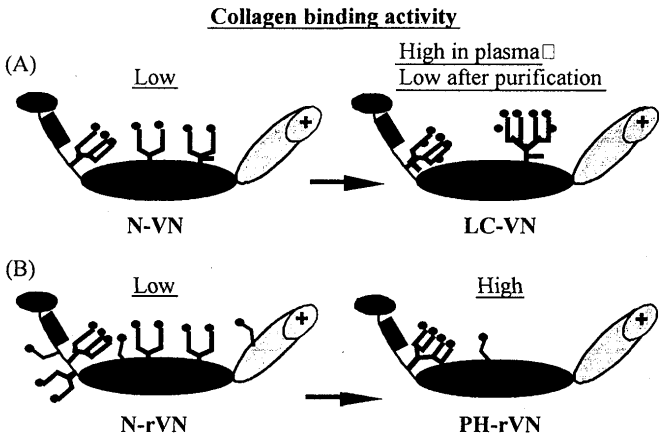

Fig. 5. Changes of glycosylation and collagen binding of LC-VN (A) and rat VN of unoperated (N-rVN) and VN produced during liver regeneration (PH-rVN) (B).

Acknowledgements

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