J. Biochem. 120, 954-960 (1996)

Porcine Vitronectin, the Most Compact Form of Single-Chain Vitronectin: The Smallest Molecular Mass among Vitronectins Was Ascribed to Deletion and Substitution of Base Pairs, and Proteolytic Trimming of the Peptide[1]

Atsuko Yoneda,* Kyoko Kojima,† Isamu Matsumoto,† Kazuo Yamamoto,‡ and Haruko Ogawa†[2]

**Doctoral Research Course in Human Culture and †Department of Chemistry, Faculty of Science, Ochanomizu University 2-1-1 Otsuka, Bunkyo-ku, Tokyo 112; and ‡Faculty of Pharmaceutical Science, The University of Tokyo, Bunkyo-ku, Tokyo 113*

Received for publication, June 6, 1996

Vitronectin is a multifunctional glycoprotein regulating the fibrinolysis, complement, and coagulation systems in plasma, besides exhibiting cell-spreading activity. Porcine vitronectin has an unusually small molecular mass among the vitronectins hitherto found, which seems to make it hard for it to retain all the known activities. In this study, the complete primary structure of porcine vitronectin was elucidated by cloned cDNA and glycoprotein analyses. A coding sequence of 459 amino acids including a signal peptide of 19 amino acids was deduced from the cDNA. The coding sequence showed 70.3% homology with that of human vitronectin, but porcine vitronectin lacked 22 amino acids in the connecting region. One amino acid substitution resulted in the loss of a potential glycosylation site in accordance with the finding on glycopeptide analyses that porcine vitronectin contained two kinds of glycosylated sequences, while human vitronectin contained three. C-Terminal analysis of porcine vitronectin indicated that an 80 amino acid fragment was completely removed from the C-terminal end on proteolytic processing. Thus, porcine vitronectin only exists in a truncated single-chain form representing the most compact functional form of vitronectin, which suggests the lack of functional necessity of the truncated C-terminal fragment.

Key words: cDNA cloning, C-terminal analysis, glycosylation site, vitronectin.

Vitronectin (VN) has long been regarded as a cell-adhesive glycoprotein present in plasma. Recently, it has been considered to be a multifunctional glycoprotein that may regulate a number of physiologically important cascade processes (reviewed in Refs. *1-4*). For example, VN may regulate the fibrinolysis system by stabilizing plasminogen activator inhibitor-1, and the complement system by inhibiting cell lysis with the complement terminal complex, besides playing a role in cell adhesion *via* interaction with various types of integrin on the cell surface. VN can also bind to different types of collagen, the thrombin-antithrombin III (AT-III) complex, and heparin-like glycosaminoglycans, and through these interactions it may be concerned in the constitution of the extracellular matrix and the coagulation system. The biological activities of vitronectin are based on its ability to interact with various molecules, and several binding sites have been mapped to functional domains of its peptide chain (*1-4*), but others remain ambiguous. The characteristics of the primary structure need to be clarified to establish the structure-function relationship of VN.

cDNA sequences have been reported so far for human (*5, 6*), rabbit (*7*), and mouse (*8*) VNs. Human, rabbit and mouse VNs showed similar molecular masses (59, 53, and 56 kDa, respectively) on Ferguson plot analyses (*9*). Their molecular masses are in good agreement with those calculated from their carbohydrate contents and cDNA sequences, which exhibited high homology throughout the molecule including the number of potential glycosylation sites (*10*). The cDNA sequences together with those obtained on amino acid sequence analyses indicated that human VN (hVN) has two forms, single-chain (59 kDa) and two-chain (50+9 kDa), the latter having a nick in the C-terminal half but having the two chains linked with a disulfide bond (*11*). The two forms of hVN are derived through a DNA polymorphism of the VN gene, one form of which codes a proteinase-resistant (single-chain) VN and

[1] This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas, No. 05274107, from the Ministry of Education, Science, Sports and Culture of Japan, the Hayashi Memorial Foundation for Female Natural Scientists, and a Sasakawa Scientific Research Grant from The Japan Science Society. The nucleotide sequence data reported in this paper have been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence databases under the accession number D63145.

[2] To whom correspondence should be addressed. Tel: +81-3-5978-5343, Fax: +81-3-5978-5344, E-mail: hogawa@hososipc.chem.ocha.ac.jp

Abbreviations: VN, vitronectin; pVN, porcine VN; hVN, human VN; cDNA, complementary DNA; AT-III, anti-thrombin III.

the other a proteinase-sensitive (two-chain) VN (*12*). There is 10% of the single-chain form in human plasma, however, even when only the sensitive type of VN gene is present, and conversely 11-29% of the two-chain form when the resistant type of VN gene is present. Thus individuals possess two forms of VN in their plasma in various ratios. Many other species possess two forms of VN too, but the functional necessity and significance of these VN chain types remain unknown. On the other hand, porcine VN (pVN) has been observed only in a single-chain form and its molecular mass was unusually small (44 kDa) among the VNs from the 27 species so far investigated (*9*). Our previous studies showed that pVN had N-terminal sequences homologous to those of other VNs (*9*), similar carbohydrate contents (9%) (*13*), and complex-type *N*-linked oligosaccharides as well as hVN (*14, 15*). Considering the conservative cDNA sequences hitherto isolated, it cannot be explained how pVN exhibits heparin-binding activity, because its molecular mass is too small for it to contain a heparin-binding region (located at 48-50 kDa from the N-terminal end of hVN). In this study, in order to elucidate its complete primary structure and to answer the above question, we performed cDNA cloning, C-terminal peptide sequence analysis and *N*-glycosylation site determination of pVN.

MATERIALS AND METHODS

Materials—PVN and hVN were prepared from porcine and human sera, respectively, as described previously (*13*). All restriction enzymes, T4 DNA ligase, M13 mp18, T4 polynucleotide kinase, alkaline phosphatase, and *Escherichia coli* DNA polymerase (Klenow fragment) were purchased from Takara Shuzo, Kyoto. The porcine and human liver cDNA libraries in λgt10 were from Clontech Lab., Palo Alto, CA, USA. [α-^{32}P]dCTP and [γ-^{32}P]dATP were from Amersham Int. plc, Buckinghamshire, UK. *N*-α-Tosyl-L-lysine chloromethylketone-treated α-chymotrypsin (bovine pancreas) and diphenyl carbamyl chloride-treated trypsin (bovine pancreas) were from Sigma Chem., St. Louis, MO, USA. Concanavalin A-agarose was from Seikagaku Kogyo, Tokyo. The μ Bondasphere 5μ C18 300A column (3.9×150 mm) was from Millipore.

Preparation of Probes—For use as a probe, a hVN cDNA was screened and isolated from a human liver cDNA library in λgt10 using two ^{32}P-labeled oligonucleotide probes, [GGAACCGTTCTTGAGGTTGGTGAAGGCGTC] [complementary oligonucleotide encoding the amino acid sequence from 143 to 152 (*5*)] and [GATGTCTGGGGCATCGAGGGCCCCATCGAT] [encoding the amino acid sequence from 179 to 188 (*5*)]. The cloned hVN cDNA was radiolabeled by means of the DNA polymerase (Klenow fragment) reaction using a random primer and [α-^{32}P]dCTP. Mixed oligonucleotides [GAT(C)TAT(C)CAT(C)GAA(G)GAA(G)AC] coding for Asp-Tyr-His-Glu-Glu-Thr (one of the sequences obtained from the digest of pVN with endoproteinase Asp-N, see Fig. 1) were synthesized and radiolabeled by means of the kinase reaction using [γ-^{32}P]dATP.

Isolation and Sequence Analysis of pVN cDNA—A porcine liver cDNA library in λgt10 was screened by the standard plaque hybridization technique using two kinds of probes (hVN cDNA and mixed oligonucleotides). The phage DNAs of the resulting positive clones were prepared by the plate lysate method, and the cDNA inserts were analyzed by Southern blotting, then subcloned into the *Eco*RI site of M13mp18. Sequence analyses of single-stranded templates were performed by the dideoxynucleotide chain termination method after heat denaturation (100°C, 15 min) using a 373S DNA sequencer (Perkin-Elmer/Cetus). The sequences were determined on both strands.

Preparation of the Glycopeptide Fraction—Digestion with proteases: PVN was digested with three proteases, as shown below. Digestion with α-chymotrypsin was performed as described previously (*14*), and with endoproteinase Asp-N following the manufacturer's standard protocol. Digestion with trypsin was performed as below. VN in 50 mM Tris-HCl (pH 7.8) containing 1 mM $CaCl_2$ and 30 mM NaCl was digested with trypsin (2%, by mass) at 37°C for 24 h. EDTA and phenylmethanesulfonyl fluoride were added to the digest to 1 mM, then the mixture was boiled for 10 min.

Concanavalin A Affinity Chromatography: The resulting digest was desialylated by mild acid treatment to decrease the microheterogeneity caused by sialylation of oligosaccharides, as described previously (*14*). Then the product was applied to a concanavalin A-agarose column (0.75×3 cm) equilibrated with 0.1 M sodium acetate buffer (pH 6.7) containing 1 mM calcium chloride and 1 mM magnesium chloride. Glycopeptides were eluted with the column buffer containing 0.1 M methyl α-D-mannoside.

Separation and Sequence Analyses of Glycopeptides—Glycopeptides in the eluate were further separated and purified by reverse-phase HPLC (μ Bondasphere 5μ C18 300A column) using three solvents, solvent A (water containing 0.05% trifluoroacetic acid), solvent B (2-propanol containing 0.05% trifluoroacetic acid), and solvent C (acetonitrile). The column was equilibrated with solvent A at the flow rate of 1.0 ml/min at 40°C. After injection of a sample, the proportions of solvents B and C were increased linearly to 42 and 18%, respectively, in 60 min. The glycopeptides obtained were analyzed with an automated protein sequencer model 476A (Perkin-Elmer/Cetus).

Isolation of the C-Terminal Fragment of pVN—The experimental procedure followed that for the automated C-terminal fragment fractionator (CTFF-1; Shimadzu) used. Briefly, pVN (400 pmol) was digested with endoproteinase Lys-C (2 pmol) for 36 h at 37°C, then the product was applied to the apparatus. The digested polypeptides were covalently immobilized through their N-terminal α- and C-terminal ϵ-amino groups on *p*-phenylene diisothiocyanate polymer beads. Only the C-terminal fragment with no Lys residue was eluted on cleavage at the first peptide bond of the immobilized peptides with trifluoroacetic acid, and it was collected automatically. The sequence of the fragment was determined with a protein sequencer (Shimadzu).

RESULTS

Isolation and Sequence Analysis of pVN cDNA—A porcine liver cDNA library in λgt10 (about 40,000 phages) was screened as described under "MATERIALS AND METHODS," two positive clones encoding the pVN open reading frame being obtained. The sequence of pVN cDNA determined is shown in Fig. 1. Two clones had the same cDNA

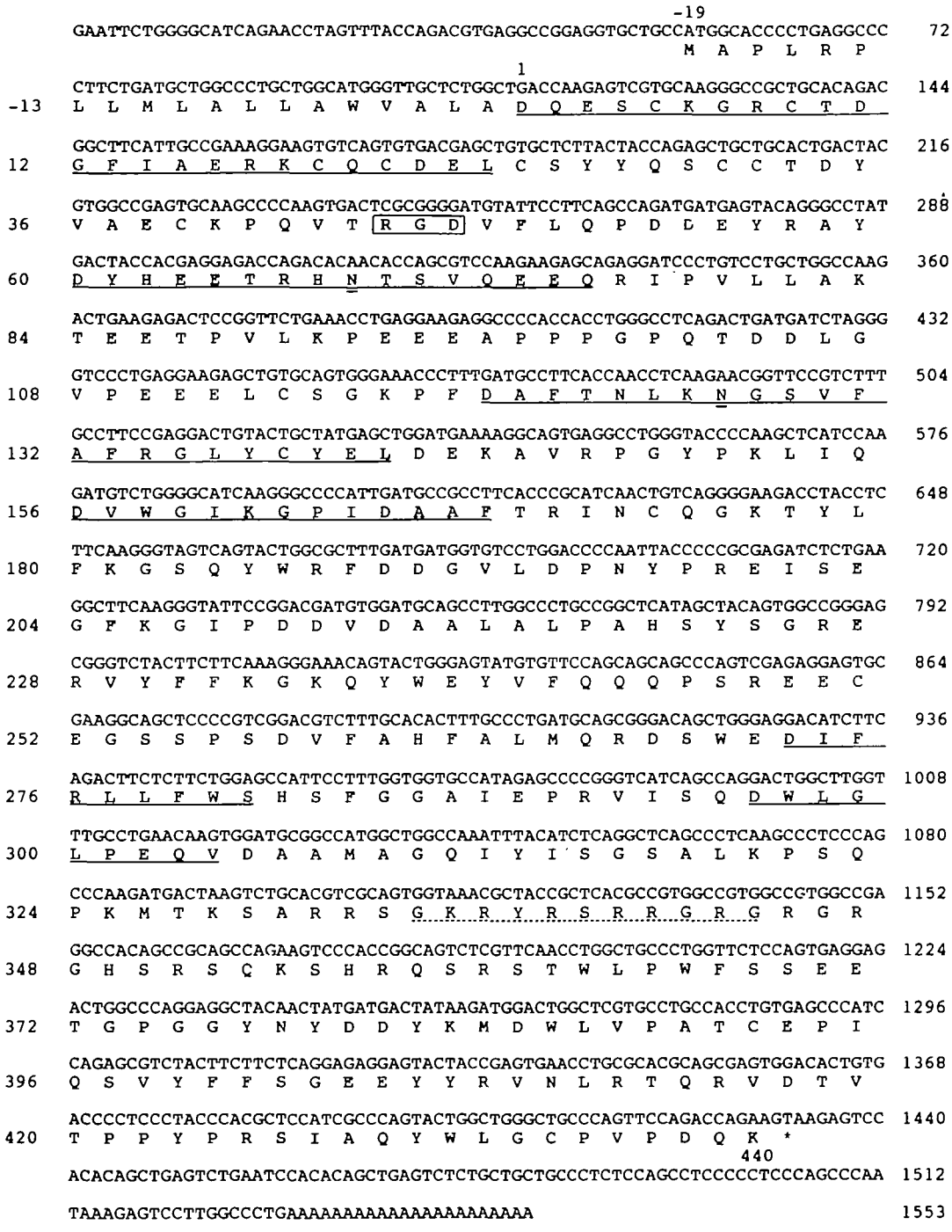

Fig. 1. **The pVN cDNA sequence and amino acid sequence deduced from pVN cDNA.** The deduced amino acid sequence is shown in a single-letter code beneath the nucleotides. The numbers on the right are the positions of nucleotides, and those on the left the positions of amino acids. The box represents a cell-attachment site, the double line a potential *N*-glycosylation site, and the broken line a heparin-binding consensus sequence (X-B-B-X-B-X, where B symbolizes a basic amino acid residue). The asterisk represents the stop codon. The sequences obtained for digests with endoproteinase Asp-N are underlined.

sequence, although the lengths of the 3′ end non-coding regions of the inserts were different. As the N-terminal amino acid sequence of the purified pVN was DQESXKGRXTDGFIAERKXQX, in a single-letter code (*9*), Asp at position 1 in Fig. 1 was assigned as the N-terminal amino acid residue. This result together with the amino acid sequence of hVN (*5, 6*) suggested that the first 19 residues (amino acids −19 to −1 in Fig. 1) comprise a signal peptide. The amino acid sequence thus deduced from the pVN cDNA sequence was composed of 459 amino acids including the 19 amino acids of the signal peptide, the molecular mass of the deduced pVN polypeptide without the signal peptide being calculated to be 50,525 Da. A cell attachment sequence, Arg-Gly-Asp (amino acids 45 to 47),

TABLE I. **Amino acid sequences of glycopeptides obtained from VNs on concanavalin A affinity chromatography.** X denotes an unidentified amino acid. Underlining indicates the consensus sequence for *N*-glycosylation. Each fragment corresponds with a peak in Fig. 3, A and B.

	Enzyme	Fragment	Sequence	Amino acids
pVN	Trypsin	A1	HXTSVQEEQ	67-75
		A2	XGSVF	127-131
hVN	α-Chymotrypsin	B1	TMPEDEYTVYDDGEEKNXATVHE	50-72
		B2	DAFTDLKXG	143-151
		B3	RFEDGVLDPDYPRXISDG	210-227

two potential *N*-glycosylation sites (Asn68 and Asn127), and a tandem repeat of the heparin-binding consensus sequence [XBBXBX, B is a basic amino acid (*16*)], amino acids 334 to 349 and 349 to 354, were found in the sequence.

DNA sequence analysis of the hVN clone indicated it was the same as that Jenne and Stanley reported (*5*), although substitutions were found only at the positions at which single codon substitutions are known to frequently occur (*2*). The substitutions found in the clone are indicated in Fig. 2.

Alignment with the Amino Acid Sequences of Other VNs—The amino acid sequence of pVN deduced from the cDNA was aligned with those of hVN (*5*), rabbit VN (*7*), and mouse VN (*8*), as shown in Fig. 2. Gaps were introduced to achieve maximum sequence homology among the four species. Comparison of the sequence with that of hVN showed 70.3% homology, which is the lowest among known VNs [homology between hVN and rabbit VN, 76% (*7*), and that between hVN and mouse VN, 74%]. While the polypeptides of hVN, and rabbit and mouse VN deduced from the respective cDNAs were composed of 478, 475, and 476 amino acid residues, respectively, pVN was composed of 459 amino acid residues, which is 16-19 amino acid residues fewer than the other three VNs. Interestingly, marked deletions were concentrated in the so-called connecting region (*2*), and such significant deletions were not found in the VNs from other species so far reported (*10*).

The amino acid residue corresponding to Asn(223) in hVN is replaced with Glu in pVN. Consequently, the number of potential *N*-glycosylation sites in pVN is decreased to two. The numbers and locations of cysteine residues are almost completely conserved among the four VNs, suggesting that cysteine residues are essential for the conformational formation of VNs. The Arg-Gly-Asp sequence for cell attachment is also conserved.

Proteolytic Processing of pVN—A single pentapeptide, His-Arg-Gln-Ser-Arg, was obtained from the purified pVN with a C-terminal fragment fractionator. The sequence coincided with amino acids 356-360. The fragment containing Lys(440), if present, cannot be obtained by this method. On the other hand, the conventional C-terminal analysis using carboxypeptidase B and A indicated the presence of Arg, Ser, and Thr after digestion for 5-24 h, but not Lys at the C-terminal end (data not shown). Thr might have originated from the contaminants. Combining the results, the C-terminal amino acid residue was determined to be Arg(360), not Lys(440) deduced from the cDNA. N-terminal analysis of pVN showed a single sequence of DQESXKGRXTDG (*9*) but not that starting with Ser(361). Furthermore, when pVN was eluted from heparin column without β-mercaptoethanol treatment, it had the same molecular mass on SDS-PAGE as that purified under the reducing conditions (data not shown), suggesting that the truncated fragment of Ser(361)-Lys(440) is not linked with disulfide bridge in purified pVN. The molecular mass of the polypeptide from amino acids 1 to 360 was estimated to be 41,149 Da. The structure of the major oligosaccharide of pVN is of the monofucosylated disialobiantennary type, its calculated molecular mass being about 2.5 kDa (*14*). Therefore, the molecular mass of pVN calculated as a glycoprotein having two oligosaccharides per mol, 46 kDa, after consideration of proteolytic processing is close with that, 44 kDa, obtained on Ferguson analysis.

As shown in Fig. 2, Arg(360)-Ser(361) of pVN correspond to Arg(379)-Ala(380) of hVN, which is the cleavage site of the two-chain form of hVN (*11*). The synthesized polypeptide of pVN must be cleaved at the Arg(360)-Ser(361) position by a trypsin-like protease during *in vivo* biosynthesis, as suggested for the two-chain type hVN. In the case of hVN, Thr(381) is essential for cleavage of the polypeptide, while an allele having Met(381) encodes a protease-resistant VN, but most people possess two forms of hVN in various ratios due to unquantitative cleavage. In the case of hVN, the cleaved 10-kDa fragment is linked with a 65-kDa polypeptide *via* a disulfide linkage between Cys(274) and Cys(453). In contrast, neither a non-truncated long chain of pVN nor a cleaved C-terminal fragment could be found in four different batches of pooled porcine sera or even in fresh plasma under either non-reducing or reducing conditions (data not shown). Both pVN cDNAs obtained in this study encoded Thr(362), a protease-sensitive type, and only the cleaved form was found on C-terminal analysis of pVN too. pVN was therefore considered to be present only in a truncated single-chain form. Plasma VN was reported to be synthesized in the liver (*8*). The results obtained here suggest that the highly active enzyme responsible for the C-terminal processing may be present in porcine liver, or the deletion of the third glycosylation site in pVN may increase the sensitivity against proteolytic processing of C-terminal peptide. Cys(251) might remain with a free sulfhydryl group in pVN as predicted for several Cys residues in hVN (*2, 11*). In VN purified by denaturation, however, thiol-disulfide exchange might have occurred by urea treatment in the purification procedure. VN purified under non-denaturing conditions is necessary to determine the native states of the cysteines.

Determination of the N-Glycosylation Sites of VNs—Ninety-six percentage of the total oligosaccharides of pVN have been shown to be of the biantennary complex-type (*14*), a suitable ligand for concanavalin A. The digest of pVN with trypsin was therefore subjected to concanavalin A affinity chromatography and the glycopeptide fraction obtained on specific elution from the column was further separated by reverse-phase HPLC. The elution pattern of glycopeptides from the tryptic digest is shown in Fig. 3A.

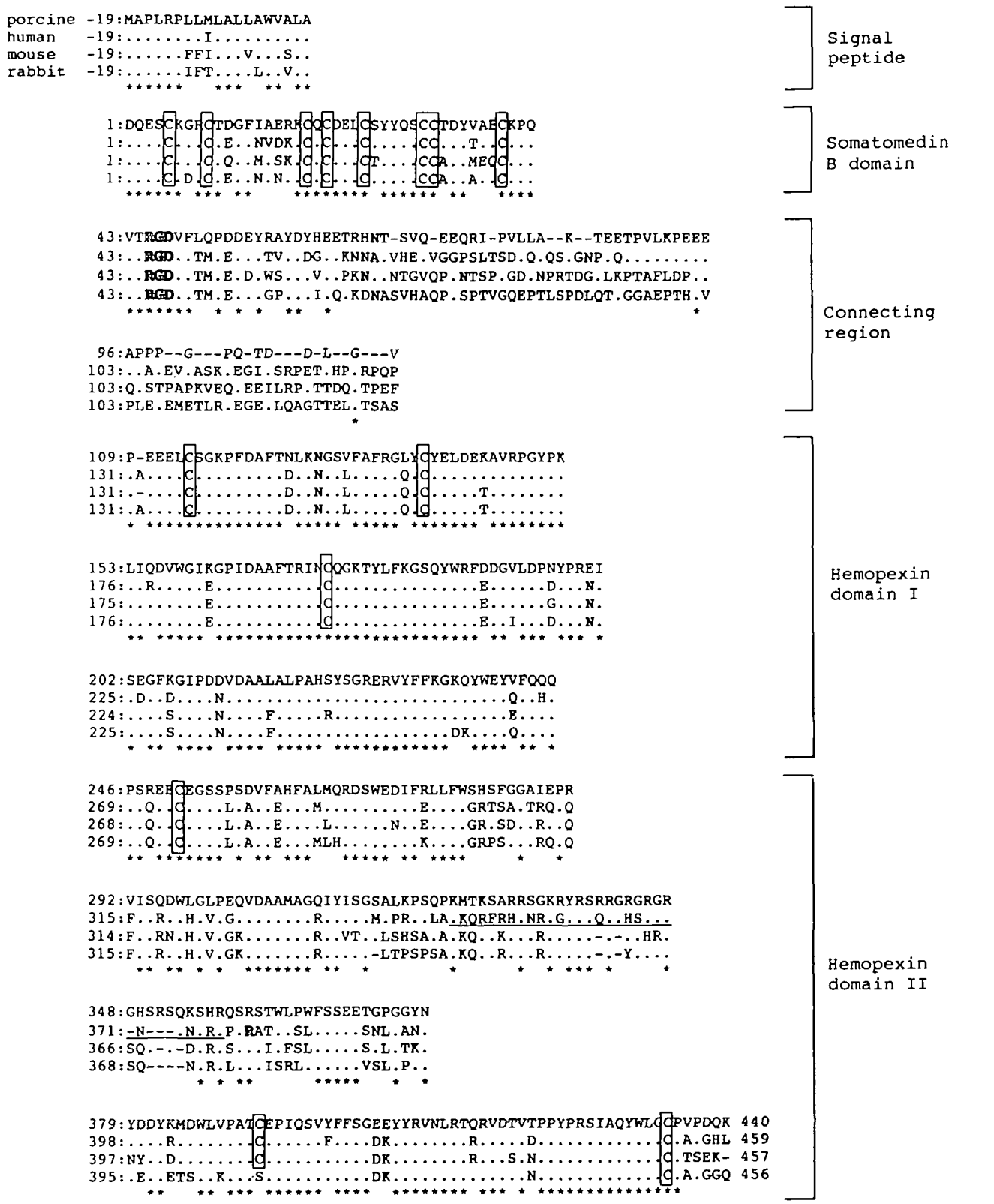

Fig. 2. **Comparison of the amino acid sequences of four VNs.** Dashes represent gaps to maximize homology. * denotes the same amino acids residues through four VNs. Dots denote the same amino acids as those in pVN; RGD, cell attachment site; N, potential *N*-glycosylation site; and boxes, conserved cysteine residues. Underlining indicates the heparin-binding region of hVN. R denotes the C-terminal amino acid residue of the 65-kDa fragment of the two-chain form of hVN. Each domain was aligned according to Ref. 2. The sequence of hVN aligned in this figure was of the clone screened in this paper, the four substitutions being Ser206, Ala347, Thr381, and Asp423.

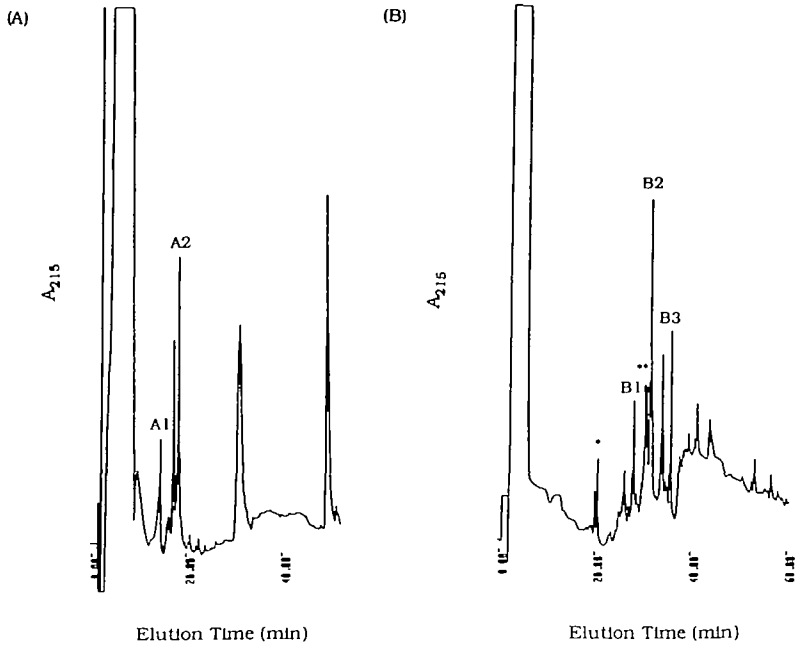

Fig. 3. **Reverse-phase HPLC of glycopeptides from pVN and hVN.** (A) Elution pattern of glycopeptides from a tryptic digest of pVN. (B) Elution pattern of glycopeptides from an α-chymotryptic digest of hVN. The materials in the peaks denoted by asterisks had the same sequence as that of B1.

The sequences of fractions A1 and A2 were analyzed. Other fractions in the pattern were also observed in the pattern of the control fraction and thus were not further analyzed. Two kinds of glycosylated sequences corresponding to separate potential sites in Fig. 1 were obtained, as shown in Table I. No sequence other than these was obtained from the digests with α-chymotrypsin and endoproteinase Asp-N. Similarly, hVN was digested with α-chymotrypsin, and the glycopeptides produced were obtained by concanavalin A affinity chromatography and separated by reverse-phase HPLC, as shown in Fig. 3B. Sequence analyses indicated that three kinds of glycosylated sequences corresponding to three potential sites deduced from the cDNA (*5, 6*) (see Fig. 2) were obtained, as shown in Table I. These results indicated that all potential *N*-glycosylation sites of pVN and hVN were actually glycosylated. The two *N*-glycosylation sites conserved among the four species might play important roles in the molecular conformation and/or activity, though the third glycosylation site lost in pVN might affect the polypeptide conformation and the C-terminal proteolytic processing in consequence.

DISCUSSION

The domain structures of pVN proposed in this study are summarized in Fig. 4A, in comparison with that of hVN (*5*). The small molecular mass of pVN was ascribed to the complete processing of the C-terminal fragment, the decrease in *N*-glycosylation sites, and the deletion in the connecting region.

In spite of its small size, pVN contained all known functional domains, *i.e.*, heparin-binding region, cell attachment sequence, and *N*-glycosylation sites. The binding sites for collagen, thrombin—AT-III, and β-endorphin have not been unambiguously identified. The function of the C-terminal fragment has been proposed in several reports (*17-19*). The results suggested that the C-terminal fragment is not functionally important in VN, considering that

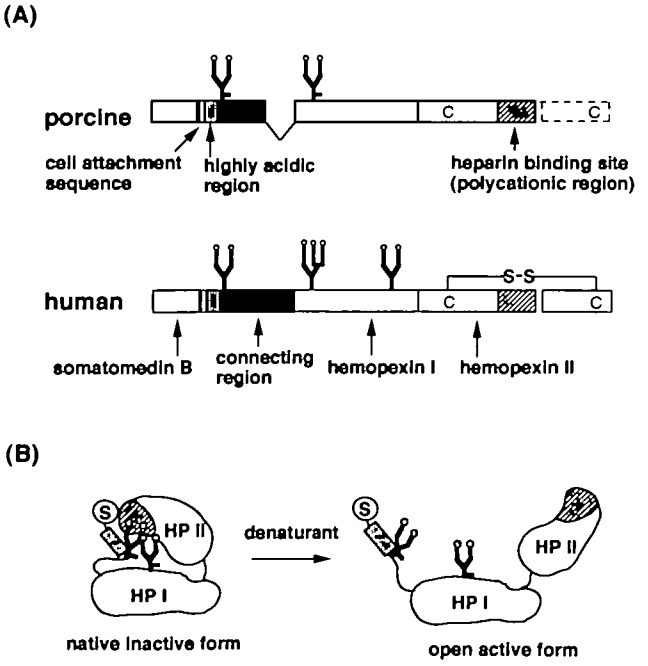

Fig. 4. **Domain structure models of VNs.** (A) Ψ and Ψ represent nonfucosylated and fucosylated biantennary disialooligosaccharides, respectively. HVN contains a high content of triantennary oligosaccharides Ψ (*15*) compared to pVN (*14*). In the case of hVN, the oligosaccharide structure does not reflect site-specific glycosylation. (B) Hypothetic conformational transition of pVN. The model illustrates the hypothetic conformational transition, with a denaturant, from the inactive form to the active form. S, somatomedin B domain; ▭, highly acidic region; HP I, hemopexin domain I; and HP II, hemopexin domain II. (+) and (−) denote positive and negative charges, respectively.

C-terminal processing occurs at an early stage of peptide processing of pVN. Rabbit and mouse VNs have been hitherto detected only in single-chain form (*9, 13*), and they have molecular mass closer to a heavy chain (50 kDa) of the two-chain form of hVN. Considering that they contain larger amounts of *O*-linked oligosaccharides than hVN and pVN (*9, 13*) in addition to three and four potential *N*-glycosylation sites for rabbit and mouse VNs, respectively (*7, 8*), a similar truncated single-chain form can be predicted though the actual cleavage sites are not clear for these VNs. Porcine, rabbit and mouse VNs commonly contain an Arg-Ser sequence at the cleavage site (possible for rabbit and mouse), while hVN contains Arg-Ala at this site. This may be related with the high susceptibility of these VNs to proteolysis.

The connecting region of hVN contains possible important functional sites: a highly acidic region (amino acids 53-64), a sulfation site(s) (Tyr56 and probably Tyr59) (*20*), a putative crosslinking site(s) (Gln93) (*21*), and one of the collagen-binding site(s) (*22, 23*). In particular, a highly acidic region and a sulfation site(s), together with the somatomedin B domain, which has an overall acidic character, may contribute to neutralization of the charge of polycationic groups present in the C-terminal portion, which can stabilize the native inactive form of VN. The presence and contribution of the glycan moiety were not so far considered in the working hypothesis of conformational transition of VN (*1*). Based on our observations obtained here and before (*14, 15*), a schematic model involving oligosaccharides on VN is presented for the conformational states of inactive and active VN in Fig. 4B. All the acidic residues responsible for the polyanionic site of hVN were found to be conserved in the connecting region of pVN, even though overall portions of it exhibit rather low homology to those of other VNs. In addition, one potential *N*-glycosylation site was conserved among VNs hitherto studied in the close vicinity of the acidic region, and was actually *N*-glycosylated in pVN and hVN (Table I). Our previous study indicated that the *N*-linked oligosaccharides of both pVN and hVN were commonly of the sialylated complex type (*14, 15*). The combined results suggested the possibility that bulky sialooligosaccharides may significantly affect the conformational formation of clustered anionic charges. The sialooligosaccharides seem to play essential roles in conformational formation of the latent molecular form and its transition to the active form: *e.g.*, negative charges of sialic acids interact with polycationic groups instead of, or cooperatively with, clustered acidic amino acids. To confirm this hypothesis, the roles of oligosaccharides and the connecting region are under investigation in our laboratory using recombinant VN mutants.

We also thank Miss Tomoko Kuriki (Shimadzu Co., Kyoto) for her kind support in the C-terminal fragment isolation.

REFERENCES

1. Preissner, K.T. (1991) Structure and biological role of vitronectin. *Annu. Rev. Cell. Biol.* **7**, 275-310
2. Tomasini, B.R. and Mosher, D.F. (1990) Vitronectin. *Prog. Hemostasis Thromb.* **10**, 269-305
3. Preissner, K.T. and Jenne, D. (1991) Structure of vitronectin and its biological role in haemostasis. *Thromb. Haemostas.* **66**, 123-132
4. Preissner, K.T. (1989) The role of vitronectin as multifunctional regulator in the hemostatic and immune systems. *Blut* **59**, 419-431
5. Jenne, D. and Stanley, K.K. (1985) Molecular cloning of S-protein, a link between complement, coagulation and cell-substrate adhesion. *EMBO J.* **4**, 3153-3157
6. Suzuki, S., Oldberg, A., Hayman, E.G., Pierschbacher, M.D., and Ruoslahti, E. (1985) Complete amino acid sequence of human vitronectin deduced from cDNA. Similarity of cell attachment sites in vitronectin and fibronectin. *EMBO J.* **4**, 2519-2524
7. Sato, R., Komine, Y., Imanaka, T., and Takano, T. (1990) Monoclonal antibody EMR1a/212D recognizing site of deposition of extracellular lipid in atherosclerosis. *J. Biol. Chem.* **265**, 21232-21236
8. Seiffert, D., Keeton, M., Eguchi, Y., Sawdey, M., and Loskutoff, D.J. (1991) Detection of vitronectin mRNA in tissues and cells of the mouse. *Proc. Natl. Acad. Sci. USA* **88**, 9402-9406
9. Nakashima, N., Miyazaki, K., Ishikawa, M., Yatohgo, T., Ogawa, H., Uchibori, H., Matsumoto, I., Seno, N., and Hayashi, M. (1992) Vitronectin diversity in evolution but uniformity in ligand binding and size of the core polypeptide. *Biochim. Biophys. Acta* **1120**, 1-10
10. Ehrlich, H.J., Richter, B., von der Ahe, D., and Preissner, K.T. (1993) Primary structure of vitronectins and homology with other proteins in *Biology of Vitronetins and Their Receptors* (Preissner, K.T., Rosenblatt, S., Kost, C., Wegerhoff, J., and Mosher, D.F., eds.) pp. 59-66, Excerpta Medica, Amsterdam
11. Dahlbäck, B. and Podack, E.R. (1985) Characterization of human S protein, an inhibitor of the membrane attack complex of complement. Demonstration of a free reactive thiol group. *Biochemistry* **24**, 2368-2374
12. Kubota, K., Hayashi, M., Oishi, N., and Sasaki, Y. (1990) Polymorphism of the human vitronectin gene causes vitronectin blood type. *Biochem. Biophys. Res. Commun.* **167**, 1355-1360
13. Kitagaki-Ogawa, H., Yatohgo, T., Izumi, M., Hayashi, M., Kashiwagi, H., Matsumoto, I., and Seno, N. (1990) Diversities in animal vitronectins. Differences in molecular weight, immunoreactivity and carbohydrate chains. *Biochim. Biophys. Acta* **1033**, 49-56
14. Yoneda, A., Ogawa, H., Matsumoto, I., Ishizuka, I., Hase, S., and Seno, N. (1993) Structures of the *N*-linked oligosaccharides on porcine plasma vitronectin. *Eur. J. Biochem.* **218**, 797-806
15. Ogawa, H., Yoneda, A., Seno, N., Hayashi, M., Ishizuka, I., Hase, S., and Matsumoto, I. (1995) Structures of the *N*-linked oligosaccharides on human plasma vitronectin. *Eur. J. Biochem.* **230**, 994-1000
16. Cardin, A.D. and Weintraub, J.H.R. (1989) Molecular modeling of protein-glycosaminoglycan interactions. *Arteriosclerosis* **9**, 21-32
17. Izumi, M., Yamada, K.M., and Hayashi, M. (1989) Vitronectin exists in two structurally and functionally distinct forms in human plasma. *Biochim. Biophys. Acta* **990**, 101-108
18. Korc-Grodzicki, B., Chain, D., Kreizman, T., and Shaltiel, S. (1990) An enzymatic assay for vitronectin based on its selective phosphorylation by protein kinase A. *Anal. Biochem.* **188**, 288-294
19. Hannan, G.N., Reilly, W., and McAuslan, B.R. (1988) Mechanisms of serum protein binding and cell anchorage to immobilized serotonin and indole analogs. *Exp. Cell Res.* **176**, 49-59
20. Jenne, D. and Tchopp, J. (1989) Molecular structure and functional characterization of a human complement cytolysis inhibitor found in blood and seminal plasma: Identity to sulfated glycoprotein 2, a constituent of rat testis fluid. *Proc. Natl. Acad. Sci. USA* **86**, 7123-7127
21. Skoorstengaard, K., Halkier, T., Hojrup, P., and Mosher, D. (1990) Sequence location of a putative transglutaminase crosslinking site in human vitronectin. *FEBS Lett.* **262**, 269-274
22. Izumi, M., Shimo-Oka, T., Morishita, N., Li, I., and Hayashi, M. (1988) Identification of the collagen-binding domain of vitronectin using monoclonal antibodies. *Cell. Struct. Funct.* **13**, 217-225
23. Ishikawa-Sakurai, M. and Hayashi, M. (1993) Two collagen-binding domains of vitronectin. *Cell. Struct. Funct.* **18**, 253-259