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Characterization of Ficolins as Novel Elastin-Binding Proteins and Molecular Cloning of Human Ficolin-1[1]

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A novel elastin-binding protein, EBP-37, was recently identified and purified from human plasma. Its partial amino acid sequences showed significant homology to porcine ficolins, which were originally purified from porcine uterus membranes as multimeric proteins with fibrinogen- and collagen-like domains. Here we report the presence of ficolins in an elastin-binding fraction of porcine plasma and the direct binding of recombinant porcine ficolin-α to elastin. In addition, a cDNA encoding a human counterpart of porcine ficolins that is composed of 319 amino acids and is different from EBP-37 was cloned and named human ficolin-1. Northern blotting of various human tissues revealed that human ficolin-1 mRNA is highly expressed in peripheral blood leukocytes. These data suggested that there are at least two kinds of ficolin-related proteins in both pig and human, and they may function as plasma proteins with elastin-binding activities.

Key words: elastin, elastin-binding protein, ficolin, plasma, transforming growth factor-β1.

Elastin is a predominant protein of mature elastic fibers and is one of the major components of the extracellular matrix of various tissues, including artery wall, lung and skin. Various constituents of blood are known to interact with elastin, and their disregulated interactions may play significant roles in diseases of human aorta, such as arteriosclerosis (*1*) and aneurysm (*2*). We have previously identified and purified a novel elastin-binding protein (EBP-37) from human plasma (*3*). Amino acid sequencing and immunoblot analysis revealed that EBP-37 is highly similar to porcine ficolin-α and -β that were originally identified as transforming growth factor-β1 (TGF-β1)-binding proteins with fibrinogen- and collagen-like domains from porcine uterus membranes (*4, 5*).

Recently, a novel human serum lectin with fibrinogen- and collagen-like domains, termed P35 (*6*), was molecularly cloned and characterized. Comparison of the amino acid sequence of EBP-37 with that of P35 indicated that these proteins are identical. In addition, a novel corticosteroid-binding protein, termed hucolin (*7*), was purified from human plasma, and its amino-terminal amino acid sequence was found to be identical to that of EBP-37 and P35. Thus, EBP-37/P35/hucolin appears to function as a human ficolin, and may play important roles in view of its binding activities to diverse substances including elastin, sugar, and corticosteroid.

In the present study, we found that a large amount of ficolins (or ficolin-like proteins) is present in the α-elastin-Sepharose-binding fraction of porcine plasma, and that recombinant porcine ficolin-α, as well as EBP-37, binds directly to elastin. Moreover, we obtained a cDNA that is closely related to, but different from, EBP-37/P35/hucolin by screening a human uterus cDNA library with porcine ficolin-α cDNA as a probe. Since the cDNA encodes a protein which is equally similar to porcine ficolin-α and -β, this clone was termed human ficolin-1. Northern blot analysis of various human tissues revealed that human ficolin-1 mRNA is highly expressed in peripheral blood leukocytes. Taken together, these observations suggested that there are at least two ficolin-related proteins in both pig and human, which may have important roles as plasma proteins, and binding activity to elastin may be a common feature of the ficolin-related proteins. The structural and functional properties of ficolins and related gene products are discussed.

MATERIALS AND METHODS

α-Elastin-Sepharose 4B Affinity Chromatography and SDS-PAGE—α-Elastin (*8, 9*) and α-elastin-Sepharose 4B (*ca.* 5 mg of α-elastin per ml of hydrated gel) (*9*) were prepared as described previously. α-Elastin-Sepharose 4B affinity chromatography of various mammalian plasmas was performed at 25°C as described previously (*3, 9*). The

[1] The nucleotide sequence reported in this paper has been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence databases (accession number D83920).

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Abbreviations: 36-kDa MAP, 36-kDa microfibril-associated glycoprotein; LTBP, latent transforming growth factor-β binding protein; MFAP4, a human microfibril-associated glycoprotein; MTN, human multiple tissue northern; PAE, porcine aortic endothelial; PVDF, polyvinylidene difluoride; TGF-β, transforming growth factor-β.

α-elastin-Sepharose-binding fraction was subjected to SDS-PAGE (8 or 10% polyacrylamide gel) as described by Laemmli (*10*). Gels were stained with Coomassie Brilliant Blue R-250 (Merck).

Amino Acid Sequencing of a 40 kDa Protein in α-Elastin-Binding Fraction of Porcine Plasma—Electroblotting of the α-elastin-binding proteins of porcine plasma from SDS-gel (10% polyacrylamide gel, nonreducing conditions) onto a polyvinylidene difluoride (PVDF) membrane (Immobilon-P, Millipore) was performed as described (*11, 12*). The membrane was stained with Coomassie Brilliant Blue R-250. Peptide bands were then excised. Internal peptide sequences of the 40 kDa protein were obtained using the peptide mapping technique of Cleveland *et al.* as described previously (*3, 13*). The 40 kDa protein was digested with *Staphylococcus aureus* V8 protease (Boehringer Mannheim). The amino acid sequences were determined by use of a pulse-liquid-phase amino acid sequencer (Model 477A protein sequencer, Applied Biosystems).

Immunoblot Analysis of Plasma Ficolins—The α-elastin-Sepharose-binding fraction of porcine plasma was mixed with SDS-sample buffer, boiled at 95°C for 4 min, and subjected to SDS-PAGE (8% polyacrylamide gel) under nonreducing or reducing conditions. After electrophoresis, the proteins were electrophoretically transferred to PVDF membranes. Immunostaining of porcine plasma ficolins was performed with an anti-EBP-37 antiserum as previously described (*3*) or an antiserum denoted AN1801, reactive with both ficolin-α and -β, that was raised against a synthetic peptide (*5*). Immunostainings of plasma ficolin-like proteins in α-elastin-Sepharose-binding fractions of various mammalian plasmas were performed with anti-EBP-37 antiserum.

Assay for Binding of Recombinant Porcine Ficolin-α to Insoluble Elastin, α-Elastin-Sepharose and Gelatin-Sepharose—Metabolic labeling of the ficolin-α-transfected porcine aortic endothelial (PAE) cells was performed for 12 h with [^{35}S]methionine and cysteine as described previously (*5*). After labeling, the medium was collected and centrifuged for 5 min at 19,000 × *g*, and the supernatant was used for the binding experiment. Autoclaved insoluble elastin (from 4 mg of dried powder, Sigma) (*9*), α-elastin-Sepharose 4B beads (20 μl of wet gel, *ca.* 5 mg of α-elastin per ml of hydrated gel) (*9*), gelatin-Sepharose 4B beads (20 μl of wet gel, *ca.* 6 mg of gelatin per ml of hydrated gel, Pharmacia Biotech) and Sepharose 4B beads (20 μl of wet gel, Pharmacia Biotech) were incubated with 1 ml of the radio-labeled medium for 12 h at 4°C. Washing was performed four times with 50 mM Tris-HCl, pH 7.4, containing 1 M NaCl and 1% Triton X-100 and once with distilled water. Bound materials were eluted by boiling for 5 min in SDS-sample buffer (100 mM Tris-HCl, pH 8.8, 0.01% bromphenol blue, 36% glycerol, 4% SDS) and analyzed by a 5 to 15% gradient SDS-PAGE under non-reducing conditions. Gels were fixed and subjected to fluorography.

Screening a Phage Library—Approximately 2×10^6 clones of a human uterus λgt10 cDNA library (Clontech) were screened with porcine ficolin-α cDNA as a probe (*5*). ^{32}P-labeling of the porcine ficolin-α cDNA was done with a Ready-To-Go DNA Labeling Kit (Pharmacia Biotech). Hybridization to duplicate nitrocellulose filters (Hybond-C extra, Amersham) was performed as described previously (*5*). Inserts of the positive clones were amplified by PCR using λgt10 forward and reverse primers, and subcloned into pGEM-T vector (Promega). Nucleotide sequencing was performed with a Sequenase Version 2.0 DNA sequencing kit (United States Biochemical).

Anchored PCR—To obtain a full-length cDNA clone of human counterparts of porcine ficolins, anchored PCR was performed by Taq DNA polymerase using the human uterus cDNA library as a template. λgt10 reverse primer, AS15S-1 primer (5′-CCCCCGGTCTAGCAGGTCCTT-3′), and AS15S-2 primer (5′-TGGCTGGGGAAATGGGGTG-AC-3′) were used as PCR primers. PCR was performed for 25 cycles of 94°C (45 s), 48 or 55°C (45 s), and 72°C (90 s). The PCR products were subcloned into pGEM-T vector and were sequenced as described above.

Northern Blot Analysis—Human multiple tissue northern (MTN) blot and MTN blot II (Clontech) were probed with ^{32}P-labeled human ficolin-1 cDNA fragment (nucleotides 262–1194) as described previously (*5*).

RESULTS

Identification of Ficolin-Like Proteins in the α-Elastin-Binding Fraction of Porcine Plasma—To identify α-elastin-binding proteins in porcine plasma, plasma was passed through a Sepharose 4B column and then applied to an α-elastin-Sepharose 4B column at 25°C. The α-elastin-Sepharose-binding fraction was subjected to SDS-PAGE and analyzed by staining with Coomassie Brilliant Blue R-250. Figure 1 shows the migration patterns of these proteins in SDS-PAGE (10% polyacrylamide gel) under reducing (Fig. 1A) and nonreducing (Fig. 1B) conditions. Several bands with different molecular sizes, including 27, 37–40, 54, 62, and 76–88 kDa, were observed under reducing conditions, and two major bands with sizes of 37–40 and 250 kDa were seen under nonreducing conditions. The doublet band of 40 and 37 kDa was found only in the eluate from the α-elastin-Sepharose 4B column, but not from the plain Sepharose 4B column under reducing and nonreducing conditions (data not shown). The migration profile of the doublet band of 40 and 37 kDa was similar to that observed

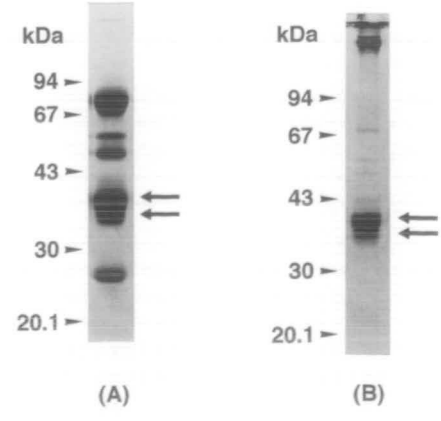


Fig. 1. **SDS-PAGE analysis of α-elastin-Sepharose-binding proteins of porcine plasma.** Aliquots (15 μl) of α-elastin-Sepharose-binding fraction, which had an absorbance at 280 nm of 1.2, were subjected to SDS-PAGE (10% polyacrylamide gel) under reducing (A) and nonreducing (B) conditions. The gels were stained with Coomassie Brilliant Blue R-250. The arrows indicate the 40 and 37 kDa proteins. The positions of the molecular weight markers are shown on the left.

in immunoprecipitates of recombinant porcine ficolin-α (*5*). In the case of human plasma, only a single band with an apparent molecular mass of 37 kDa was observed (*3*). As described below, sequencing studies indicated that the upper 40 kDa protein of the doublet band under nonreducing conditions is identical or closely related to porcine ficolin-α. However, two-dimensional gel electrophoresis revealed that several isoelectric variants appear to exist in unequal amounts in the 40 and 37 kDa proteins (data not shown).

Immunoblot Analysis of Porcine Plasma Ficolins and Distribution of Plasma Ficolin-Like Proteins in α-Elastin-Sepharose-Binding Fractions of Various Mammalian Plasmas—The α-elastin-Sepharose-binding fraction of porcine plasma was subjected to SDS-PAGE under nonreducing and reducing conditions, and then immunoblot analysis was carried out by using anti-EBP-37 antiserum (*3*) (Fig. 2, A and B) and AN1801 antiserum (*5*) (Fig. 2C and D). As shown in Fig. 2 (A-D), similar patterns were obtained with both antisera. High-molecular-weight components of more than 200 kDa and 3 different molecules with sizes of about 75, 40, and 37 kDa reacted with the anti-EBP-37 antiserum and AN1801 under nonreducing conditions; the high-molecular components were all converted to 40 and 37 kDa molecules upon reduction. A similar immunoblot pattern was obtained with recombinant ficolin-α (*5*). These results indicate that the ficolin-like molecules exist as disulfide-linked multimers and dimers as well as monomers in the α-elastin-Sepharose-binding fraction of porcine plasma. Most of the 40 kDa molecules found in porcine plasma were converted to 37 kDa molecules by *N*-glycosidase F (Boehringer Mannheim) treatment (data not shown). Similarly, human EBP-37 was shifted from 37 to 34.5 kDa by *N*-glycosidase F treatment (data not shown).

We investigated the plasma ficolin-like proteins in α-elastin-Sepharose-binding fractions of various mammalian plasmas by immunoblot analysis. As shown in Fig. 2E, strong signals were detected in porcine and human

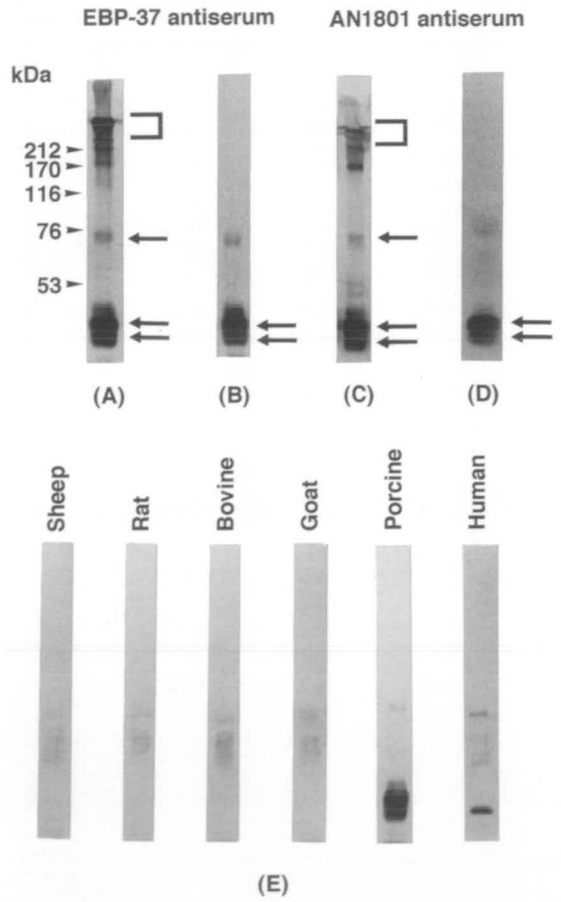
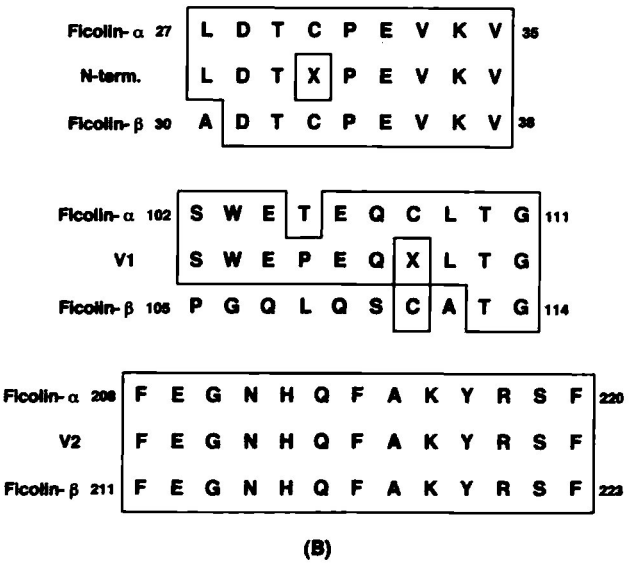


Fig. 2. **Immunoblot analysis of porcine plasma ficolins and α-elastin-Sepharose binding fractions of various mammalian plasmas.** Aliquots (7 μl) of α-elastin-Sepharose-binding fraction of porcine plasma, which had an abeorbance at 280 nm of 0.5, were subjected to SDS-PAGE (8% polyacrylamide gel) under nonreducing (A, C) and reducing (B, D) conditions followed by transfer to PVDF membranes. The membranes were incubated with the EBP-37 antiserum (A, B) and AN1801 antiserum (C, D) and immunoreactive proteins were visualized using horseradish peroxidase-conjugated secondary antibody as described previously (*3*). The arrows indicate monomer and dimer of ficolins, and the bracket, multimeric forms. The positions of the molecular weight markers are shown on the left. Immunoblot analyses of α-elastin-Sepharose-binding fractions of various mammalian plasmas were performed with the same method under reducing conditions by using the EBP-37 antiserum (E).

	Sequence
N-term.	L D T X P E V K V v g l e
V1	S W E P E Q X L T G p r
V2	F E G N H Q F A K Y R S F

(A)



(B)

Fig. 3. **Amino acid sequencing of the 40 kDa protein from α-elastin binding fraction of porcine plasma and sequence comparison between the 40 kDa protein and porcine ficolins.** (A) Amino-terminal amino acid sequence (N-term.), amino acid sequences of V8 protease digestion fragments 1 (V1) and 2 (V2) of the 40 kDa protein are shown. X denotes an unidentified residue. The sequence shown in small letter code involves some ambiguity. (B) Comparison of the amino acid sequences of ficolin-α (I) and -β (II) with those obtained from N-term., V1 and V2. Identical amino acid residues are boxed.

plasmas, but no signal of ficolin-like proteins was detected in the α-elastin-Sepharose-binding fractions of plasmas from sheep, rat, bovine, and goat.

Amino Acid Sequencing of the 40 kDa Protein in α-Elastin-Binding Fraction of Porcine Plasma—Determination of the amino-terminal amino acid sequence of the 40 kDa protein revealed that it is identical to that of porcine ficolin-α (Fig. 3, A and B, N-term.) (*5*). The internal amino acid sequences of the 40 kDa protein (V1, V2) were determined after V8 protease digestion. The sequence of peptide V1 was also identical to that of ficolin-α except the 4th amino acid residue, which was Pro instead of Thr as reported in a previous study (*5*). There are at least two possible reasons for this difference: one is that the sequence of peptide V1 may come from a ficolin-like peptide chain other than ficolin-α and -β, and the other is that this difference may reflect protein polymorphism or difference among the porcine race. The sequence of peptide V2 was identical to both ficolin-α and -β (*5*). These results strongly suggested that the 40 kDa protein identified in the α-elastin-binding fraction is porcine ficolin-α and/or closely related molecules.

Elastin-Binding Activity of Recombinant Ficolin-α—We reported previously that EBP-37 from human plasma bound most efficiently to α-elastin, compared with gelatin (derived from type I collagen), type II collagen, fibronectin, and fibrinogen (*3*). In order to study whether porcine ficolin-α interacts with elastin, binding assay was performed using metabolically labeled porcine ficolin-α which was produced from the cells transfected with the ficolin-α cDNA. As shown in Fig. 4, recombinant porcine ficolin-α bound to insoluble elastin as well as α-elastin-Sepharose, as a 37-40 kDa monomer, 70-80 kDa dimer, and several higher molecular weight multimers. It also bound to gelatin-Sepharose, but the amount was much less than that recovered from the insoluble elastin or α-elastin-Sepharose. Ficolin-α did not bind to the plain Sepharose beads. These results further suggested a direct interaction of recombinant porcine ficolin-α with elastin. Furthermore, we found an efficient association of partially purified EBP-37 from human plasma with α- and κ-elastin, and laminin, and to a lesser extent with gelatin, but only marginally with types I, II, III, and IV collagen, fibronectin and vitronectin

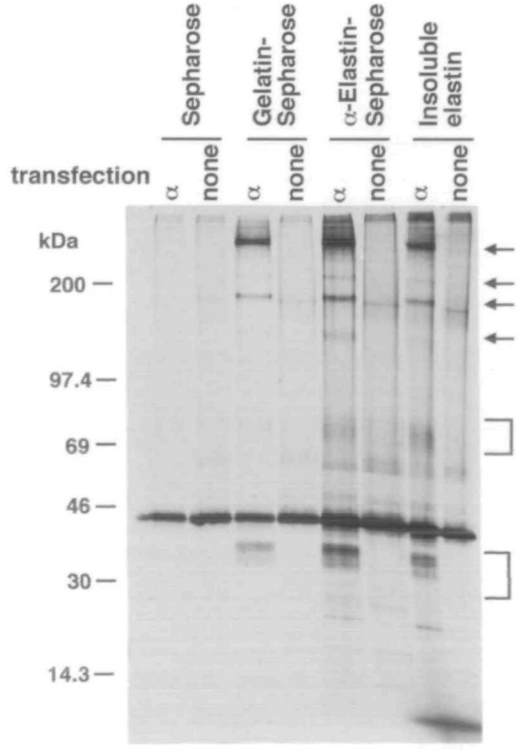


Fig. 4. **Binding of recombinant porcine ficolin-α to elastin.** Control PAE cells (none) and PAE cells transfected with ficolin-α (α) were metabolically labeled with [^{35}S]methionine and cysteine. The media were then subjected to the binding assay (see text). Specific bands present in the medium of transfected PAE cells are indicated by arrows and brackets. The migration distances of molecular weight markers are indicated on the left.

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CTGAGTGGAGCCACCATGGCCCGGGGGCTCGCTGTCCTGCTAGTCTTGTTCCTGCATATC  60
                M  A  R  G  L  A  V  L  L  V  L  F  L  H  I   15

AAGAACCTGCCTGCCCAGGCTGCGGACACATGTCCAGAGGTGAAGGTGGTGGGCCTGGAG 120
K  N  L  P  A  Q  A  A  D  T  C  P  E  V  K  V  V  G  L  E   35

GGCTCTGACAAGCTCACCATTCTCCGAGGCTGCCCGGGGCTGCCCGGGGCCCCAGGGCCA 180
G  S  D  K  L  T  I  L  R  G  C  P  G  L  P  G  A  P  G  P   55

AAGGGAGAGGCAGGTGTCATTGGAGAGAGAGGAGAACGCGGTCTCCCTGGAGCCCCTGGA 240
K  G  E  A  G  V  I  G  E  R  G  E  R  G  L  P  G  A  P  G   75

AAGGCAGGACCAGTGGGGCCCAAAGGAGACCGAGGAGAGAAGGGGATGCGTGGAGAGAAA 300
K  A  G  P  V  G  P  K  G  D  R  G  E  K  G  M  R  G  E  K   95

GGAGACGCTGGGCAGTCTCAGTCGTGTGCGACAGGCCCACGCAACTGCAAGGACCTGCTA 360
G  D  A  G  Q  S  Q  S  C  A  T  G  P  R  N  C  K  D  L  L  115

GACCGGGGGTATTTCCTGAGCGGCTGGCACACCATCTACCTGCCCGACTGCCGGCCCCTG 420
D  R  G  Y  F  L  S  G  W  H  T  I  Y  L  P  D  C  R  P  L  135

ACTGTGCTCTGTGACATGGACACGGACGGAGGGGGCTGGACCGTTTTCCAGCGGAGGATG 480
T  V  L  C  D  M  D  T  D  G  G  G  W  T  V  F  Q  R  R  M  155

GATGGCTCTGTGGACTTCTATCGGGACTGGGCCGCATACAAGCAGGGCTTCGGCAGTCAG 540
D  G  S  V  D  F  Y  R  D  W  A  A  Y  K  Q  G  F  G  S  Q  175

CTGGGGGAGTTCTGGCTGGGGAACGACAACATCCACGCCCTGACTGCCCAGGGAAGCAGC 600
L  G  E  F  W  L  G  N  D  N  I  H  A  L  T  A  Q  G  S  S  195

GAGCTCCGTGTAGACCTGGTGGACTTTGAGGGCAACCACCAGTTTGCTAAGTACAAATCA 660
E  L  R  V  D  L  V  D  F  E  G  N  H  Q  F  A  K  Y  K  S  215

TTCAAGGTGGCTGACGAGGCAGAGAAGTACAAGCTGGTACTGGGAGCCTTTGTCGGGGGC 720
F  K  V  A  D  E  A  E  K  Y  K  L  V  L  G  A  F  V  G  G  235

AGTGCGGGTAATTCTCTAACGGGCCACAACAACAACTTCTTCTCCACCAAAGACCAAGAC 780
S  A  G  N  S  L  T  G  H  N  N  N  F  F  S  T  K  D  Q  D  255

AATGATGTGAGTTCTTCGAATTGTGCTGAGAAGTTCCAGGGAGCCTGGTGGTACGCCGAC 840
N  D  V  S  S  S  N  C  A  E  K  F  Q  G  A  W  W  Y  A  D  275

TGTCATGCTTCAAACCTCAATGGTCTCTACCTCATGGGACCCCATGAGAGCTATGCCAAT 900
C  H  A  S  N  L  N  G  L  Y  L  M  G  P  H  E  S  Y  A  N  295

GGTATCAACTGGAGTGCGGCGAAGGGGTACAAATATAGCTACAAGGTGTCAGAGATGAAG 960
G  I  N  W  S  A  A  K  G  Y  K  Y  S  Y  K  V  S  E  M  K  315

GTGCGGCCCGCCTAGACGGGCCAGGACCCCTCCACATGCACCTGCTAGTGGGGAGGCCAC 1020
V  R  P  A  *                                               319

ACCCACAAGCGCTGCGTCGTGGAAGTCACCCCATTTCCCCAGCCAGACACACTCCCATGA 1080

CGCCCACAGCTGCCCCTTTGCCCCCAGCTCAGTCAAGCCGCCACATGCCCACAACCTCAC 1140

CAGAGGGAGAATTATGTTTCTAAATATGTTTACTTTGGGACAGAAAAAAAAAAA 1194
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Fig. 5. **Nucleotide and deduced amino acid sequences of human ficolin-1 cDNA.** The deduced amino acid sequence of human ficolin-1 cDNA is shown below the nucleotide sequence. The putative signal sequence according to the rules of von Heijne (*15*) is underlined with a thick line. The collagen-like domain is underlined. The fibrinogen-like domain (arrows) and a potential *N*-linked glycosylation site (box) are shown.

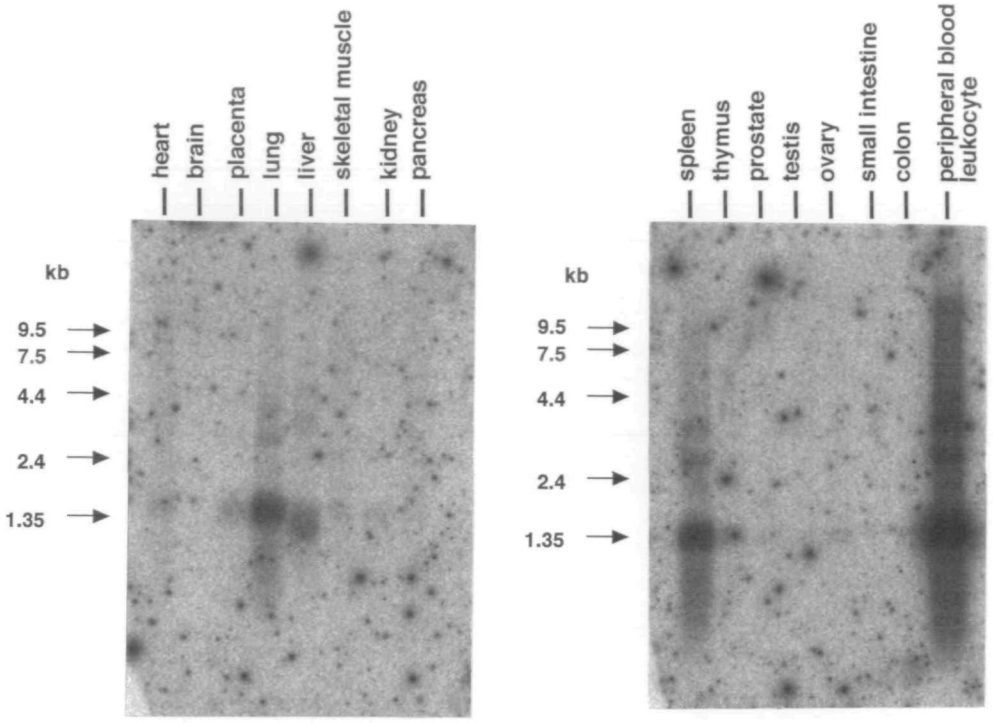

Fig. 6. Northern blot analysis of human ficolin-1. Human multiple tissue northern (MTN) blot and MTN blot II (Clontech) were probed with the human ficolin-1 cDNA fragment. Each lane contained 2 μg of polyadenylated RNA from the indicated tissues. Size markers are indicated on the left.

by using an ELISA method (our unpublished data). Thus, elastin-binding may be a common feature or function of the ficolin and EBP-37 group.

Cloning of a Human Ficolin cDNA—A human uterus cDNA library was screened with the porcine ficolin-α cDNA as a probe. The primary screening yielded 33 positive clones. Three of the longest clones, denoted 15S7, 8S, and 11S, were sequenced entirely. The sequence of 15S7 clone was found to encode an incomplete amino acid sequence of human ficolin-1 (Fig. 5, cDNA sequence corresponding to nucleotides 262–1194). The sequences of other clones (8S, 11S) were found to encode a human microfibril-associated glycoprotein (MFAP4); the gene for MFAP4 is deleted in Smith-Magenis syndrome (*14*). To clone the complete amino-terminal fragment of human ficolin-1, anchored PCR was performed. The largest PCR product was found to encode the amino-terminal part of human ficolin-1 (Fig. 5, cDNA sequence corresponding to 1–369), and thereby the complete human ficolin-1 cDNA was obtained (Fig. 5). A 1,194 bp nucleotide sequence has an ATG start codon at position 16, followed by a 957 bp open reading frame which encodes a peptide with 319 amino acids. A hydrophobic signal sequence was observed after the ATG start codon; the 3′-untranslated sequence contained a poly(A) tail.

The primary structure of human ficolin-1 has two distinct features, *i.e.*, a collagen-like domain following a short N-terminal peptide sequence, and a C-terminal fibrinogen-like structure. A potential *N*-glycosylation site was found at Asn-298.

The overall amino acid sequence of human ficolin-1 showed 74.3% identity to porcine ficolin-α, and 78.1% to porcine ficolin-β by using the Clustal computer alignment program (*16*) of the Lasergene program (DNASTAR). Amino acid sequence comparison did not reveal whether the human ficolin-1 is a human counterpart of porcine ficolin-α or ficolin-β. Therefore, the human clone was termed ficolin-1.

The deduced amino acid sequence of human ficolin-1 was essentially identical or closely related to that of the human ficolin which was very recently cloned from a human uterus cDNA library (*17*). Two amino acid residues at 126 and 280 are different from those of the reported sequence, *i.e.* Thr at 126 and Asn at 280 in the present study were Asn and Ser, respectively, in the reported sequence (*17*). The cDNA sequence differences were observed at nucleotide positions 264, 392, 393, 852, 854, and 1121, *i.e.* A at 264, C at 392, C at 393, A at 852, A at 854, and C at 1121 in the present study were G, A, T, G, G, and T, respectively in the reported sequence (*17*). Moreover, the cDNA sequence (5′-TTTGGG-3′) corresponding to residues 1174–1179 in the present study was TTTTGGGG in the reported sequence (*17*).

Expression of Human Ficolin-1 mRNA—The distribution of human ficolin-1 mRNA was investigated using poly(A)$^+$ RNA obtained from various human tissues (Fig. 6). The human ficolin-1 probe (15S7 clone, nucleotides 262–1194) hybridized to a 1.4 kb mRNA in peripheral blood leukocytes, spleen, lung, and placenta, suggesting that blood cells may be a major source for human ficolin-1. Two additional larger transcripts of 3.7 and 3.0 kb were also observed in the same tissues, which might represent alternative splicing variants or cross-hybridization to other related gene products such as MFAP4. The liver expressed a 1.3 kb transcript, which is slightly smaller than the major 1.4 kb transcript in peripheral blood leukocytes. Since P35 was mainly expressed in liver with various sizes of transcript including 1.3, 3.0, 3.2, and 4.0 kb (*6*), the 1.3 kb message may be a cross-hybridized signal to P35.

DISCUSSION

In this study, we found ficolin-like proteins in an α-elastin-Sepharose-binding fraction of porcine plasma. As described previously, we have identified and purified EBP-37 from human plasma as a human homologue of porcine ficolins (*3*). Two other candidates for the human ficolin gene products have recently been identified. One is P35, a novel human serum lectin that functions as an opsonin (*6*). The other is hucolin, which was isolated as a corticosteroid-binding protein from human plasma (*7*). The partial amino acid sequences obtained from the purified EBP-37 (*3*) and hucolin (*7*) matched the deduced amino acid sequence of P35 (*6*), indicating that EBP-37, P35, and hucolin are identical. In contrast, human ficolin-1 in this report is closely related to, but different from, EBP-37/P35/hucolin. Thus, there are at least two kinds of ficolin-related proteins in human, as in the case of pig.

Porcine ficolin-like proteins and human EBP-37 are present in α-elastin-Sepharose-binding fraction of plasma. In addition, human ficolin-1 was mainly produced from peripheral blood leukocytes. The deduced amino acid sequence of human ficolin-1 has the features of a secreted protein, starting with a hydrophobic signal sequence (Fig. 5). Human ficolin-1 may be secreted into the circulation and other body fluids. These results suggested ficolin and EBP-37 group may function as plasma proteins with an elastin-binding activity. We could not detect ficolin-like proteins in α-elastin-binding fractions of sheep, rat, bovine, and goat plasmas by immunoblot analysis. The reasons for the negative results are currently unknown; however, plasma ficolin-like proteins might exist in significant amounts only in porcine and human plasmas, as in the case of conglutinin, which is a serum lectin that is present in significant amounts only in members of the *Bovidae* (*18*). Alternatively, anti-EBP-37 antiserum might have species specificities in its epitope recognition.

We found that recombinant porcine ficolin-α as well as EBP-37 interacted directly with elastin, which is a core protein of elastic fibers together with surrounding microfibrils, a complex of glycoproteins (*19*). Bovine 36-kDa microfibril-associated glycoprotein (36-kDa MAP) has a fibrinogen-like domain (*20*) and shares a high sequence homology with MFAP4, which is encoded by a candidate gene responsible for Smith-Magenis syndrome. MFAP4 shows 51 to 52% amino acid sequence identity to porcine ficolins in their fibrinogen-like domain (*14*), and the 36 kDa MAP is 41 to 42% similar to ficolins in the 127 amino acid sequence available for the 36 kDa MAP (*14*). cDNAs for MFAP4 were isolated by screening the cDNA library using a ficolin-α cDNA probe in the present study. Thus, the structural relationship between ficolins and the 36-kDa MAP and MFAP4, and the binding activity of ficolins to elastin suggest that ficolins may also associate with elastin-microfibrils *in vivo*, and function as one of the extracellular matrix constituents.

Large glycoproteins of 350 kDa, called fibrillins, are also major components of elastin-associated microfibrils (*21*). Fibrillins are composed of two types of cysteine-rich repeat sequences, *i.e.*, an epidermal growth factor-like motif and an eight-cysteine motif (*22*). Abnormalities in the fibrillin genes result in certain connective tissue disorders, such as Marfan syndrome (*22-24*). The overall structures of fibrillins are similar to those of latent TGF-β binding proteins (LTBP) (*22, 25*), which bind to the N-terminal precursor parts of TGF-β *via* a disulfide bridge. Among the three isoforms of LTBPs, LTBP-1 and -2 have been shown to be components of microfibrils (*26*). Interestingly, bovine LTBP-2 was immunolocalized to the elastin-associated microfibrils (*27*). In view of the fact that ficolins were identified and purified as TGF-β1 binding proteins and possibly colocalize with LTBPs, they may modulate the activity of TGF-β *in vivo*.

Moreover, recent evidence has suggested that TGF-β in blood may regulate atherogenesis (*28*) and it was proposed that TGF-β is a key inhibitor of atherosclerosis (*29*). To understand the functions of porcine ficolin-like proteins and EBP-37 in blood, it is necessary to investigate the interactions of porcine ficolin-like proteins and EBP-37 with TGF-β.

Another important function of ficolins has been suggested by the recent cloning and characterization of P35 as a human serum Ca^{2+}-dependent lectin (*6*). P35 was shown to enhance phagocytosis of *Salmonella typhimurium* strain TV119 by polymorphonuclear neutrophils. It was suggested that P35 recognizes a large number of nonreducing terminal GlcNAc residues exposed on the surface. However, since such oligosaccharides are not present on elastin and the binding of EBP-37 to the α-elastin involves hydrophobic interaction (*3*), the binding of ficolins to elastin may occur *via* a different binding site. Together, the current data suggest potential important functions of ficolins *in vivo; i.e.*, they are produced by blood leukocytes and bind to elastin, and they may function as an opsonin. Identification of the functional domain that mediates the binding to elastin and comparison with the binding site to GlcNAc may be important for understanding the *in vivo* function of ficolins.

There could be microheterogeneity in porcine plasma ficolin-like proteins and EBP-37, based on the results of two-dimensional gel electrophoresis (data not shown). It is possible that the alteration of a peptide portion and the post-translational processing of ficolins alter the structure of the whole molecule. In order to understand the *in vivo* functions of the ficolin and EBP-37 group, it will be important to analyze the related gene products and the mechanism of polymerization, and to investigate the localization of the molecules in the extracellular matrix.

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REFERENCES

1. Adams, C.W.M. (1959) Role of lipids of aortic elastic fibers in atherogenesis. *Lancet* **i**, 1075-1076
2. Campa, J.S., Greenhalgh, R.M., and Powell, J.T. (1987) Elastin degradation in abdominal aortic aneurysms. *Atherosclerosis* **65**, 13-21
3. Harumiya, S., Omori, A., Sugiura, T., Fukumoto, Y., Tachikawa, H., and Fujimoto, D. (1995) EBP-37, a new elastin-binding protein in human plasma: Structural similarity to ficolins, transforming growth factor-β1-binding proteins. *J. Biochem.* **117**, 1029-1035

4. Ichijo, H., Rönnstrand, L., Miyagawa, K., Ohashi, H., Heldin, C.-H., and Miyazono, K. (1991) Purification of transforming growth factor-β1 binding proteins from porcine uterus membranes. *J. Biol. Chem.* **266**, 22459-22464
5. Ichijo, H., Hellman, U., Wernstedt, C., Gonez, L.J., Claesson-Welsh, L., Heldin, C.-H., and Miyazono, K. (1993) Molecular cloning and characterization of ficolin, a multimeric protein with fibrinogen- and collagen-like domains. *J. Biol. Chem.* **268**, 14505-14513
6. Matsushita, M., Endo, Y., Taira, S., Sato, Y., Fujita, T., Ichikawa, N., Nakata, M., and Mizuochi, T. (1996) A novel human serum lectin with collagen- and fibrinogen-like domains that functions as an opsonin. *J. Biol. Chem.* **271**, 2448-2454
7. Edgar, P.F. (1995) Hucolin, a new corticosteroid-binding protein from human plasma with structural similarities to ficolins, transforming growth factor-β1-binding proteins. *FEBS Lett.* **375**, 159-161
8. Partridge, S.M., Davis, H.F., and Adair, G.S. (1955) The chemistry of connective tissues. 2. Soluble proteins derived from partial hydrolysis of elastin. *Biochem. J.* **61**, 11-21
9. Harumiya, S., Jung, S.-K., Sakano, Y., and Fujimoto, D. (1993) Interaction of human plasma fibronectin with α-elastin. *J. Biochem.* **113**, 710-714
10. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685
11. Towbin, H., Staehelin, T., and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**, 4350-4354
12. Matsudaira, P. (1987) Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. *J. Biol. Chem.* **262**, 10035-10038
13. Cleveland, D.W., Fischer, S.G., Kirschner, M.W., and Laemmli, U.K. (1977) Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. *J. Biol. Chem.* **252**, 1102-1106
14. Zhao, Z., Lee, C.-C., Jiralerspong, S., Juyal, R.C., Lu, F., Baldini, A., Greenberg, F., Caskey, C.T., and Patel, P.I. (1995) The gene for a human microfibril-associated glycoprotein is commonly deleted in Smith-Magenis syndrome patients. *Hum. Mol. Genet.* **4**, 589-597
15. von Heijne, G. (1984) How signal sequences maintain cleavage specificity. *J. Mol. Biol.* **173**, 243-251
16. Higgins, D.G. and Sharp, P.M. (1988) CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. *Gene* **73**, 237-244
17. Lu, J., Tay, P.N., Kon, O.L., and Reid, K.B.M. (1996) Human ficolin: cDNA cloning, demonstration of peripheral blood leucocytes as the major site of synthesis and assignment of the gene to chromosome 9. *Biochem. J.* **313**, 473-478
18. Lu, J., Laursen, S.B., Thiel, S., Jensenius, J.C., and Reid, K.B.M. (1993) The cDNA cloning of conglutinin and identification of liver as a primary site of synthesis of conglutinin in members of the Bovidae. *Biochem. J.* **292**, 157-162
19. Parks, W.C., Pierce, R.A., Lee, K.A., and Mecham, R.P. (1993) Elastin in *Advances in Molecular and Cell Biology* (Kleinman, H.K., ed.) Vol. 6, pp. 133-181, JAI Press, London
20. Kobayashi, R., Mizutani, A., and Hidaka, H. (1994) Isolation and characterization of a 36-kDa microfibril-associated glycoprotein by the newly synthesized isoquinolinesulfonamide affinity chromatography. *Biochem. Biophys. Res. Commun.* **198**, 1262-1266
21. Sakai, L.Y., Keene, D.R., and Engvall, E. (1986) Fibrillin, a new 350-kDa glycoprotein, is a component of extracellular microfibrils. *J. Cell Biol.* **103**, 2499-2509
22. Maslen, C.L., Corson, G.M., Maddox, B.K., Glanville, R.W., and Sakai, L.Y. (1991) Partial sequence of a candidate gene for the Marfan syndrome. *Nature* **352**, 334-337
23. Lee, B., Godfrey, M., Vitale, E., Hori, H., Mattei, M.-G., Sarfarazi, M., Tsipouras, P., Ramirez, F., and Hollister, D.W. (1991) Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. *Nature* **352**, 330-334
24. Dietz, H.C., Cutting, G.R., Pyeritz, R.E., Maslen, C.L., Sakai, L.Y., Corson, G.M., Puffenburger, E.G., Hamosh, A., Nanthakumar, E.J., Curristin, S.M., Stetten, G., Myers, D.A., and Francomano, C.A. (1991) Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. *Nature* **352**, 337-339
25. Kanzaki, T., Olofsson, A., Moren, A., Wernstedt, C., Hellman, U., Miyazono, K., Claesson-Welsh, L., and Heldin, C.-H. (1990) TGF-β1 binding protein: A component of the large latent complex of TGF-β1 with multiple repeat sequences. *Cell* **61**, 1051-1061
26. Dallas, S.L., Miyazono, K., Skerry, T.M., Mundy, G.R., and Bonewald, L.F. (1995) Dual role for the latent transforming growth factor-β binding protein in storage of latent TGF-β in the extracellular matrix and as a structural matrix protein. *J. Cell Biol.* **131**, 539-549
27. Gibson, M.A., Hatzinikolas, G., Davis, E.C., Baker, E., Sutherland, G.R., and Mecham, R.P. (1995) Bovine latent transforming growth factor β1-binding protein 2: Molecular cloning, identification of tissue isoforms, and immunolocalization to elastin-associated microfibrils. *Mol. Cell. Biol.* **15**, 6932- 6942
28. Grainger, D.J., Kemp, P.R., Liu, A.C., Lawn, R.M., and Metcalfe, J.C. (1994) Activation of transforming growth factor-β is inhibited in transgenic apolipoprotein(a) mice. *Nature* **370**, 460-462
29. Grainger, D.J., Kemp, P.R., Metcalfe, J.C., Liu, A.C., Lawn, R.M., Williams, N.R., Grace, A.A., Schofield, P.M., and Chauhan, A. (1995) The serum concentration of active transforming growth factor-β is severely depressed in advanced atherosclerosis. *Nature Med.* **1**, 74-79