Purification, Cloning, and Characterization of a Profibrinolytic Plasminogen-binding Protein, TIP49a*

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The plasminogen receptors responsible for enhancing cell surface-dependent plasminogen activation expose COOH-terminal lysines on the cell surface and are sensitive to proteolysis by carboxypeptidase B (CpB). We treated U937 cells with CpB, then subjected membrane fractions to two-dimensional gel electrophoresis followed by ligand blotting with 125I-plasminogen. A 54-kDa protein lost the ability to bind 125I-plasminogen after treatment of intact cells and was purified by two-dimensional gel electrophoresis and then sequenced by mass spectrometry. Two separate amino acid sequences were obtained and were identical to sequences contained within human and rat TIP49a. The cDNA for the 54-kDa protein matched the human TIP49a sequence, and encoded a COOH-terminal lysine, consistent with susceptibility to CpB. Antibodies against rat TIP49a recognized the plasminogen-binding protein on two-dimensional Western blots of U937 cell membranes. Human 125I-Glu-plasminogen bound specifically to TIP49a protein, and binding was inhibited by e-aminoacproic acid. A single class of binding sites was detected, and a $K_d$ of 0.57 ± 0.14 μM was determined. TIP49a enhanced plasminogen activation 8-fold compared with the BSA control, and this was equivalent to the enhancement mediated by plasmin-treated fibrinogen. These results suggest that TIP49a is a previously unrecognized plasminogen-binding protein on the U937 cell surface.

Assembly of fibrinolytic molecules on the cell surface promotes plasminogen activation and the association of plasmin with cell surfaces (reviewed by Plow et al. in Ref. 1). This proteolytic activity on the cell surface participates in physiological processes in which cells must degrade extracellular matrices to migrate. The binding of plasminogen to cell surfaces is required for enhanced plasminogen activation (2–5), and inactivation of plasminogen binding sites eliminates the cell-dependent enhancement of activation (4, 6). Monocytoid U937 cells have a high capacity for plasminogen (~1.6 × 10^7 sites/cell; Ref. 7), and no single molecule can account for the entire capacity to bind plasminogen. Both protein and nonprotein (gangliosides; Refs. 8 and 9) molecules have been identified as plasminogen-binding molecules present on cell surfaces. Treatment of cells with carboxypeptidase B (CpB) reduces plasminogen binding to proteinaceous receptors on the cell surface (5, 10–12). COOH-terminal lysyl, rather than arginyl, residues are implicated as plasminogen binding sites because plasminogen has a much higher affinity for lysine than arginine. Although CpB treatment reduces plasminogen binding to U937 monocytoid cells by ~60%, the cell surface-dependent enhancement of plasminogen activation is reduced by >95% (5). This suggests that the class of plasminogen binding sites with COOH-terminal lysine residues accessible to CpB is predominantly responsible for the cell surface-dependent enhancement of plasminogen activation.

α-Enolase is a candidate monocytoid cell plasminogen receptor with a COOH-terminal lysine (10, 13). However, the number of molecules of α-enolase present on the surface of U937 cells is only 10% of the number of plasminogen binding sites. In the current study, we have utilized susceptibility to CpB treatment as a means to identify a previously unrecognized plasminogen-binding protein that exposes a COOH-terminal lysine in an accessible orientation on the cell surface. To discriminate between such proteins and proteins with inaccessible COOH-terminal lysines, whole cells were treated with CpB to remove exposed COOH-terminal lysines. Cell membranes were subsequently prepared and analyzed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) followed by ligand blotting with 125I-plasminogen. Comparison of ligand blots of membranes of untreated intact cells with membranes of CpB-treated intact cells revealed the plasminogen-binding proteins that exposed COOH-terminal lysines on the cell surface, i.e. the class of plasminogen-binding proteins that is predominantly responsible for the cell surface-dependent enhancement of plasminogen activation.

Using this methodology we have purified and identified a previously unrecognized plasminogen-binding protein present on the surface of U937 monocytoid cells. We obtained its cDNA sequence, which was identical to human TIP49a. TIP49a was characterized previously as a nuclear protein that has single-stranded DNA-stimulated ATPase and ATP-dependent DNA helicase activity (14) and is present in the cytosol as well (15). Here, we identify a new subcellular location for TIP49a and

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The abbreviations used are: CpB, carboxypeptidase B; 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; BSA, bovine serum albumin; HBSS, Hanks’ balanced salt solution supplemented with 20 mM HEPES; HRP, horseradish peroxidase; IEF, isoelectric focusing; MACS buffer, PBS containing BSA, EDTA, and heat-inactivated human serum; PBS, phosphate-buffered saline; PBST-80, PBS containing 0.025% Tween 80; PAGE, polyacrylamide gel electrophoresis; TBST, Tris-buffered saline with 0.1% Tween 20; TBP, TATA-binding protein; PCr, polymerase chain reaction; EST, expressed sequence tag; THC, tentative human consensus; CAPS, 3-(cyclohexylamino)propanesulfonic acid; tPA, tissue plasminogen activator.

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demonstrate that TIP49a binds plasminogen and enhances plasminogen activation.

**EXPERIMENTAL PROCEDURES**

**Proteins**—Glu-plasminogen was obtained from plasma prepared from fresh human blood collected into 3 ml EDTA, 3 ml benzamidine, 100 KIU/ml Trasylol (Miles, Kankakee, IL), and 100 µg/ml soybean trypsin inhibitor (Sigma). Glu-plasminogen was purified from the plasma by affinity chromatography on lysine-Sepharose (16) in 0.01 M sodium phosphate, pH 7.3, 0.15 M NaCl (phosphate-buffered saline (PBS)) containing 1 mM benzamidine, 3 mM EDTA, and 0.02% NaN3, followed by gel filtration on Bio-Gel A-5m (Bio-Rad) as described previously (17). The concentration of purified Glu-plasminogen was determined at 280 nm using an extinction coefficient of 1.61 E0.1% 190 (18). Glu-plasminogen was iodinated to specific activities of 0.34–0.45 µCi/µg using the IODINGEN method (19).

Recombinant rat TIP49a was expressed as a fusion protein containing an NH2-terminal histidine tag in BL21(DE3)pLysS er’s instructions. For every 107 cells, the cell pellet was resuspended in lysis buffer (50 mM Tris-Cl, pH 7.5, 5% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin G, and 0.2 M KCl, 1 mM CaCl2, and the protease inhibitors above). The current never exceeding 15 mA. The IEF gels were fixed in 12% trichloroacetic acid and washed six times with 50 ml of H2O. Individual lanes were excised from the gels and soaked in reduced sample buffer to remove SDS. The gels were then subjected to two-dimensional gel and overlaid with molten 0.5% agarose in 120 mM Tris, pH 6.8. Second dimension SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (23).

**Ligand Blotting**— Samples were subjected to 2D-PAGE and transferred to Immobilon P (Millipore, Bedford, MA) at 100 mA for 18 h in either 25 mM Tris-HCl containing 192 mM glycine and 10% methanol or 10 mM CAPS buffer, pH 11, with 10% methanol. Blots were blocked for 2 h in 2% BSA in PBS containing 0.1% Tween 20 (PBS-BSA), then incubated with 50 nM 125I-plasminogen in PBS-BSA for 2 h at 22 °C. The blots were washed seven times with PBS-BSA containing 0.5 M NaCl, then dried and subjected to autoradiography. Control blots were incubated with 50 nM 125I-plasminogen in the presence of 0.1 mM aminocaproic acid. Autoradiography was performed using BioMax MS film (Eastman Kodak Co.).

**Western Blotting**—Western blotting was performed on the Immobilon P membranes after probing with ligand blots with 125I-plasminogen. The membranes were blocked for 1 h in bovine lactotransferrin technique optimiser (5% dry nonfat milk in PBS with 0.03% antifree A and 2.5 mM phenylmethylsulfonyl fluoride (BLOTTO) (24). The primary antibody was added for 1 h, and the membranes were washed three times in Tris-buffered saline (0.01 M Tris-HCl, pH 7.2, 0.15 M NaCl) containing 0.1% Tween 20 (TBST). Goat anti-rabbit IgG-horseradish peroxidase (HRP) conjugate (Pierce) was added for 1 h in TBST. The blots were washed three times with TBST and then subjected to chemiluminescent detection using either ECL (Amersham Pharmacia Biotech) or Supersignal CL-HRP (Pierce).

**Plasminogen Binding Assays**—Nunc Maxisorp 96-well plates were coated with either 5 µg/ml TIP49a or 10 mg/ml BSA in 0.1 M NaHCO3, pH 8.5, for 2 h at 37 °C. All wells were postcoated with 10 mg/ml BSA in PBS containing 0.025% Tween 80 (PBST-80) for 1 h at 22 °C, and then washed three times with 1 mg/ml BSA in PBST-80. The immobilized TIP49a or the control BSA-coated wells were incubated with increasing concentrations of 125I-plasminogen diluted to PBS-BSA containing 1 mg/ml BSA for 4 h at 22 °C. The wells were washed four times with PBST-80, the bound 125I-plasminogen was solubilized with 10% SDS, and the radioactivity was quantitated by counting in a γ-counter (Isotope Data Inc., Palatine, IL). Nonspecific binding was determined as the amount of 125I-plasminogen bound to BSA, and specific binding was calculated by subtracting nonspecific binding from total binding. The binding kinetics were calculated by weighted least squares regression using the program LIGAND (25).

**Plasminogen Activation Assay**—Nunc Maxisorp 96-well plates were coated with either 5 µg/ml TIP49a or 10 mg/ml BSA in 0.1 M NaHCO3, pH 8.5, for 2 h at 37 °C, or with 10 µg/ml fibrinogen in PBS for 90 min at 37 °C. The fibrinogen-coated wells were washed once with 50 mM Tris-Cl, pH 7.5, containing 150 mM NaCl, 0.01% Tween 80, 1 mg/ml BSA, 1 mg/ml gelatin, and 50 units/ml Trasylol, and treated with 60 mg/ml plasmin (Kabi Pharmacia Inc., Franklin, OH) in PBS for 30 min at 37 °C. The plasm was inactivated with 0.1 M p-nitrophenyl-p'-guanidino benzate and 0.5 mM 4-(2-aminoethoxyl)benzenesulfonyl fluoride in PBS for 20 min at 25°C. All wells were postcoated with 10 mg/ml BSA in PBST-80 for 1 h at 22 °C and washed three times with 1 mg/ml BSA in PBST-80. The immobilized TIP49a and plasmin-treated fibrinogen were incubated with varying concentrations of 125I-plasminogen diluted into PBST-80 containing 1 mg/ml BSA for 4 h at 22 °C. The wells were then washed four times with PBST-80. A duplicate 96-well plate was included to determine the amount of 125I-plasminogen bound to TIP49a and plasmin-treated fibrinogen. The 125I-plasminogen bound in the duplicate wells was solubilized with 10% SDS, and the radioactivity was quantitated by counting in a γ-counter. The amount of 125I-plasminogen equivalent to the amount bound to the TIP49a-coated wells was added to the BSA-coated wells so that the effects of plasminogen could be compared with plasminogen bound to either TIP49a or plasmin-treated fibrinogen. The plasmin substrate, S-2251 (DiaPharma Group Inc., Franklin, OH), was added to all wells to a final concentration of 0.5 mM and t-PA was added to 15 mM, then A405 was
fractions from intact cells treated with CpB (Fig. 1, panel C), showed that a prominent plasminogen-binding protein (mass – 54 kDa, pI 6.5) was undetectable after CpB treatment of intact cells (arrow), indicating that it lost the ability to bind plasminogen. Cytoplasmic proteins did not show changes in plasminogen binding following CpB treatment of intact cells (Fig. 1, compare panels B and D), suggesting that proteolysis by CpB did not occur in the interior of intact cells. (One plasminogen-binding protein at –28 kDa was present in both membrane (panel A) and cytoplasmic (panel B) fractions and did show a decrease in 125I-plasminogen binding in the membrane fraction, and an anomalous decrease in 125I-plasminogen binding in the cytoplasmic fraction following CpB treatment. Since only this single protein showed a decrease in 125I-plasminogen binding following CpB treatment in both membrane and cytoplasmic fractions, it most likely represents a contamination of the cytoplasmic preparation by this membrane protein). We examined whether the 54-kDa CpB-sensitive plasminogen-binding protein corresponded to either α-enolase or cytokeratin 8, since these known plasminogen-binding proteins are in the size range of the 54-kDa protein. Western blotting with an anti-α-enolase antibody was performed on the same blots used for ligand blotting of control membranes and cytosol. Two spots reacting with anti-α-enolase were observed on the Western blots at a similar size as the 54-kDa protein, but at different pI values (Fig. 1, panels E and F), consistent with the reported distribution of multiple α-enolase isoforms with distinct pI values (27). Cytokeratin 8 was not detected in U937 membranes or cytosol by Western blotting (data not shown), consistent with its reported pattern of expression (28, 29). These data suggest that a previously unrecognized 54-kDa membrane-associated plasminogen-binding protein exposed a COOH-terminal lysine in an accessible orientation on the extracellular face of the cell.

We examined whether the 54-kDa plasminogen-binding protein was up-regulated during cell death. Cells were treated with 10 μg/ml cycloheximide for 18 h, resulting in an increase in the percentage of dead cells from 2% to 23% (as determined by propidium iodide incorporation measured by fluorescence-activated cell sorting). The 125I-plasminogen ligand blotting intensity of the 54-kDa protein increased ~4-fold following the cycloheximide treatment (data not shown).

**Purification and Identification of the Plasminogen-binding Protein**—To purify and identify the 54-kDa plasminogen-binding protein, we first examined whether the protein could be concentrated by solubilization of the U937 membranes in Triton X-100 containing protease inhibitors. The Triton X-100 insoluble material was subsequently solubilized in 0.5% SDS, and both soluble and insoluble fractions were subjected to two-dimensional ligand blotting with 125I-plasminogen. The 54-kDa protein was present in the Triton X-100 insoluble fraction in ~3-fold higher concentration than in the Triton X-100-soluble fraction (Fig. 2). The concentration by Triton X-100 solubilization was sufficient to allow purification of the novel plasminogen-binding protein directly from two-dimensional gels.

We sought to purify the 54-kDa plasminogen-binding protein directly from two-dimensional gels. 300 μg of the Triton X-100-insoluble fraction from U937 membranes were subjected to 2D-PAGE and replicate gels were either ligand-blotted with 125I-plasminogen (Fig. 3, panel A) or stained with colloidal Coomassie (Fig. 3, panel B). Two replicate colloidal Coomassie-stained gels were aligned with the 125I-plasminogen ligand blot and the 54-kDa plasminogen-binding protein was excised from the gels. The protein was eluted from the gels and digested with trypsin. A capillary reverse-phase chromatograph coupled

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**RESULTS**

**Exposure of Carboxyl-terminal Lysines on the U937 Cell Surface**—We examined U937 membrane-associated proteins for the presence of COOH-terminal lysines exposed to the extracellular environment. Intact U937 cells were incubated in either the presence or absence of 100 units/ml CpB prior to preparing membrane and cytoplasmic fractions as described under “Experimental Procedures.” The fractions were subjected to 2D-PAGE and ligand blotted with 125I-plasminogen. Comparison of ligand blots from membrane fractions of intact control cells (Fig. 1, panel A) with ligand blots of membrane

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**Fig. 1.** 2D-PAGE analysis of plasminogen-binding proteins that expose COOH-terminal lysines on the cell surface. Intact U937 cells were treated either in the absence (A and B) or presence (C and D) of 100 units/ml CpB for 30 min at 37 °C prior to fractionation into membrane and cytosolic fractions. 2D-PAGE was performed on 100 μg of membrane proteins (A and C) or 10 μg of the cytoplasmic proteins (B and D), followed by ligand blotting with 50 nm 125I-plasminogen. The PVDF membranes probed as 125I-plasminogen ligand blots in panels A and B were re-probed as Western blots using an anti-α-enolase antibody on both the membrane (E) and cytosolic (F) fractions. First dimension IEF using 2% Bio-Lytes, pH 6–8, is along the horizontal axis of the gel, and second dimension SDS-PAGE is along the vertical axis. The arrow indicates the location of the 54-kDa plasminogen-binding protein. The spot in panel A at ~34 kDa was an artifact of ligand blotting and was not observed in any other experiments.
were solubilized with Triton X-100 containing 2 mM peptatin A, 50 KIU/ml Trypsylod, 8 mM 2-guanidinoethylmercaptosuccinic acid, and 2 mM EDTA, and the insoluble material was pelleted by centrifugation at 10,000 × g for 5 min. 100 μg of protein from the Triton X-100-soluble fraction (A) or Triton X-100 insoluble fraction (B) were subjected to 2D-PAGE and replicate gels were either ligand-blotted with 125I-plasminogen (A) or Triton X-100 insoluble fraction (B). First dimension IEF using 2.0% Ampholine, pH 6–8, is along the horizontal axis of the gel, and second dimension SDS-PAGE is along the vertical axis. The arrow indicates the location of the 54-kDa plasminogen-binding protein.

Fig. 2. Concentration of the novel plasminogen-binding protein from U937 membranes using Triton X-100. U937 membranes were solubilized with Triton X-100 containing 2 μM leupeptin, 1.5 mM pepstatin A, 50 KIU/ml Trypsylod, 8 mM 2-guanidinoethylmercaptosuccinic acid, and 2 mM EDTA, and the insoluble material was pelleted by centrifugation at 10,000 × g for 5 min. 100 μg of protein from the Triton X-100-soluble fraction (A) or Triton X-100 insoluble fraction (B) were subjected to 2D-PAGE and ligand blotted with 50 nM 125I-plasminogen. First dimension IEF using 2.0% Ampholine, pH 6–8, is along the horizontal axis of the gel, and second dimension SDS-PAGE is along the vertical axis.

Fig. 3. Purification of the 54-kDa plasminogen-binding protein. 300 μg of the Triton X-100-insoluble fraction of U937 membranes was subjected to 2D-PAGE and replicate gels were either ligand-blotted with 125I-plasminogen (A) or stained with colloidal Coomassie (B). First dimension IEF using 2.0% Ampholine, pH 6–8, is along the horizontal axis of the gel, and second dimension SDS-PAGE is along the vertical axis.

to the electrospray ionization source of a Finnigan LCQ quadrupole ion trap mass spectrometer was used to obtain two peptide sequences, 11 and 18 residues, from the tryptic digest of the protein (Table I). The exact peptides were found in a rat protein, TATA-binding protein-interacting protein (TIP49a) by searching the nonredundant protein sequence data base (30). When we obtained these sequences, they were not present in the National Center for Biotechnology Information nonredundant human protein data base.

BLAST searches of the nonredundant human EST data base yielded three ESTs whose calculated translation products were an exact match to the 11-residue peptide sequence from the 54-kDa protein, and one EST whose calculated translation product was an exact match to the 18-residue peptide sequence. The EST that matched the 18-residue peptide sequence also contained cDNA sequence encoding the 11-residue peptide sequence in frame with the other peptide sequence. These ESTs were used to search the TIGR Human Gene Index data base (available via the World Wide Web) for tentative human consensus (THC) sequences (31). The ESTs used to assemble the THC sequence were then used to search GenBank for additional ESTs with significant sequence identity. Alignment of the ESTs revealed that there were significant amounts of sequence not contained within the original THC, and that the aligned ESTs extended over two THC sequences, THC 171358 and THC 153501 (Fig. 4). All ESTs mapped to this alignment, suggesting that only one molecule could account for the peptide sequences generated. A consensus sequence was generated from the EST alignment, and the predicted translation product of the consensus sequence was identical to the rat TIP49a protein except for five amino acid substitutions (Fig. 5).

Cloning the Plasminogen-binding Protein—One EST clone (185194) was screened for a possible full-length cDNA insert by performing PCR with primers made to the cDNA sequence outside of the calculated open reading frame of the full-length consensus cDNA. This EST clone contained sequence that corresponded to the 5’ region of the consensus cDNA sequence, and was a close match to the consensus cDNA. EST 185194 yielded a PCR product 1.6 kilobase pairs in length, and the entire 1734-base pair cDNA insert was sequenced. This EST clone contained a full-length human TIP49a cDNA that was identical to the human TIP49a cDNA recently reported by Makino et al. (22), and whose predicted translation product differed from the predicted translation product of the consensus sequence by 4 amino acid residues (Fig. 5). The predicted translation products of the human and rat TIP49a cDNAs were identical except for one amino acid at position 291, which is isoleucine in the human sequence and valine in the rat sequence, as published previously (22). Both molecules have a lysine at the carboxyl terminus, suggesting that these molecules are capable of binding plasminogen.

To verify that TIP49a corresponded to the 54-kDa plasminogen-binding protein, two-dimensional 125I-plasminogen ligand blots of U937 and monocyte membrane proteins were re-probed as Western blots using anti-rat TIP49a antibody (Fig. 6). The immunoreactive protein observed with the anti-rat TIP49a antibody directly overlaid the 54-kDa 125I-plasminogen-binding spot observed at pl 6.5. TIP49a was present in both U937 and peripheral blood monocyte membranes.

Plasminogen Binding to TIP49a—We tested whether plasminogen could bind directly to TIP49a. Recombinant rat TIP49a was immobilized on wells of microtiter plates and incubated with increasing concentrations of 125I-Glu-plasminogen. The binding was specific and saturable. At an input concentration of 0.25 μM 125I-plasminogen, 15.8 μg unlabeled plasminogen inhibited the binding by 94%, and there was no competition with a 1:180 dilution of rabbit serum. Plasminogen binding was dose-dependent over a concentration range of 16 nM to 2 μM 125I-plasminogen and reached saturation at 2 μM, with a binding maximum of 0.83 pmol (Fig. 7). When the data in the binding isotherm in Fig. 7 were subjected to weighted least squares regression, a single class of binding sites with a dissociation constant in the range of the dissociation constant reported for the interaction of plasminogen with U937 cells (Kd = 0.5–0.8 μM; Refs. 32 and 33).

Functional Consequences of the Interaction between Plasminogen and TIP49a—We examined the ability of TIP49a to enhance plasminogen activation. The wells of microtiter plates were coated with recombinant rat TIP49a and incubated with increasing concentrations of 125I-Glu-plasminogen to generate
differing amounts of bound plasminogen. Unbound 125I-plasminogen was removed. In duplicate wells, the amount of 125I-plasminogen bound was determined and an equivalent amount of 125I-Glu-plasminogen was added to BSA-coated wells to determine the rate of plasmin formation from soluble plasminogen. The rate of plasmin formation from 125I-plasminogen bound to plasmin-treated fibrinogen was determined in parallel. The cleavage of S-2251 after the addition of 15 nM tPA was measured and plasmin formation was determined using a plasmin standard curve. Plasmin formation increased with the amount of 125I-plasminogen bound to either TIP49a or plasmin-treated fibrinogen, but changed little with increasing concentrations of 125I-plasminogen in solution. When 0.78 pmol of 125I-Glu-plasminogen were bound to immobilized rat TIP49a, tPA-dependent activation was enhanced 8-fold compared with the same amount of soluble plasminogen, and was equivalent to the enhancement observed with plasminogen bound to plasmin-treated fibrinogen (Fig. 8).

DISCUSSION

The purpose of our study was to examine monocytoid cells for the presence of profibrinolytic plasminogen-binding proteins. Only the subset of plasminogen-binding proteins that is accessible to CpB on the cell surface accounts for the majority of the ability of monocytoid cells to stimulate plasminogen activation (5). To identify members of this subpopulation of plasminogen-binding proteins, we used treatment of intact U937 cells with CpB followed by 2D-PAGE and 125I-plasminogen ligand blotting of cell membranes to discriminate between CpB-accessible and inaccessible plasminogen-binding proteins on the surface of monocytoid cells. Comparison of membranes from untreated cells with those of CpB-treated cells showed a loss of ligand blotting activity by proteins that were accessible to CpB on the cell membrane. One protein was observed to be particularly sensitive to CpB treatment of the intact cells and was purified by 2D-PAGE. Based on mass spectrometry of tryptic digests, the protein was identified as TIP49a. We obtained a full-length clone from a human EST clone, and the cDNA sequence predicted a protein with a COOH-terminal lysine, consistent with a plasminogen-binding protein. Recombinant TIP49a exhibited properties of a profibrinolytic plasminogen receptor because it bound plasminogen and promoted plasminogen activation.

TIP49a was originally identified as a nuclear protein and was isolated as a TATA-binding protein (TBP)-interacting protein by chromatography of rat liver nuclear extracts on a histidine tagged TATA-binding protein affinity column (21). TIP49a is present in a macromolecular complex with TBP in nuclear extracts (21), and was later found to directly bind TBP (34). TIP49a exhibits partial homology to RuvB bacterial recombination factors, including regions with a high degree of homology to Walker A and B motifs that are characteristic of DNA/RNA helicases (21), and was subsequently demonstrated to have single-stranded DNA-stimulated ATPase and ATP-de-
pendent DNA helicase activity (14). Human TIP49a cloned from a human cDNA library has a predicted sequence that differs from rat TIP49a at a single residue Val291 (rat) to Ile291 (human) (22).

Other studies using different purification strategies have identified proteins with a cDNA sequence identical to human TIP49a. RuvB-like protein (RUVBL1) was identified using the 14-kDa subunit of replication protein A as bait in a yeast two-hybrid system (35). RUVBL1 (TIP49a) was found to coimmunoprecipitate with at least three unidentified proteins, and was demonstrated to be essential for yeast viability (35). An ubiquitously occurring nuclear matrix protein (NMP238) with a pI of 6.5 and migrating with a mass of 54 kDa was detected by systematic comparison of two-dimensional electrophoretic patterns of nuclear membrane proteins from a variety of human tissues and cell lines (15). Pontin52 was isolated by affinity binding of SW480 cell lysates with recombinant β-catenin (34). RuvBL1, NMP238, and Pontin52 have cDNA sequences identical to TIP49a (15, 34, 35).

Subsequent studies indicate that TIP49a is distributed in multiple subcellular compartments in addition to the nucleus. A cytoplasmic localization of TIP49a has been demonstrated by
immunofluorescence microscopy and immunoblot analysis (15). Furthermore, a cytoplasmic protein with TIP49a sequence, ECP-54 (standing for erythrocyte cytosolic protein), was identified by affinity chromatography of erythrocyte cytosol with the integral membrane protein stomatin (36). TIP49a is similar to subunits of T-complex protein 1 and may function as a chaperone in the cytosol (15). In the current study, we found TIP49a associated with the membranes of U937 monocyte cells and peripheral blood monocytes. Because membrane TIP49a was identified by treating intact cells with CpB, under conditions where cytoplasmic proteins were unaffected, the membrane localization could not be attributed to cytoplasmic contamination of the membrane preparations. Thus, TIP49a appears to be a “moonlighting” protein, a member of a growing list of proteins that are recognized as identical gene products exhibiting multiple functions at distinct cellular and extracellular sites through “gene sharing” (37–39).

The mechanism by which TIP49a is exposed on the cell membrane is unknown. The cDNA sequence of TIP49a does not predict a classical signal sequence. Nonetheless, TIP49a acts as an autoantigen in sera of some patients with autoimmune diseases such as poly-myositis/dermatitis and autoimmune hepatitis (22). Thus, mechanisms for release of TIP49a and its membrane localization or direct translocation to the cell membrane must exist. Several extracellular proteins lacking cleavable signal sequences are well known (40). Interestingly, other candidate plasminogen receptors, α-enolase (10), annexin II (41), cytokektarin 8 (42), actin (43), and amphoterin (44), in mammalian cells and bacterial glyceraldehyde-3-phosphate dehydrogenase (45, 46) also lack cleavable signal sequences.

The interaction of plasminogen with TIP49a mimicked the interaction of plasminogen with cells. The Kd for the interaction of TIP49a with plasminogen was 0.57 μM, similar to that which we have measured previously for the interaction of plasminogen with U937 cells (Kd = 0.8 μM; Ref. 32). Plasminogen bound to TIP49a was more readily activated (8-fold) than plasminogen in solution, as shown for plasminogen bound to cells and cell lines (reviewed in Ref. 7).

TIP49a is widely distributed and is present in normal human tissues and cells and in transformed cells. TIP49a mRNA and/or protein have been detected in heart, brain, placenta, lung, liver, lung, kidney, spleen, and testes (15, 34, 36). Within the testes, TIP49a was specifically localized in a subset of the germ cells at the stage of pachyteny (stage IX–X) to round spermatids (14). TIP49a has been detected in MCF7, HeLa, TCL598, Jurkat, KB, SW480, AN3-CA cancer cells, 293 T-transformed embryonic kidney cells, and WI38 primary fibroblasts (15, 34, 35). By Western blotting with rat anti-TIP49a (21), we have also identified TIP49a in membranes of 293s, COS, and Raw 264 cells.2

The subcellular distribution and function of TIP49a may vary between cell types. Nonetheless, on U937 monocyte cells, TIP49a exhibits a membrane-associated subcellular location, in an orientation in which TIP49a may directly interact with plasminogen and promote plasminogen activation.

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