ADAM 12, a Disintegrin Metalloprotease, Interacts with Insulin-like Growth Factor-binding Protein-3*

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Insulin-like growth factor-binding protein (IGFBP)-3 binds the insulin-like growth factors with high affinity and modulates their actions. Proteolytic cleavage of IGFBP-3 may regulate insulin-like growth factor bioavailability. IGFBP-3 is extensively degraded in serum during pregnancy; however, as yet the pregnancy-specific protease, or proteases, have not been identified. We utilized a yeast two-hybrid assay and a human placental cDNA library to investigate IGFBP-3-interacting proteins. A disintegrin and metalloprotease-12 (ADAM 12), a member of a family of metalloprotease disintegrins that is highly expressed in placental tissue, was identified as interacting with IGFBP-3. This interaction involved the cysteine-rich domain of ADAM 12. Unlike other members of this family of disintegrin metalloproteases that are membrane proteins, ADAM 12 exists as an alternatively spliced soluble secreted protein. To verify the interaction between ADAM 12 and IGFBP-3, an expression construct containing an ADAM 12-S cDNA was transfected into COS-1 cells. Co-purification was observed when conditioned medium was analyzed by immunoprecipitation with an antibody against either ADAM 12 or IGFBP-3 followed by Western blotting with anti-IGFBP-3 or anti-ADAM 12. Although minimal proteolysis of IGFBP-3 was observed in conditioned medium from control cells, this was increased ~4-fold in conditioned medium from ADAM 12-S-transfected cells. Recombinant ADAM 12-S partially purified from conditioned medium on a heparin-Sepharose column also proteolyzed IGFBP-3. The degradation pattern was similar to that seen with pregnancy serum, and the presence of ADAM 12-S in serum during pregnancy was confirmed. The data suggest that ADAM 12-S has IGFBP-3 protease activity, and it may contribute to the IGFBP-3 protease activity present in pregnancy serum.

The majority of the insulin-like growth factor (IGF)1 binding capacity in rodent and human serum is attributable to insulin-like growth factor-binding protein-3 (IGFBP-3) (1, 2). This binding protein is present in serum as a complex of ~150–200 kDa. This ternary complex is composed of IGF-I or IGF-II, an 85-kDa acid-labile subunit and IGFBP-3 (2). The ternary complex represents a relatively slowly turning over the reservoir of IGF, which is regulated largely by growth hormone. Both the acid-labile subunit and IGFBP-3 are growth hormone-dependent, either directly or via IGF-1 (3, 4).

Because IGFBP-3 has an affinity for IGF-I and -II comparable, or greater than, their receptors and is present in high concentrations in serum and biological fluids, the mechanism whereby IGF is liberated from this complex to interact with the respective membrane receptors is unclear at present. Proteolytic degradation of IGFBP-3 generates fragments with reduced binding affinity for IGF-I and consequently has been regarded as one potential mechanism whereby delivery of IGF-I to the receptor is facilitated (5–7).

Under normal circumstances the IGFBP-3 present in the plasma is largely intact, although some IGFBP-3 protease activity is detectable. In serum from pregnant women and to a lesser extent serum from poorly controlled diabetic patients and patients recovering from surgery or severe illness, much of the immunoreactive IGFBP-3 is present as smaller proteolytic fragments that have reduced binding affinity for IGF-I (5–7). It is thought that this proteolytic degradation of IGFBP-3 enhances IGF-I and -II bioavailability in these situations. Evidence from size fractionation suggests that IGFBP-3 protease activity present in third trimester serum is likely to be because of more than one IGFBP-3 protease (8). This IGFBP-3 protease activity disappears rapidly after parturition and is thought to arise from the placenta (5, 8). IGFBP-3 protease activity has also been identified in a variety of other situations such as in conditioned medium from MCF-7 breast cancer cells (9), skin blister fluid (10), and in ovarian follicular fluid (11). The relationship between each of these protease activities is not clear at this point in time. Partial purification of a major IGFBP-3 protease from late pregnant serum indicates that the protease also has activity against IGFBP-4 and -5, is present in fractions containing proteins of apparent molecular size of 50–100 kDa, and is a gelatinase-like protease that is recognized by an anti-disintegrin domain antibody (5). This latter observation suggests that this protease may possibly be a soluble disintegrin metalloprotease, that is, a member of the ADAM family. Although this gelatinase-like protease appears to be the major IGFBP-3 protease present in pregnancy serum, there is evidence of other abundant proteases in sera from pregnant women that have slightly different specificity (8).

We utilized a yeast two-hybrid system to identified placental proteins that interact with IGFBP-3. ADAM 12-S, a secreted disintegrin metalloprotease, was identified (12). Furthermore our results suggest that IGFBP-3 is a substrate for ADAM 12-S proteolytic activity.

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§ The abbreviations used are: IGF, insulin-like growth factor; IGFBP-3, insulin-like growth factor-binding protein-3; ADAM 12, a disintegrin and metalloprotease-12; ADAM 12-S, ADAM short soluble form; kb, kilobase; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; PIPES, 1,4-piperazinediethanesulfonic acid; ADAM 12-L, ADAM long membrane-bound form.
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EXPERIMENTAL PROCEDURES

DNA Constructs and Library Screening—The plasmid, pBlueScript/human IGFBP-3, containing a 2.4-kb full-length human IGFBP-3 cDNA was digested with HindIII. The 1.9-kb HindIII fragment was recovered and cut with AvaI, resulting in a 1.0-kb fragment containing a sequence encoding 30–264-amino acid residues with a 27-amino acid signal sequence deleted. The AvaI fragment was blunted with klenow enzyme, and ligated into a blunt-ended vector pAS1 of the yeast two-hybrid system, resulting in an in-frame fusion of human IGFBP-3 cDNA downstream of GAL4 DNA binding domain. The correct in-frame sequence was verified by sequence analysis. The expression of the fusion protein with molecular mass of 48 kDa was confirmed by Western blot in the yeast cell strain CG1945 transfected with this construct. A human placental cDNA library in the pACT2 plasmid (CLONTECH Laboratories, Inc.) was screened with the human IGFBP-3 pAS1/BP-3 construct at a scaled large secretional polyethylene glycol/lithium acetate transformation method according to the manufacturer’s instructions. Yeast cells of the CG1945 strain containing pAS1/hBP-3 were transformed with 20–40 µg of library DNA/transformation. The transformed cells were spread on 40 SD/Trp-Leu-His plates containing 5 µM 3-amino-1,2,4-triazole and incubated 5 days at 30 °C. Plasmid DNAs were extracted from yeast cells cultured in SD/Trp-Leu-His/3-amino-1,2,4-triazole medium. Separating the pAS1/BP-3 plasmid from the AD/library plasmid, and selecting AD/library plasmids was carried out by transformation of Escherichia coli HB101 carrying a leuB mutation and plating cells on M9 agar medium containing 50 µg/ml ampicillin, 40 µg/ml proline, and 1 µM thiamine-HCl. The plasmids were analyzed by restriction enzyme digestion and polyacrylamide gel electrophoresis. The interaction of IGFBP-3 with the positive cDNA clones was verified by mating yeast. Yeast Y187 cells were transformed with pAS1/ BP-3 and the yeast CG1945 cells were transformed with AD-positive plasmids prepared from E. coli HB101. These two yeast cells were mated and plated on SD/Trp-Leu-His/5 µM 3-amino-1,2,4-triazole medium. Plasmids were sequenced on an ABI automated DNA sequencer with an M13 forward primer and reverse primer 5’-GTTGAAGTGAACTTGGAGTGTAATG-3’; the polymerase chain reaction products were cloned into the EcoRI/XhoI site of the pACT2 vector, and the open-reading frame of the fusion protein was confirmed by DNA sequence analysis. Yeast Y187 cells were co-transformed with pAS1/hBP-3 and the various deletion constructs of ADAM 12-S and grown on SD/Trp-Leu-His plates. The interaction of BP-3 with the various ADAM 12-S domains was determined by the detection of β-galactosidase activity using a chemiluminescent detection kit (CLONTECH Laboratories Inc., Palo Alto, CA).

Transfection, Immunoprecipitation, and Western Blotting—Biotinylated goat anti-human IGFBP-3 antibody was obtained fromDiagnostic Systems Laboratories, Webster, TX. The rabbit ADAM 12 antibody, rb 119, was raised against the disintegrin domain of ADAM 12, amino acids 411–557 (13). The rb 122 antibody was raised in rabbits against the N- and C-terminal regions of ADAM 12-S (13). An expression construct containing the full-length human ADAM 12-S cDNA (GenBank accession number AF023477 (12, 13)) was transfected into a COS-1 cell (ATCC, Rockville, MA) using Lipofectin (Life Technologies, Inc.) according to manufacturer’s instructions. After 24 h the transfected cells were washed twice with serum-free Dulbecco’s modified Eagle’s medium and cultured for a further 48 h. The conditioned medium was centrifuged at 1100 x g for 10 min in a Sorvall RC-5B centrifuge (Invitrogen, Carlsbad, CA) at 4 °C overnight and then with peroxidase-conjugated goat anti-rabbit IgG (Life Technologies, Inc.). Detection was performed using an ECL Western blotting detection kit from Amersham Pharmacia Biotech.

Partial Purification of Recombinant ADAM 12-S—The ADAM 12-S cDNA was cloned in between the PvuII and NotI sites of the expression vector (Invitrogen, Carlsbad, CA). The 293-EBNA cell line (Invitrogen, Carlsbad, CA) was transfected with this expression plasmid using LipofectAMINE (Life Technologies, Inc.). Transfected cells were grown in Dulbecco’s modified Eagle’s medium with Glutamax I and 10% fetal bovine serum (Life Technologies, Inc.) with 100 µg/ml hygromycin B (Roche Molecular Biochemicals) to select for cells carrying the expression plasmid. For partial purification of the ADAM 12-S protein, cells were grown in Dulbecco’s modified Eagle’s medium/F12 medium, and the conditioned medium was diluted with two volumes of 20 mM Tris (pH 8.0) and subjected to chromatography on a HiTrap column (Amersham Pharmacia Biotech). Elution was performed with a 50–300 mM NaCl gradient in 20 mM Tris (pH 8.0) and 0.2% CHAPS. Fractions containing the ADAM 12-S protein were identified by Western blotting. Containing fractions from 13-chromatography of medium from untransfected 293-EBNA cells served as a negative control. IGFBP-3 Protease Activity—The IGFBP-3 protease activity was assessed as described previously (9). Samples were incubated with recombinant human IGFBP-3 in the buffer containing 100 mM NaCl, 50 mM Tris-HCl (pH 7.4), 10 mM CaCl2, and 0.05% CHAPS, 2 mM CaCl2, and 1 µM ZnCl2 unless otherwise stated. Subsequently, the samples were subjected to 12% SDS-polyacrylamide gel electrophoresis under nonreducing conditions, and the proteolytic fragments were detected by Western blot as described above. Serum from normal nonpregnant women and women in the third trimester of pregnancy was obtained with informed consent from Department of Obstetrics and Gynecology, University of Manitoba and by Dr. J. Bock, Department of Obstetrics and Gynecology, University Hospital of Copenhagen, Denmark.

Inhibition of IGFBP-3 Protease Activity by EDTA and 1,10-Phenanthroline—The heparin-Sepharose fraction of conditioned medium from 293-EBNA transfected with the ADAM 12-S cDNA was stripped of endogenous metalloprotease with 2 mM EDTA, 0.2 mM 1,10-phenanthroline. After 10 min at 20 °C the chelating reagents were removed using a MicroSpin G-25 column (Amersham Pharmacia Biotech). The result fraction was tested in the IGFBP-3 protease assay in the presence and absence of 2 mM CaCl2 and 1 µM ZnCl2.

RESULTS

Two-hybrid Screening of the Placental cDNA Library—A total of 5 x 108 tryptophan and leucine auxotrophic transformants were screened resulting in the identification of 53 positive colonies. Three colonies were chosen at random for further study. The plasmids were rescued from these colonies, expanded in E. coli, and re-introduced into yeast cells to confirm the interaction with the pAS1/hBP-3 bait plasmid. The inserts present in the three AD-positive plasmids were sequenced and found to be identical to the previously reported sequence of human TAPI (14), fibronectin (15), and ADAM 12 also known as meltrin α (GenBank™ accession number AF023477 (12)). Because ADAM 12 is an active metalloprotease (12) and highly expressed in placenta (13), we chose to examine the interaction of ADAM 12 and IGFBP-3 in detail.

The GALA-DNA activation domain fusion protein identified in the yeast two-hybrid screen contains the ADAM 12 sequence starting at amino acid number 546, that is, downstream of the disintegrin domain and upstream of the fusion peptide-like sequence in the cysteine-rich domain (Fig. 1). The clone identified in the yeast two-hybrid screen contained a 1.5-kb insert including 0.8-kb 3'-untranslated sequence. Sequence information derived from the 3'-end of the insert indicated that the DNA sequence was that of ADAM 12-S, lacking the trans-
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Membrane domain and cytoplasmic tail that is present in the long form, ADAM 12-L. In frame 5'-deletions were made using polymerase chain reaction and the mutant clones tested for interaction in the yeast system. Deletion of amino acids 546–619 reduced the expression of β-galactosidase by ∼10-fold (Fig. 1). Deletion of a further 60 amino acid residues reduced β-galactosidase activity to baseline.

**ADAM 12-S Is Present in Pregnancy Serum**—Sera from nonpregnant and pregnant women were analyzed by immunoblot using polyclonal rabbit serum and monoclonal antibody generated against ADAM 12. ADAM 12-S was not detected in serum from nonpregnant women, whereas the 68-kDa protease was easily detected with either antibody in serum from a pregnant woman (Fig. 2). An immunoreactive band of 250 kDa was also apparent with both antibodies and may represent ADAM 12-S complexed to larger proteins such as α2-macroglobulin (12).

**IGFBP-3 Interacts with ADAM 12**—To confirm the interaction of ADAM 12 with IGFBP-3, COS-1 cells were transfected with an expression vector containing a full-length ADAM 12-S cDNA (12). Expression of recombinant ADAM 12-S in COS-1 cells was confirmed by Western blotting using antisera raised against ADAM 12 (13). Although both the 92- and 68-kDa protease and the mature 68-kDa protease were observed in conditioned medium, the 68-kDa form predominated (data not shown).

Although COS-1 express IGFBP-3 at a low level, additional exogenous human IGFBP-3 was added to demonstrate interaction with ADAM 12. Immunoprecipitation with antibody against IGFBP-3 precipitated ADAM 12 from conditioned medium (Fig. 3, lane 1). In immunoprecipitates from conditioned medium from ADAM 12-S-transfected cells, four bands were apparent, the 92- and 68-kDa forms of ADAM 12-S and a faint slowly migrating band and a prominent band with an apparent size of 50 kDa. We assume that these bands correspond to the immunoglobulin fragments, because they are present in immunoprecipitates of conditioned medium from vector-transfected cells (lane 2). Both the 92- and 68-kDa forms of ADAM 12-S were observed in the immunoprecipitates; however, because of the relatively higher abundance of the 68-kDa form of ADAM 12-S in conditioned medium, this form predominated in the immunoprecipitates. The specificity of these immunoreactive bands was confirmed by the absence in conditioned medium from COS-1 cells transfected with the empty vector. Only the immunoglobulin-derived bands were apparent in the immunoprecipitates of the control conditioned medium (Fig. 3, lane 2). Furthermore protein A-Sepharose beads coated with normal rabbit serum did not precipitate ADAM 12-S (Fig. 3, lane 4). A complementary experiment was performed to confirm the interaction. In this case conditioned medium from ADAM 12-S-transfected cells together with conditioned medium from control cells were analyzed by immunoprecipitation with antibody to ADAM 12, and the IGFBP-3 present in the immunoprecipitates was identified by immunoblotting with antibody to IGFBP-3 (Fig. 4). IGFBP-3 was easily identified in the anti-ADAM 12 immunoprecipitates from ADAM 12-S-transfected cells but was not observed when nonimmune normal rabbit serum was used (Fig. 4, lane 1 compared with lane 3). In these experiments, a biotinylated anti-IGFBP-3 antibody was used together with a streptavidin-horseradish peroxidase conjugate, and consequently the immunoglobulin bands are not apparent. A very weak IGFBP-3 band was observed when conditioned medium from control cells were subjected to immunoprecipitation with antibody to ADAM 12 and normal rabbit serum (Fig. 4).
4, lanes 2 and 4) and also in conditioned medium from ADAM 12-S-transfected cells immunoprecipitated with normal rabbit serum (Fig. 4, lane 3). This most probably reflects residual, nonspecifically bound IGFBP-3 not completely removed during the washing steps.

**IGFBP-3 Proteolysis by ADAM 12**—To determine whether ADAM 12 had any proteolytic activity directed against IGFBP-3, we examined the levels of endogenous IGFBP-3 in conditioned medium from COS-1 cells transfected with ADAM 12-S or the empty vector. Although the endogenous IGFBP-3 secreted by the COS-1 cells was largely intact in ADAM 12-S containing conditioned medium, the 30-and 19-kDa IGFBP-3 proteolytic fragments were easily seen. Whereas in conditioned medium from control cells only a small amount of the 30-kDa fragment was observed (Fig. 5).

Because this IGFBP-3 proteolysis could have occurred at the cell membrane rather than in the conditioned medium, we tested the ability of the conditioned medium to degrade IGFBP-3 in the absence of the cell monolayer. For these experiments recombinant IGFBP-3 was incubated with control or ADAM 12-S containing conditioned medium. As a positive control human third trimester pregnancy serum was included in the analysis. Proteolytic degradation of IGFBP-3 was apparent with conditioned medium from the COS-1 cell transfected with ADAM 12-S but not with conditioned medium from cells transfected with the empty vector (Fig. 6A, lane 4 and 5 compared with lane 7). The major immunoreactive proteolytic fragments of IGFBP-3 generated by the ADAM 12-S containing conditioned medium had similar apparent molecular masses, ~30 and 19 kDa, as that apparent in human pregnancy serum (Fig. 6, lane 3).

The time course of proteolytic degradation is shown in Fig. 7. A small amount of degraded IGFBP-3 was apparent in the zero time sample possibly as a result of degradation of endogenously expressed IGFBP-3 or because of some minimal degradation of exogenous IGFBP-3 that occurs during the storage of this sample of ~20 °C for the 24-h period. In samples incubated at 37 °C there was a time-dependent increase in the amount of IGFBP-3 degraded. However even after 24 h the majority of the IGFBP-3 remained intact. When the immunoreactive bands were analyzed by densitometry, the ratio of the sum of 17- and 30-kDa IGFBP-3 fragment to intact IGFBP-3 increased from 0.001 to 0.258.

The ADAM 12-S was partially purified from COS-1 and 293-EBNA cell-conditioned medium by heparin-Sepharose chromatography. The heparin-Sepharose fraction from trans-
fected cells was devoid of any endogenous IGFBP-3 (Fig. 8A, lane 4) had marked protease activity against IGFBP-3, whereas no activity was apparent in the heparin-Sepharose fraction of conditioned medium from untransfected cells (Fig. 8). The proteolytic activity of the ADAM 12-S-containing heparin-Sepharose fraction was partially inhibited by EDTA and 1,10-phenanthroline (Fig. 8A). To investigate the effect of divalent cations further, the partially purified ADAM 12-S was preincubated with EDTA and 1,10-phenanthroline and then excess EDTA and 1,10-phenanthroline was removed by Sephadex G25 chromatography. In the absence of divalent cations, the resulting ADAM 12-S containing fraction was without activity against IGFBP-3 (Fig. 8B, lane 4). However, this fraction had full activity in the presence of 2 mM CaCl2 and 1 mM ZnCl2 (Fig. 8B, lane 5).

**DISCUSSION**

Utilizing the yeast two-hybrid system and IGFBP-3 as a bait, we identified ADAM 12, TAP1, and fibronectin cDNAs in a human placental library. TAP1 is a member of the ABC transporter superfamily and is associated with the export of proteins from the cytosol to the endoplasmic reticulum lumen (16). The functional significance of the association between TAP1 and IGFBP-3 is unclear, and no attempt as yet has been made to confirm this interaction in mammalian cells. Although no direct interaction between IGFBP-3 and fibronectin has been reported, the closely related binding protein, IGFBP-5, binds to fibronectin (17). Thus it is likely that IGFBP-3 also interacts with fibronectin.

We chose to examine the interaction between ADAM 12 and IGFBP-3 in further detail because a component of the IGFBP-3 protease activity in pregnancy serum appears to be because of metalloprotease (5, 8). ADAM 12, also known as meltrin α, is a member of a large family of disintegrin metalloproteases that are widely distributed and developmentally regulated and conserved across species (18). They are usually membrane-anchored proteins that serve multiple functions including cell-cell and cell-matrix adhesion as well as proteolysis (18). These disintegrin metalloproteases are involved in a diverse array of physiological processes including lymphocyte adhesion, wound healing, hemostasis, sperm attachment, invasion, and metastasis (18). ADAM 12 is abundantly expressed in the human placenta (12). In addition to the membrane-bound form or long form of this protein, ADAM 12-L, an alternatively spliced secreted form is expressed in the placenta (12). ADAM 12-S differs from ADAM 12-L at the C-terminal end, is devoid of the transmembrane and cytoplasmic domains, and as demon-
strated here is present in the maternal circulation. ADAM 12-S, which is not active in its 96-kDa form, contains a prodomain at the N terminus, upstream of a furin cleavage site. Removal of the prodomain converts ADAM 12-S into a ~68-kDa active zinc-dependent metalloprotease (12). Downstream of the metalloprotease domain is the disintegrin domain followed by a cysteine-rich domain. The GAL4-DNA binding domain fusion protein identified in the yeast two-hybrid screen contains the ADAM 12-S sequence starting at amino acid number 546, that is, downstream of the disintegrin domain and within the cysteine-rich domain. By deletion analysis we determine that IGFBP-3 interacts with the proximal part of the region from residue 546 to 679. This sequence is also present in the extracellular region of the membrane-bound form ADAM 12-L.

When a full-length ADAM 12-S was expressed in the COS-1 or 293-EBNA cells both the 92- and 68-kDa forms were apparent indicating that these cells express furin or a similar protease capable of activating the ADAM 12-S proenzyme. Interestingly, lower amounts of endogenous intact IGFBP-3 and correspondingly more IGFBP-3 degradation products were observed in cells transfected with the ADAM 12-S expression vector than the empty vector.

Interaction of ADAM 12-S with IGFBP-3 was confirmed in the mammalian cell system by reciprocal immunoprecipitation and immunoblotting. It is also likely, although as yet unproven, that IGFBP-3 also interacts with the membrane-bound ADAM 12-L. Thus it is possible that the membrane bound form, ADAM 12-L, may be responsible for one, or another, of the IGFBP-3 membrane binding sites previously identified in various cell lines (19, 20).

The majority of the IGFBP-3 binding capacity in rodent and human serum is attributable to IGFBP-3 (1, 2). This binding protein is present in a ternary complex of ~150 kDa, composed of IGFBP-3, and an 85-kDa acid-labile subunit, and IGFBP-3 (1). It represents a relatively slowly turning over reservoir of IGF (21). Because IGFBP-3 has an affinity for IGF-I and II comparable or greater than their receptors and is present in high concentrations in serum and biological fluids, the mechanism whereby IGF is liberated from this complex to interact with the respective membrane receptors is unclear at present. Proteolytic degradation of IGFBP-3 generates fragments with reduced binding affinity for IGF-I and consequently may be one mechanism whereby delivery of IGF-I to the receptor is facilitated.

Under normal circumstances the IGFBP-3 present in the plasma is largely intact, although some IGFBP-3 protease activity is detectable. In serum from pregnant women and to a lesser extent serum from poorly controlled diabetic patients and patients recovering from surgery or severe illness, a variable proportion of the immunoreactive IGFBP-3 is present as smaller proteolytic fragments. It is thought that this proteolytic degradation of IGFBP-3 enhances IGF-I and -II bioavailability in these situations.

Evidence from size fractionation of serum from late pregnancy suggests that IGFBP-3 protease activity is likely to be because of more than one IGFBP-3 protease (8). IGFBP-3 protease activity largely disappears after parturition suggesting that much of IGFBP-3 protease arises from the placenta, decidual tissue, or trophoblasts (8, 22, 23). In late pregnancy serum, protease activity directed against IGFBP-3 is present in fractions containing proteins of 50–100 kDa (5). Zymography indicates that the major IGFBP-3 protease activity is because of a gelatinase-like protease that is recognized by an antidisintegrin domain antibody (5). This latter observation would suggest that this protease was likely to be a soluble disintegrin metalloprotease, and as such ADAM 12-S, which is highly expressed in the placenta and present in serum from pregnant women, is a possible candidate. ADAM 12-S was not detectable by Western blotting in serum from nonpregnant women.

In addition to demonstrating the interaction of IGFBP-3 and ADAM 12-S, we provide evidence that IGFBP-3 is a substrate for ADAM 12-S. Conditioned medium from cells transfected with ADAM 12-S cDNA when incubated with excess recombinant IGFBP-3 resulted in the generation of IGFBP-3 fragments similar to that seen in late pregnancy serum. This was not the case with conditioned medium from cells transfected with the empty vector. Furthermore, the concentration of intact IGFBP-3 in conditioned medium COS-1 cells, as determined by Western blotting, was less than that in conditioned medium from vector-transfected cells. Although a number of mechanisms may be responsible for this reduction in IGFBP-3 concentration in conditioned medium from ADAM 12-S-expressing cells, it is likely that enhanced proteolysis and degradation of IGFBP-3 is at least partly responsible. Despite the reduced amount of endogenous IGFBP-3 in ADAM 12-S containing conditioned medium, the smaller molecular weight IGFBP-3 fragments were more abundant suggesting that degradation of endogenously expressed IGFBP-3 in COS-1 cells was enhanced by overexpression of ADAM 12-S in these cells.

Using heparin-Sepharose, we were able to partially purify the ADAM 12-S from conditioned medium of COS-1 and 293-EBNA cells and show that this fraction had IGFBP-3 protease activity. In contrast the equivalent heparin-Sepharose fraction from conditioned medium from untransfected cells had no activity. Furthermore the protease activity of the partially ADAM 12-S fraction was inhibited by EDTA and 1,10 phenanthroline. ADAM 12-S has previously been shown to bind to heparin-Sepharose and to be a zinc-dependent metalloprotease (12).

These data provide evidence that ADAM 12-S is able to interact with IGFBP-3, and the interaction results in proteolysis of IGFBP-3. ADAM 12-S may represent only one of the components of circulating IGFBP-3 protease activity present in pregnancy serum, because the evidence suggests that additional proteases are present in this situation (8). It remains to be determined what the relative contribution of the protease activity of ADAM 12-S is to the total IGFBP-3 protease activity present in pregnancy serum. The interaction of IGFBP-3 with ADAM 12-S reported here raises the possibility that IGFBP-3 may also interact with the membrane-bound ADAM 12-L. Further studies are required to determine whether IGFBP-3 interacts with ADAM 12-L.

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