Frank A. Hills*, Ray K. Iles and Mark H. Sullivan

Differential proteolysis of insulin-like growth factor binding protein-1 (IGFBP-1) in pregnancy

Abstract: The insulin-like growth factors and their binding proteins are important for placental and foetal growth. In this study, we have investigated the presence of proteolytic activity directed against insulin-like growth factor binding protein-1 (IGFBP-1) in pregnancy. In addition, the effect of protease activity on IGFBP-1 immunoreactivity and IGF binding was characterised. ^{125}I-IGFBP-1 was incubated with maternal and foetal serum, amniotic fluid and placental extracts. Breakdown of ^{125}I-IGFBP-1 was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and autoradiography. The size distribution of endogenous IGFBP-1 was determined by Western immunoblotting. Protease inhibitor studies characterised the proteolytic activity, and Western ligand blotting with ^{125}I-IGF-I was used to determine IGF binding capacity of proteolysed IGFBP-1. Amniotic fluid samples collected after labour onset contained proteolytic activity that generated 12- and 19-kDa IGFBP-1 fragments that did not bind to ^{125}I-IGF-I. This activity was not detected in amniotic fluid collected prior to labour onset or in other tissues. Activity was blocked by aprotinin, leupeptin, phenyl methyl sulphonyl fluoride, and Kunitz soybean trypsin inhibitor but not by ethylene diamine tetraacetic acid or pepstatin. Incubation of IGFBP-1 with trypsin generated fragments of a similar size to the amniotic fluid protease. In conclusion, we have demonstrated the presence *in vivo* of a trypsin-like proteolytic activity that alters the IGF-binding function of IGFBP-1 in pregnancy.

Keywords: Amniotic fluid; IGF binding protein-1; insulin-like growth factor; proteolysis.

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Introduction

The insulin-like growth factors (IGFs) carry out a range of actions at the cellular level in many tissues including the stimulation of cell growth, cell survival and differentiation. These actions are regulated by a series of specific binding proteins [insulin-like growth factor binding proteins (IGFBPs)], which may inhibit or enhance IGF activity [7]. Changes in the production of IGFs or IGFBP, as well as differential metabolism, may lead to net changes in the effects of the IGF-IGFBP axis on tissue biology.

There is clear evidence that the IGFs and IGFBP family are implicated in the regulation of pregnancy. Abnormal production of IGFs and IGFBP-1 are associated with disorders of pregnancy such as intrauterine growth restriction and pre-eclampsia [1, 11, 13]. Indeed, low levels of IGF-I and raised levels of IGFBP-1 are associated with placental insufficiency [33]. Throughout pregnancy, there is an abundance of IGF mRNA, particularly IGF-II mRNA in cytotrophoblast tissue, with the highest gene expression found at the invading front of trophoblast columns [12]. IGFBP-1 is the major product of the maternal decidua [32]. It is postulated therefore that the IGF system is involved in placental development and that abnormal expression or levels of any component of this system may contribute to disorders of pregnancy.

In addition to changes in production of IGFs or IGFBPs, degradation of IGFBPs can decrease their binding to IGFs and hence increase net IGF levels. Proteolysis of IGFBP-3 during pregnancy has been studied extensively, and proteolytic activity directed against IGFBP-2, -4 and -5 has also been described in pregnancy tissues [4, 6, 10, 14, 15, 22]. These activities result in a decrease in IGF binding capacity, and this functional modification is therefore thought to allow increased bioavailability of IGFs to tissues and may serve to meet the extra metabolic demands of pregnancy [25]. Similar findings for IGFBP-3 have been reported, although most data are from non-pregnancy tissues [2]. Evidence for the presence of proteolytic activity directed against IGFBP-1 *in vivo* is equivocal. IGFBP-1 appears to be relatively resistant to proteolysis [10, 25], and proteases that cleave other IGFBPs have no effect on IGFBP-1 breakdown [8, 17, 24, 27]. One group has reported

the presence of proteolytic activity directed against ^{125}I-IGFBP-1 in amniotic fluid and cultured decidual cells, but the extent of proteolysis *in vivo* was not investigated [9]. The decidual activity against IGFBP-1 includes matrix metalloproteinases-3, -8 and -9 [5, 23]. However, the impact of this metabolism on determination of IGFBP levels is not certain.

The aims of this study were to investigate the metabolism of IGFBP-1 in pregnancy tissues including amniotic fluid, pregnancy serum and placenta; to characterise the enzyme responsible; and to investigate the effect of IGFBP-1 breakdown on the determination of IGFBP-1 levels by immunoassay.

Materials and methods

Tissues

Samples of maternal serum (n=40), umbilical cord serum (n=40), amniotic fluid (n=148) and placenta (n=40) were collected prior to the onset of labour, following uncomplicated labour and labour complicated by foetal distress (abnormal cardiotocogram) in the presence or absence of meconium staining. Samples of placenta (1–2 g wet weight) were suspended in 0.1 M phosphate buffered saline (PBS) and homogenised. The suspension was centrifuged to remove debris and unbroken cells. Supernatants were separated and stored at –20°C until assay. Tissue collection was approved by the local ethics committee, and informed consent was obtained from each woman.

Methods

Preparation of ^{125}I-IGFBP-1

IGFBP-1 from amniotic fluid collected between 16 and 20 weeks' gestation was purified by anion exchange, hydrophobic interaction and gel filtration chromatography, and iodinated using the chloramine T method [23]. Following iodination, radioactive peptides were separated on a Sephadex G-75 size exclusion column eluted with 50 mM PBS (GE Healthcare, Uppsala, Sweden) containing 1% (w/v) bovine serum albumin.

Proteolysis of ^{125}I-IGFBP-1

Proteolysis of ^{125}I-IGFBP-1 was investigated using a method similar to that used by Lamson et al. [20] to investigate ^{125}I-IGFBP-3 proteolysis. Intact ^{125}I-IGFBP-1 (2 μL) was added to samples of amniotic fluid, serum or placental extracts (2 μL). Mixtures were incubated at 37°C. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer (2 μL) was added to each sample, and samples were then applied to a discontinuous SDS-PAGE gel and ^{125}I visualised by autoradiography.

Preliminary studies were carried out to establish the presence of ^{125}I-IGFBP-1 breakdown in the different tissues tested. For these experiments, the labelled protein was incubated with pregnancy fluids described above (n=8 for each fluid and n=2 for each delivery type) for 16 h at 37°C. In order to detect intermediate breakdown products and determine the incubation time required for ^{125}I-IGFBP-1 proteolysis, samples in which proteolytic activity was apparent after 16 h of incubation were incubated for 0, 5, 10, 20, 30 and 60 min with ^{125}I-IGFBP-1 as described above prior to SDS-PAGE and autoradiography. A larger group of samples (n=8 for each delivery type per tissue) was tested to confirm our findings.

Detection of immunoreactive IGFBP-1 fragments

The size of immunoreactive IGFBP-1 and IGFBP-1 fragments was assessed by SDS-PAGE and Western immunoblotting. Samples of amniotic fluid, serum or placental extracts (2 μL) were mixed with incubation buffer (6 μL) and SDS-PAGE sample buffer (2 μL) and run on SDS-PAGE gel as described above. Proteins were transferred onto polyvinylidene difluoride (PVDF) membranes. Transfer was achieved by the application of 400 mA for 1 h at room temperature. After drying, the PVDF membranes were incubated with Tris-buffered saline (TBS) containing 1% bovine serum albumin for 1 h to block any subsequent non-specific binding. These membranes were then incubated with polyclonal sheep anti-IGFBP-1 antibody at a dilution of 1 in 10,000 for 16 h at room temperature. After three 15-min washings, the membranes were incubated for a further 1 h with horseradish peroxidase-labelled rabbit anti-sheep IgG (1 in 1000 dilution). The immunoreactive proteins were visualised by the addition of TBS containing 0.03% hydrogen peroxide and 0.05% diaminobenzidene. Colour development was stopped by washing with distilled water.

Protease inhibitor studies

A series of protease inhibitors were used to identify the nature of the IGFBP-1 proteolytic activity present in amniotic fluid. Ethylene diamine tetraacetic acid (EDTA, 100 mM), phenyl methyl sulphonyl fluoride (PMSF, 4 mM), aprotinin (10 mg/mL), leupeptin (5 mg/mL), pepstatin (3 mg/mL) and Kunitz soybean trypsin inhibitor (STI, 1 mg/mL) were used in these studies. Concentrations of this order are reported to be effective in the inhibition of breakdown of other IGFBPs [4, 6]. ^{125}I-Labelled IGFBP-1 (2 μL, 30,000 cpm) was incubated with amniotic fluid (2 μL), TBS (2 μL) and protease inhibitor at 37°C for 1 h. At the end of this incubation period, SDS-PAGE sample buffer (2 μL) was added. Samples were applied to a discontinuous SDS-PAGE gel under the conditions described above. Gels were then dried and radioactivity visualised by exposure to X-ray film.

Digestion of IGFBP-1 by trypsin

IGFBP-1 purified from amniotic fluid (50 ng) was incubated for 1 h at 37°C with increasing concentrations of bovine trypsin (Sigma-Aldrich, Gillingham, UK) in the range 0.2–6.4 U/mL in a total volume of 7.5 μL. This preparation contained <0.008 U of chymotrypsin activity per milligram of protein. Samples were then subjected to

SDS-PAGE, Western immunoblotting and visualisation using diaminobenzidene as described above.

Effect of proteolysis on IGF binding

Samples of amniotic fluid that were found to contain immunoreactive IGFBP-1 fragments were run on SDS-PAGE and transferred onto PVDF membranes as described above. Following transfer, membranes were incubated with ^{125}I-IGF-I (30,000 cpm/mL) for 16 h at room temperature. Radioactive IGF-I was visualised by autoradiography.

Effect of proteolysis on IGFBP-1 immunoassay

The effect of protease activity on the IGFBP-1 radioimmunoassay (RIA) was investigated by studying parallelism following sample dilution. Samples of amniotic fluid containing intact (n=4) or proteolysed IGFBP-1 (n=4), as identified from Western immunoblotting, were initially diluted 1:10 in horse serum and then double diluted in horse serum to a final dilution of 1:640. Diluted samples were then assayed for IGFBP-1 using the radioimmunoassay described previously [34].

Results

Western analysis of amniotic fluid, serum and placental extracts

Amniotic fluid from elective section deliveries contained IGFBP-1 detectable by Western blotting (Figure 1A). Amniotic fluid obtained from pregnancies in labour or with foetal distress showed intact IGFBP-1, as well as smaller immunoreactive products of approximately 19 and 12 kDa. Meconium-stained amniotic fluid contained little or no immunoreactive IGFBP-1 (Figure 1A). Maternal and foetal serum (Figure 1B and C, respectively) and placental extracts (not shown) contained immunoreactive IGFBP-1,

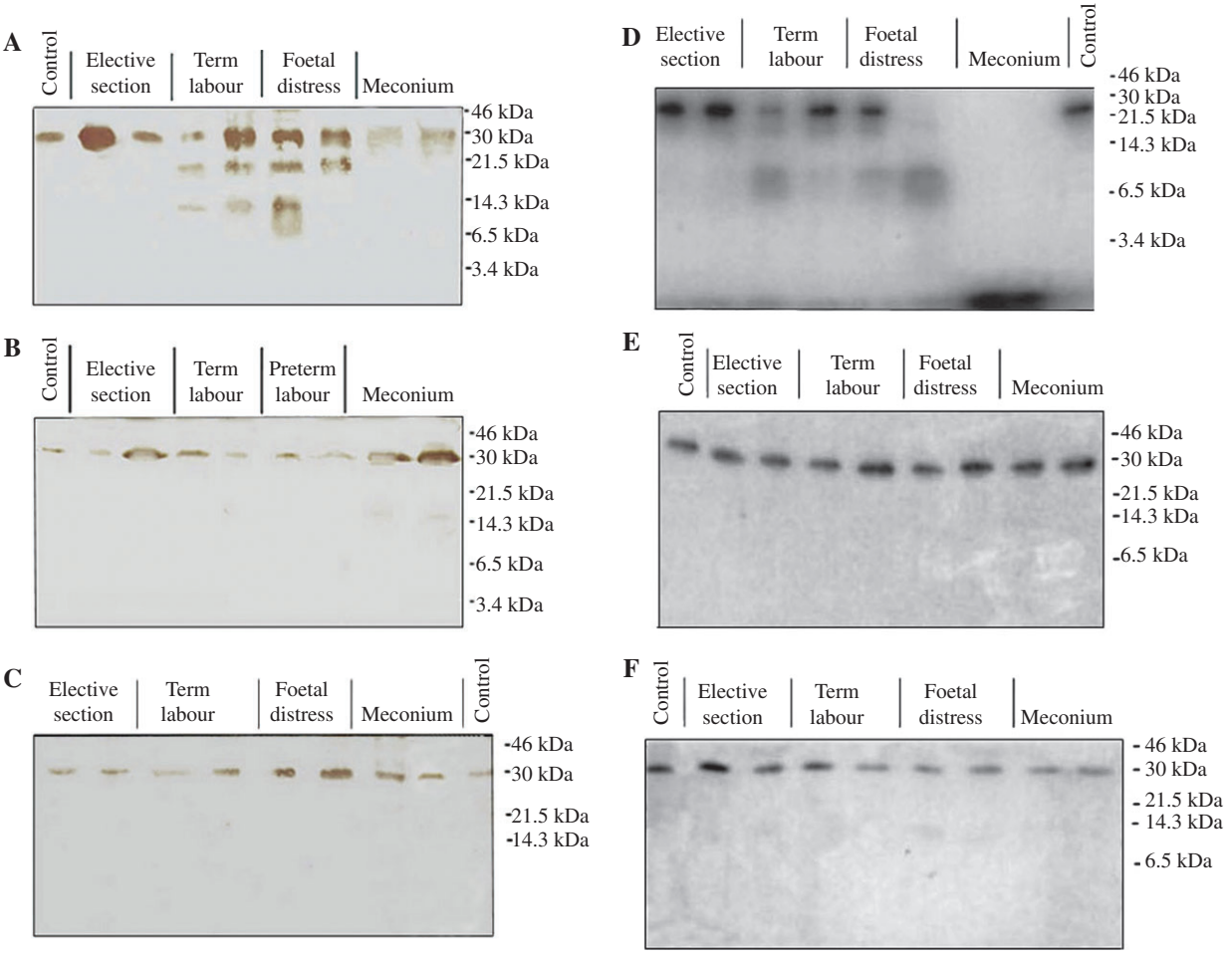

Figure 1 Western immunoblots showing the size distribution of immunoreactive IGFBP-1 present in pregnancy tissues (A–C), and autoradiographs showing the size distribution of ^{125}I-IGFBP-1 after overnight incubation at 37°C with the same samples (D–F). Tissues used were amniotic fluid (A, D), maternal sera (B, E) and umbilical cord sera (C, F) collected at delivery with clinical conditions indicated. Control lane contained unlabelled purified IGFBP-1 in PBS (A–C) or ^{125}I-IGFBP-1 (D–F).

but no smaller peptides, and the integrity of the IGFBP-1 was not affected by the type of delivery.

Proteolysis of ^{125}I-IGFBP-1 by amniotic fluid, serum and tissue extracts

The same samples used in Figure 1A–C were incubated with ^{125}I-IGFBP-1 and analysed by PAGE and autoradiography. No metabolism of ^{125}I-IGFBP-1 was detected in amniotic fluid from elective caesarean sections (Figure 1D). However, a single smaller band of ~12 kDa was detected in the presence of amniotic fluid from pregnancies in labour or with foetal distress, but there was no band seen at 19 kDa. All the radioactivity in the meconium-stained amniotic fluid was at the dye front (Figure 1D), suggesting complete breakdown of the ^{125}I-IGFBP-1. Maternal and foetal serum (Figure 1E and F, respectively) did not metabolise ^{125}I-IGFBP-1. A further eight samples of each of the four types of amniotic fluid (prior to labour, term labour, foetal distress and meconium-stained) were analysed for ^{125}I-IGFBP-1 breakdown and for the presence of immunoreactive IGFBP-1 fragments. These produced similar results to those described above and indicated that the metabolism of IGFBP-1 varied between samples of amniotic fluid.

We therefore analysed the metabolism of ^{125}I-IGFBP-1 by 146 further samples from different pregnancies. No radioactive fragments were observed with amniotic fluid taken before labour (caesarean section) (n=22), whereas metabolism of ^{125}I-IGFBP-1 was found in 11 (22%) of 50 samples taken during labour, 17 (42%) of 41 samples from pregnancies with foetal distress and 27 (77%) of 35 meconium-stained samples. These differences were statistically significant ($P<0.001$ by χ^2 analysis).

Effect of incubation time on ^{125}I-IGFBP-1 proteolysis

The proteolysis of ^{125}I-IGFBP-1 in Figure 1D–F indicated partial metabolism with amniotic fluids from labour or foetal distress pregnancies, but almost complete breakdown with meconium-stained fluids. It was therefore unclear whether the nature of the protease responsible was the same in these samples, so a more detailed time course was investigated. Figure 2 shows the degradation of ^{125}I-IGFBP-1 following incubation between 5 min and 1 h with amniotic fluid taken from deliveries following labour associated with foetal distress in the presence or absence of meconium staining. Following incubation with unstained fluid, a faint band at 12 kDa was present after 5 min of incubation (Figure 2A). This became more intense as the incubation time increased. There was a reduction of intensity of the intact 30-kDa band such that it was barely visible after 60 min of incubation (Figure 2A). Breakdown of ^{125}I-IGFBP-1 was much more rapid in the presence of meconium-stained fluid in that, after 5 min of incubation at 37°C, the intact ^{125}I-IGFBP-1 was no longer visible and a ^{125}I-labelled fragment at approximately 12 kDa could be clearly seen (Figure 2B).

Characterisation of proteolytic activity against IGFBP-1

The nature of the protease was characterised by the incubation with protease inhibitors. The metabolism of ^{125}I-IGFBP-1 by meconium-stained or clear amniotic fluid was unaffected by EDTA or pepstatin, whereas PMSF, aprotinin, leupeptin and STI limited the metabolism of ^{125}I-IGFBP-1 (Figure 3).

Trypsin digestion of IGFBP-1

Incubation of IGFBP-1 purified from amniotic fluid with trypsin resulted in a strikingly similar pattern of fragmentation of immunoreactive IGFBP-1 as that described above. At trypsin concentrations of 0.8–6.4 U/mL, IGFBP-1 proteolysis was evident by the dose-dependent appearance of two immunoreactive fragments of approximately 19 and 12 kDa. A comparison of the effects of trypsin and amniotic fluid on IGFBP-1 is shown in Figure 4. The pattern of immunoreactive products is very similar, such that the main bands are 30 kDa (intact protein), 19 kDa and 12 kDa in both cases (Figure 5).

Effect of proteolysis on IGF binding

IGFBP-1 fragments from amniotic fluid samples in which we had detected proteolytic activity and immunoreactive IGFBP-1 fragments (see Figure 1A and F) were assessed for ^{125}I-IGF-I binding by Western ligand blotting. ^{125}I-IGF-I bound to intact IGFBP-1 in these samples, but we were not able to detect any binding to lower-molecular-weight IGFBP-1 fragments (Figure 6).

Effect of proteolysis on IGFBP-1 RIA

The effect of proteolysis on the parallelism of dilution in the RIA is shown in Figure 7. When the results were adjusted for sample dilution, the IGFBP-1 level in non-proteolysed

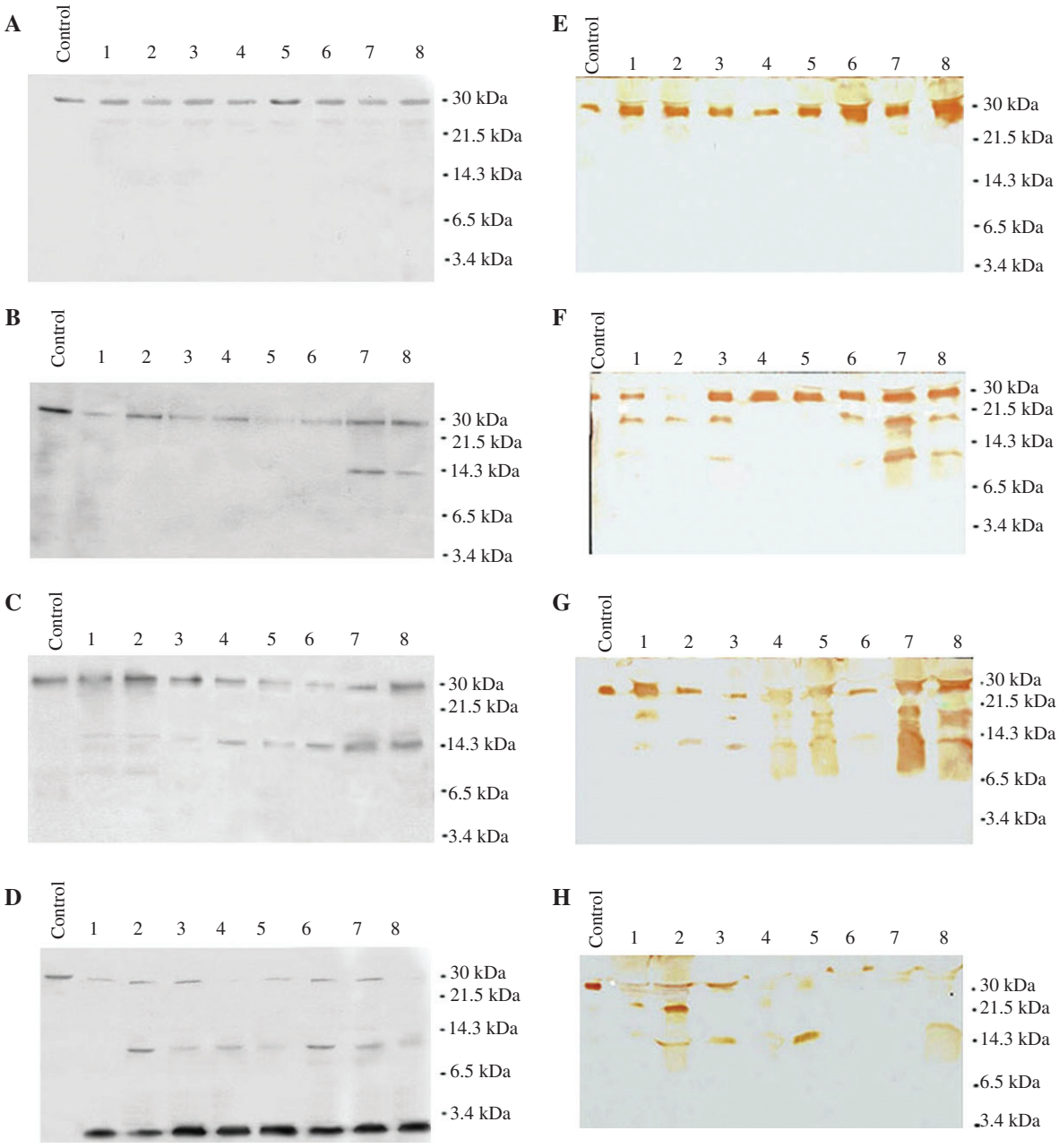

Figure 2 Breakdown of ^{125}I-IGFBP-1 (A–D) and endogenous IGFBP-1 (E–H) by amniotic fluid from deliveries prior to labour onset (A, E), following uncomplicated labour (B, F) and labour complicated by foetal distress in the absence (C, G) or presence (D, H) of meconium staining. In each blot, the numbers 1–8 represent eight different samples.

amniotic fluid samples remained constant as the samples were diluted. However, proteolysed samples did not dilute in parallel and the apparent concentration increased with increasing dilution.

Discussion

In this study, we have demonstrated that term amniotic fluid contains proteolytic activity directed against IGFBP-1. This proteolytic activity was not confined to amniotic fluid samples with obvious meconium staining. Although IGFBP-1 breakdown was not observed in any samples taken prior to labour onset, it was observed in most samples taken after normal labour and labour complicated by foetal distress. The rate of ^{125}I-IGFBP-1 breakdown was greater in meconium-stained than in clear amniotic fluid, but the fact that the breakdown fragments were the same size and were inhibited in the same way suggests that the same proteolytic mechanism may be responsible.

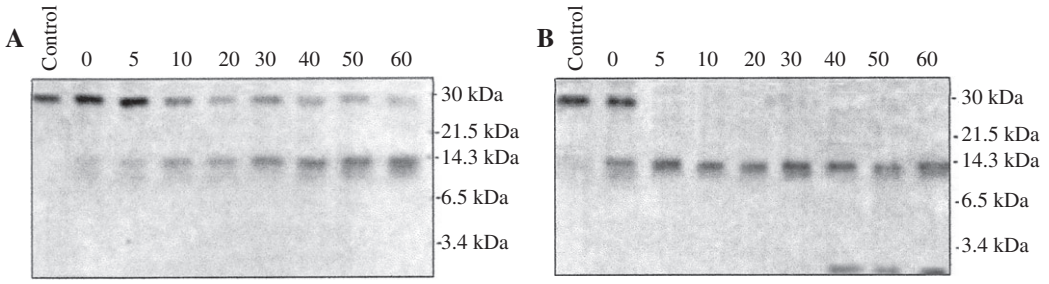

Figure 3 Autoradiograph showing the size distribution of ^{125}I-IGFBP-1 after incubation at 37°C with clear (A) and meconium-stained (B) amniotic fluid for the times indicated (minutes). Control sample ^{125}I-IGFBP-1 only with no incubation.

The immunoreactive IGFBP-1 fragment sizes of 12 and 19 kDa suggest that the protein is initially cut at a single site. However, the complete loss of immunoreactivity in some samples implies further digestion. This is consistent with our radiolabelled IGFBP-1 studies where we observed the appearance of a ^{125}I-labelled fragment of 12 kDa after 5 min of incubation with amniotic fluid. After 16 h of incubation, all radioactivity was present in very low molecular weight fragments.

Only the intact ^{125}I-IGFBP-1 and the 12-kDa product were seen in our labelled IGFBP-1 studies, suggesting that only this fragment was labelled with ^{125}I. Examination of the amino acid sequence of IGFBP-1 shows that all of the tyrosines available for labelling by ^{125}I are located towards the C-terminal end of the protein (Figure 7). Therefore, it seems likely that the smaller 12-kDa fragment seen in our labelled IGFBP-1 studies is the C-terminal fragment.

Proteolysis of labelled IGFBP-1 was inhibited by PMSF, aprotinin and leupeptin but not by pepstatin. This indicates that, like the IGFBP-3 protease in pregnancy [6], the enzyme activity responsible for the breakdown of IGFBP-1 in amniotic fluid is a serine protease. However, unlike the IGFBP-3 protease, EDTA had no inhibitory effect on IGFBP-1 protease activity, indicating that the enzyme activity is not cation dependent.

Trypsin and chymotrypsin are likely candidates for the IGFBP-1 proteolysis seen in amniotic fluid because not only are they cation independent, but also are present in significant amounts in meconium [30]. Amniotic fluid trypsin levels are particularly raised in pathologies associated with foetal compromise [21, 29]. In addition, the finding of markedly reduced amniotic fluid trypsin inhibitor levels after the onset of labour [19] is consistent with the increased trypsin-mediated IGFBP-1 proteolysis seen in these samples. Furthermore, protease activity was completely inhibited by the Kunitz STI, an inhibitor of trypsin and, to a lesser extent, chymotrypsin. However, the most compelling evidence that the IGFBP-1 proteolysis described results from trypsin activity is the observation that proteolysis of IGFBP-1 by a purified trypsin

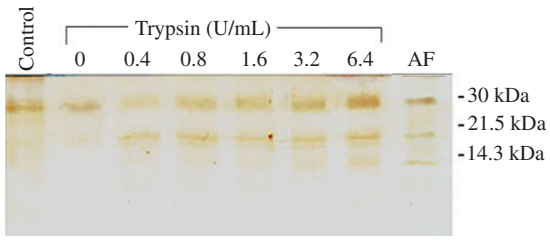

Figure 4 Western immunoblot showing the comparison of IGFBP-1 breakdown following 1-h of incubation at 37°C with amniotic fluid and with trypsin at the concentrations indicated (U/mL).

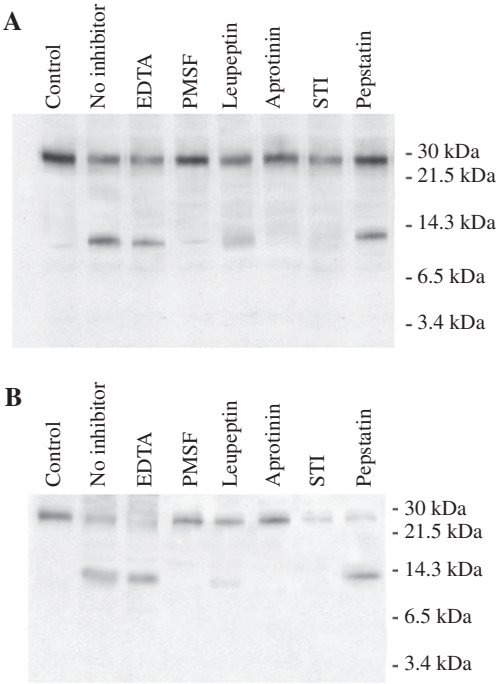

Figure 5 Effect of protease inhibitors on ^{125}I-IGFBP-1 proteolysis by clear (A) and meconium-stained (B) amniotic fluid. Final concentrations of inhibitors were as follows: EDTA 33 mM, PMSF 3.3 mM, leupeptin 1.7 mM, aprotinin 3.3 mg/mL, STI 3.3 mg/mL and pepstatin 1 mg/mL.

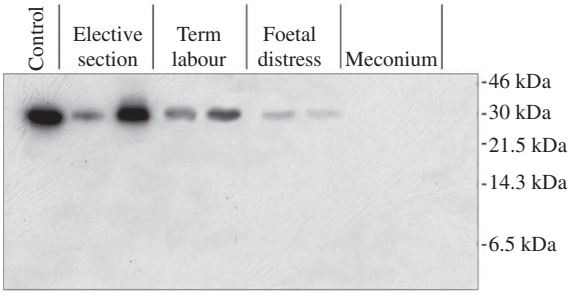

Figure 6 Western ligand blot showing binding of ^{125}I-IGF-I to IGFBP-1 species present in amniotic fluid collected from deliveries as indicated.

preparation results in immunoreactive fragments of the same size as those present in amniotic fluid.

The IGFBPs are composed of highly conserved cysteine-rich C- and N-terminal regions with respective sequence homology of 58% and 34% [16]. Structural studies have shown that, despite differences in the pairing of these cysteines, all form disulphide bonds within each of these regions and there are no disulphide linkages between the two domains [26]. This structural arrangement suggests that proteolysis of IGFBP-1 within either of these regions is unlikely to result in the fragmentation pattern observed here.

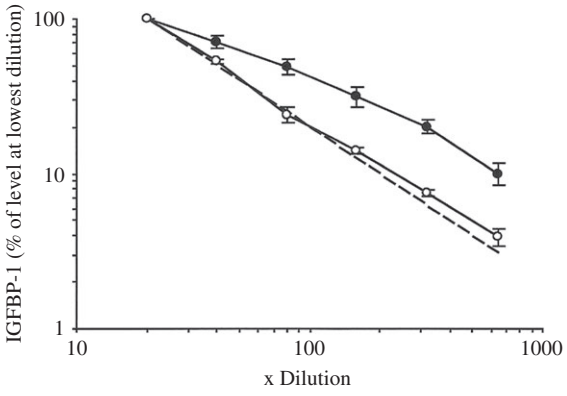

Figure 7 Effect of IGFBP-1 proteolytic activity on apparent levels of IGFBP-1 determined by RIA after serial dilution. IGFBP-1 levels were determined in samples of amniotic fluid with (●) or without (○) evidence of IGFBP-1 proteolytic activity (n=4 in each group). Because of the large range of absolute levels of IGFBP-1 in these samples, IGFBP-1 is expressed as a percentage of the value obtained from the lowest dilution. Dotted line denotes expected levels after serial dilution.

The C- and N-terminal regions are separated by a more variable midregion [18, 26] with relatively few cysteines (Figure 8).

This seems to be the likely region for IGFBP-1 cleavage in the present study. Cleavage here is consistent with the fragmentation pattern observed. In addition, previous studies where sequencing information is available have shown that IGFBP cleavage sites are all within this variable region reviewed by Firth and Baxter [7].

Trypsin is a highly specific protease that preferentially cleaves proteins on the C-terminal side of the basic amino acids lysine (k) and arginine (r). There is a lysine-rich region within the midregion of the molecule at a point approximately 40% from the C-terminal (amino acids 137, 145, 146 and 148), and cleavage here is consistent with the fragmentation observed (Figure 9). In addition, a previous study has demonstrated a cleavage site within this region following trypsin digestion *in vitro* [26].

The IGFBP-1 proteolysis described here is functionally important because it appears to dramatically reduce IGF binding capacity. This is consistent with the functional effect of IGFBP-3 proteolysis [22]. Protease activity in amniotic fluid appears to break down both labelled and unlabelled IGFBP-1. Under conditions of partial breakdown, the 12- and 19-kDa fragments retain some immunoreactivity. However, the proteolytic activity clearly has an effect on apparent IGFBP-1 levels determined by immunoassay. Thus, although the RIA antibody can recognise IGFBP-1 proteolytic fragments, proteolytic activity has a considerable effect on levels obtained with this assay and this makes such measurements unreliable.

Recently, a study by Lee et al. indicated the presence of IGFBP-1 proteolytic activity in pregnancy by matrix metalloproteases (MMPs) [23]. This activity produced IGFBP-1 fragments of a similar size to those seen in the present study. However, this is unlikely to be the source of the activity we observed in amniotic fluid as this activity was not inhibited by EDTA, a known MMP inhibitor.

Finally, the clinical consequences of this activity must be considered. The reduction of IGF binding affinity associated with the proteolysis of other IGFBPs is believed to be an important means of regulating the tissue availability of IGFs. In the context of pregnancy, circulating IGFBP-3 protease activity is thought to be a means by which the

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1   apwqcapcsa eklalcppvs  ascsevtrsa  gcgccpmcal plgaacgvat arcarglscr
61  alpgeqqplh altrgqgacv  qesdasapha  aeagspespe steiteeell  dnfhlmapse
121 edhsilwdai stydgskalh  vtnikkwkep  crielyrvve slakaqetsg eeiskfylpn
181 cnkngfyhsr qcetsmdgea glcwcvypwn gkripgspei  rgdpncqiyf nvqn
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Figure 8 IGFBP-1 amino acid sequence highlighting cysteines involved in disulphide bridge formation (c), tyrosines which may be iodinated (y) and amino acids preferentially cleaved by trypsin (r and k).

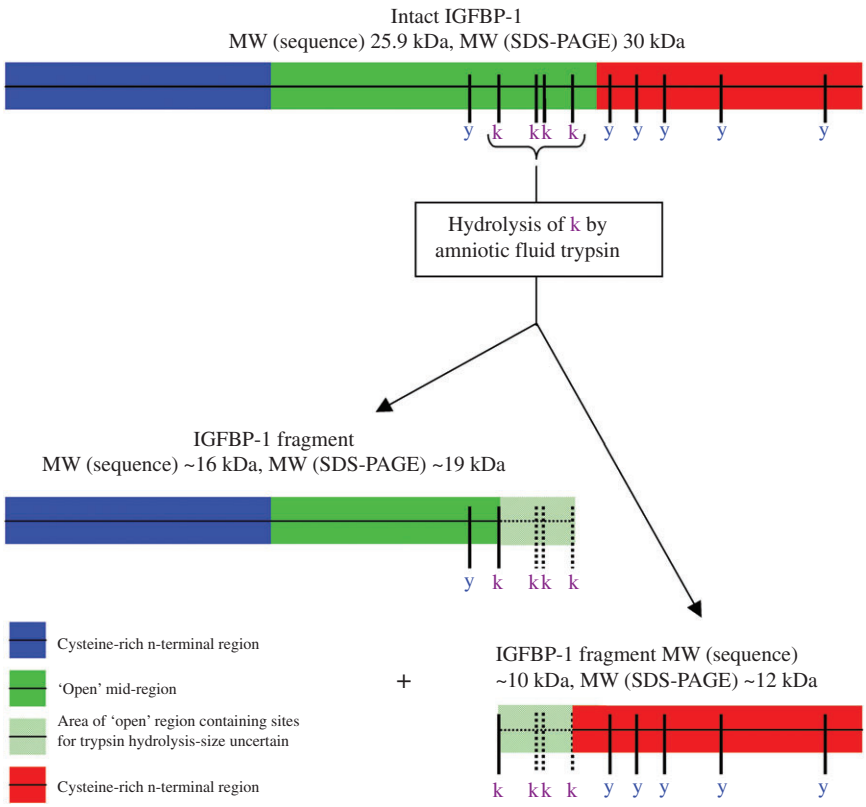

Figure 9 Proposed mechanism of IGFBP-1 proteolysis by trypsin derived from amniotic fluid. Cysteine-rich terminal regions, cleavage sites (k) for trypsin within the "open" region and the distribution of tyrosines (y) within the protein are indicated.

increased IGF availability can alter maternal metabolism to meet the extra demands of pregnancy [6, 25]. Although proteolysis was detected only in amniotic fluid collected at the very end of pregnancy, it is possible that such activity exists in early pregnancy. Such activity may be significant and a useful topic to investigate in future studies. Although the function of IGFBP-1 in amniotic fluid is uncertain, the presence of high concentrations here suggests that it may be a potent inhibitor of IGF action [28]. Modification by proteolysis may significantly affect this activity. In addition, elevated second trimester amniotic fluid IGFBP-1 levels have been reported in subjects with intrauterine growth restriction (IUGR) at term [3]. Depending on the methodology used, this apparent increase may be in part due to the effect of proteolytic activity acting on IGFBP-1.

Immunometric methods to detect IGFBP-1 in vaginal secretions have been developed as a test for premature rupture of membranes [31]. Clearly, this method may prove unreliable where IGFBP-1 proteolytic activity is present. Likewise, measurement of amniotic fluid IGFBP-1 levels should take account of trypsin-like proteolytic activity present in these samples.

Altered concentrations of IGFBP-1 in disorders of pregnancy such as IUGR and pre-eclampsia [1, 13, 33] suggest that IGFBP-1 measurement may be a useful biochemical marker. The finding of IGFBP-1 proteolysis in association with the pathology of foetal distress and physiological stress of labour suggests that the presence of IGFBP-1 fragments may be of use as a marker of foetal well-being.

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References

[1] Anime-Nyame N, Hills FA, Sooranna SR, Steer PJ, Johnson MR. A longitudinal study of maternal plasma insulin-like growth factor binding protein-1 concentrations during normal pregnancy and pregnancies complicated by pre-eclampsia. Hum Reprod. 2000;15: 2215–9.

[2] Bunn RC, Fowlkes JL. Insulin-like growth factor binding protein proteolysis. Trends Endocrinol Metab. 2003;14:176–81.
[3] Chevalier B, Lagarde A, Degrelle H, Belaisch-Allart J, Giraudet P, Gallet JP. Insulin-like growth factor binding protein-1 level in amniotic fluid: correlation with birth weight. Biol Neonate. 1998;73:404–6.
[4] Claussen M, Zapf J, Braulke T. Proteolysis of insulin-like growth factor binding protein-5 by pregnancy serum and amniotic fluid. Endocrinology. 1994;134:1964–6.
[5] Coppock HA, White A, Aplin JD, Westwood M. Matrix metallo-protease-3 and -9 proteolyze insulin-like growth factor-binding protein-1. Biol Reprod. 2004;71:438–43.
[6] Davies SC, Holly JMP, Coulson VJ, Cotterill AM, Abdulla AF, Whittacker PG, et al. The presence of cation-dependent proteases for insulin-like growth factor binding proteins (IGFBPs) does not alter the size distribution of insulin-like growth factors in pregnancy. Clin Endocrinol. 1991;34:501–6.
[7] Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev. 2002;23:824–54.
[8] Frost VJ, Macaulay VM, Wass JA, Holly JM. Proteolytic modification of insulin-like growth factor-binding proteins: comparison of conditioned media from human cell lines, circulating proteases and characterized enzymes. J Endocrinol. 1993;138:545–54.
[9] Gibson JM, Aplin JD, White A, Westwood M. Regulation of IGF bioavailability in pregnancy. Mol Hum Reprod. 2001;7:79–87.
[10] Giudice LC, Farrell EM, Pham H, Lamson G, Rosenfeld RG. Insulin-like growth factor binding proteins in maternal serum throughout gestation and in the peurperium: effects of a pregnancy-associated serum protease activity. J Clin Endocrinol Metab. 1990;71:806–16.
[11] Giudice LC, Martina NA, Crystal RA, Tazuke S, Druzin M. Insulin-like growth factor binding protein-1 at the maternal-fetal interface and insulin-like growth factor-I, insulin-like growth factor-II, and insulin-like growth factor binding protein-1 in the circulation of women with severe preeclampsia. Am J Obstet Gynecol. 1997;176:751–7.
[12] Han VKM, Bassett N, Walton J, Challis JR. The expression of insulin-like growth factor (IGF) and IGF binding protein (IGFBP) genes in the human placenta and membranes: evidence for IGF-IGFBP interactions at the feto-maternal interface. J Clin Endocrinol Metab. 1996;81:2680–93.
[13] Hills FA, English J, Chard T. Circulating levels of IGF-I and IGF binding protein-1 throughout pregnancy: relation to birthweight and maternal weight. J Endocrinol. 1996;148:303–9.
[14] Holmes RP, Holly JMP, Soothill PW. Maternal serum insulin-like growth factor binding protein-2 and -3 and fetal growth. Hum Reprod. 1999;14:1479–84.
[15] Hossenlopp P, Segovia B, Lassarre C, Roghani M, Bredon M, Bionoux M. Evidence of enzymatic degradation of insulin-like growth factor binding proteins in the “150 K” complex during pregnancy. J Clin Endocrinol Metab. 1990;71:797–805.
[16] Hwa V, Oh Y, Rosenfeld RG. The insulin-like growth factor binding protein (IGFBP) superfamily. Endocr Rev. 1999;20:761–87.
[17] Irwin JC, Suen LF, Cheng BH, Martin R, Cannon P, Deal CL, et al. Human placental trophoblasts secrete a disintegrin metalloproteinase very similar to the insulin-like growth factor binding protein-3 protease in human pregnancy serum. Endocrinology. 2000;141:666–74.
[18] Kalus W, Zweckstetter M, Renner C, Sanchez Y, Georgescu J, Grol M, et al. Structure of the IGF-binding domain of the insulin-like growth factor binding protein-5 (IGFBP-5: implications for IGF and IGF-I receptor interactions). EMBO J. 1998;17:6558–72.
[19] Kobayashi H, Suzuki K, Sugino D, Terao T. Urinary trypsin inhibitor levels in amniotic fluid of normal human pregnancy: decreased levels observed at parturition. Am J Obstet Gynecol. 1999;180:141–7.
[20] Lamson G, Giudice LC, Rosenfeld RG. A simple assay for proteolysis of IGFBP-3. J Clin Endocrinol Metab. 1991;72:1391–3.
[21] Laroche D, Herlicoviez M, Pasquet C, Travert G, Fernandez Y. Amniotic fluid immunoreactive trypsin in pregnancies with normal and pathological outcomes. Acta Obstet Gynecol Scand. 1987;66:587–90.
[22] Lassare C, Binoux M. Insulin-like growth factor binding protein-3 is functionally altered during pregnancy. Endocrinology. 1994;134:1254–62.
[23] Lee SE, Han BD, Park IS, Romero R, Yoon BH. Evidence supporting proteolytic cleavage of insulin-like growth factor binding protein-1 (IGFBP-1) protein in amniotic fluid. J Perinat Med. 2008;36:316–23.
[24] Loechel F, Fox JW, Murphy G, Albrechtsen R, Wewer UM. ADAM 12-S cleaves IGFBP-3 and IGFBP-5 and is inhibited by TIMP-3. Biochem Biophys Res Commun. 2000;278:511–5.
[25] Maile LA, Holly JMP. Insulin-like growth factor binding protein (IGFBP) proteolysis: occurrence, identification, role and regulation. Growth Horm IGF Res. 1999;9:85–95.
[26] Neumann GM, Bach LA. The N-terminal disulphide linkages of human insulin-like growth factor-binding protein-6 (hIGFBP-6) and hIGFBP-1 are different as determined by mass spectrometry. J Biol Chem. 1999;274:14587–94.
[27] Nunn SE, Peehl DM, Cohen P. Acid-activated insulin-like growth factor binding protein protease activity of cathepsin D in normal and malignant prostatic epithelial cells and seminal plasma. J Cell Physiol. 1997;171:196–204.
[28] Okajima T, Iwashita M, Takeda Y, Sakamoto S, Tanabe T, Yasuda T, et al. Inhibitory effects of insulin-like growth factor (IGF)-binding proteins-1 and -3 on IGF-activated glucose consumption in mouse BALB/c 3T3 fibroblasts. J Endocrinol. 2003;136:457–70.
[29] Pocknee RC, Abramovich DR. Origin and levels of trypsin in amniotic fluid throughout pregnancy. Br J Obstet Gynaecol. 1982;89:142–4.
[30] Robinson PG, Smith PA, Elliott RB. A simple method for the quantitative determination of stool trypsin and chymotrypsin. Clin Chim Acta. 1975;62:225–9.
[31] Rutanen EM, Karkkainen TH, Lehtovirta J, Uotila JT, Hinkula MK, Hartikainen AL. Evaluation of a rapid strip test for insulin-like growth factor binding protein-1 in the diagnosis of ruptured fetal membranes. Clin Chim Acta. 1996;253:91–101.
[32] Rutanen E-M, Koistinen R, Wahlstrom T, Bohn H, Ranta T, Seppala M. Synthesis of placental protein 12 by human decidua. Endocrinology. 1985;116:1304–9.
[33] Sifakis S, Akolekar R, Kappou D, Mantas N, Nicolaides KH. Maternal serum insulin-like growth factor-binding protein-1 (IGFBP-1) at 11-13 weeks in pre-eclampsia. Prenat Diagn. 2011;31:196–201.
[34] Wang HS, Perry LA, Kanisius J, Iles RK, Holly JMP, Chard T. Purification and assay of insulin-like growth factor binding protein-1: measurement of circulating levels throughout pregnancy. J Endocrinol. 1991;128:161–8.

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