Identification of Key Functional Amino Acids of the Mouse Fertilin β (ADAM2) Disintegrin Loop for Cell-Cell Adhesion during Fertilization*

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Fertilin β (also known as ADAM2) is a cell adhesion molecule on the surface of mammalian sperm that participates in sperm-egg membrane binding. Fertilin β is a member of the molecular family known as ADAMs or MDCs. These proteins have a disintegrin domain with homology to integrin ligands found in snake venoms; several of these snake proteins have an RGD tripeptide presented on an extended “disintegrin loop.” However, fertilin β lacks an RGD tripeptide and instead has the consensus sequence X(D/E)ECD (QDECD in mouse fertilin β) in its putative disintegrin loop, and there is controversy over which amino acids comprise the active site of the fertilin β disintegrin loop. We have used point-mutated versions of the sequence AQDECDVT and two bioassays to identify the key functional amino acids of this sequence from the mouse fertilin β disintegrin domain. Amino acid substitutions for the terminal aspartic acid residue of the QDECD sequence result in dramatically reduced activities in the two assays for protein function, implicating the terminal aspartic acid residue as critical for protein function. Substitutions for the glutamic acid and the cysteine residues in the QDECD sequence result in slight reductions in activity, whereas substitution of the first aspartic acid has virtually no effect. These data suggest that the conserved ECD sequence of the mouse fertilin β disintegrin domain, especially the terminal D residue, contributes more to the protein’s activity than does the QDE sequence that aligns with the RGD tripeptide in other disintegrins.

The interactions of gamete plasma membranes are mediated by multiple cell adhesion molecules on the surfaces of the sperm and egg. One of these cell adhesion molecules is the mammalian sperm protein fertilin β (previously known as PH-30; also known as ADAM2). The involvement of fertilin β in sperm-egg adhesion during fertilization is well established based on studies with synthetic peptides, antibodies, recombinant proteins, and the subfertile phenotype of male fertilin β knock-out mice (summarized in Ref. 1). Of particular interest is the domain of fertilin β that has homology to a family of integrin ligands originally identified in snake venoms. This domain of fertilin β, known as the disintegrin domain, has been implicated in the interactions of fertilin β with the egg membrane in a variety of species (2–9).

A large number of disintegrin domain-containing proteins have been identified. These include members of the molecular family known as ADAMs (for A disintegrin and A metalloprotease) or MDCs (for metalloprotease/disintegrin/cysteine-rich) (10, 11), of which fertilin β is a member. Disintegrin domains are also present in a number of snake venom proteins, which are called simply disintegrins, reprolysins, or snake venom metalloproteases. These snake venom polypeptides range in size from small peptide chains of <50 amino acids to multidomain proteins of several hundred amino acids that are similar in domain structure to ADAM proteins, having a metalloprotease domain and a cysteine-rich domain as well as a disintegrin domain (12–15). The best characterized of the disintegrin-containing proteins are the small disintegrins (48–84 amino acids long (12)), which are called “true” disintegrins (14, 16) because they mimic traditional integrin ligands such as fibrinogen and vitronectin through the presentation of the adhesion-mediating tripeptide sequence, RGD, at the end of an extended loop, called the disintegrin loop (DL)4 (17, 18). In contrast, the larger, multidomain snake venom metalloproteases and the ADAM proteins have disintegrin domains that have been termed “disintegrin-like,” since they lack the RGD tripeptide and contain at least one additional cysteine residue (14, 16).

The interactions of snake venom metalloproteases and ADAMs with their target cell surfaces appear to be more complicated than are those of the small disintegrins. In some cases, the peptide sequence aligning near the predicted disintegrin loop appears to mediate molecular interactions (6, 16, 19, 20). However, in other instances, domains other than the disintegrin loop appear to be involved (7, 21–23).

In the case of fertilin β, the disintegrin domain appears to be very important for this protein’s function as a cell adhesion molecule (6, 7), but there is debate over which amino acids of the fertilin β disintegrin loop are critical for function. There are primarily two sides to this debate. Like other snake venom metalloproteases and ADAM proteins with disintegrin-like domains, fertilin β does not have an RGD tripeptide in its putative disintegrin loop. In the position of the RGD tripeptide, the

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1 The abbreviations used are: DL, disintegrin loop; βDL, fertilin β DL; βDCE, fertilin β disintegrin/cysteine-rich/epidermal growth factor-like repeat; MBP, maltose-binding protein; BAP, bacterial alkaline phosphatase; ZP, zona pellucida; βD, fertilin β disintegrin domain.

2 For simplicity and for consistency with previously published reports on fertilin, we will generally refer to this domain of fertilin β as the disintegrin domain, although it should be emphasized that fertilin β and other members of the ADAM family have disintegrin-like domains.

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consensus sequence in fertilin β (based on cDNA clones from seven species) is X(D/E)E, where X is any amino acid (Table I). This has been proposed to be the tripeptide sequence involved in sperm-egg interactions. However, the sequence ECD that follows the X(D/E)E is completely conserved in all fertilin β homologues and in some other ADAM/MDCs (Table I), thus prompting the suggestion that this ECD sequence is involved in sperm-egg interactions (24, 25), similar to what has been suggested for an ECD-containing snake disintegrin-like domain (19). It is unclear which amino acids (X(D/E)E and/or ECD) comprise the active site of the putative fertilin β disintegrin loop (see Table I), because several variations of these peptide sequences inhibit sperm-egg binding: (a) peptides that include the full X(D/E)E/ECD sequence (2, 5, 6), (b) peptides that include the X(D/E)E but truncate (2, 4) or point-mutate (3) the ECD sequence, and (c) peptides that include only the ECD sequence (26).

The purpose of this study was to identify which amino acids were critical for fertilin β disintegrin function. We chose to focus specifically on the disintegrin domain in these studies, since other domains of fertilin β appear to be capable of participating in cell adhesion to a limited extent (7). We demonstrate that the fertilin β disintegrin loop β (βD) presented as a short amino acid sequence (AQDECDVT) at the terminus of a bacterial fusion protein can bind to eggs and inhibit sperm-egg binding. Then, to determine which amino acids of the fertilin β disintegrin loop is important for the function of the fusion protein-presented peptide, we tested nine different mutated forms of the fertilin β disintegrin loop pentapeptide (QDECD) as fusion protein-presented peptides in two different bioassays for protein function. We find that mutations of the second aspartic acid (QDECD) greatly affected the ability of the protein to bind to eggs and to inhibit the binding of recombinant fertilin β (MBP-βDCE) to eggs. Substitutions for the second aspartic acid (QDECD) result in dramatic losses of function in these two bioassays. Substitutions for the glutamic and the cysteine residues in the QDECD sequence result in slight reductions in activities in these two bioassays, whereas mutations of the first aspartic acid (QDECD) has virtually no effect. These data suggest that the conserved ECD sequence of the mouse fertilin β disintegrin loop, especially the terminal D residue, contributes more to the protein’s activity than does the peptide sequences inhibit sperm-egg binding: (a) peptides that include the full X(D/E)E/ECD sequence (2, 5, 6), (b) peptides that include the X(D/E)E but truncate (2, 4) or point-mutate (3) the ECD sequence, and (c) peptides that include only the ECD sequence (26).

For expression of BAP-presented βD peptides, a 500-mI culture of DH5α E. coli carrying the appropriate plasmid was induced with 1 mM isopropyl-1-thio-D-galactopyranoside at 30 °C for 8 h. The cells were pelleted, and the cell pellet was lysed with 20 ml of B-Per detergent (Pierce) according to the manufacturer’s instructions. The cell lysate was applied to a 1-ml nickel-agarose column (Pierce or Novagen, Madison, WI) equilibrated with column buffer (50 mM Tris-HCl, pH 8.0, 200 mM NaCl) by gravity. The column was then washed stepwise with column buffer containing 5, 10, and 25 mM imidazole sequentially (10 ml/wash). The fusion protein was eluted with column buffer containing 100 mM imidazole. The fusion protein-containing eluates were pooled and dialyzed against WHITCO buffer (109.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 7 mM NaHCO3, 1.5 mM HEPES) for 36–48 h with at least four buffer changes. The dialyzed protein was concentrated (Microcon microconcentrators; Amicon, Beverly, MA) to 2–10 mg/ml; protein concentration was determined by a BCA assay (Pierce). In preliminary experiments, the control BAP fusion protein and the BAP-βDCE fusion protein were cleaved with enterokinase (New England Biolabs) to remove the His-tag and PCR fragments (27). Cleaved and uncleaved proteins were compared for their abilities to bind to eggs and to inhibit the binding of MBP-βDCE (assays described below). However, no difference was observed between the cleaved and uncleaved proteins, indicating that the presence of the His-tag and PCR fragments (i.e., inhibit or enhance) the function of BAP-βD or the lack of function of control BAP in these assays. Therefore, for subsequent experiments, uncleaved fusion proteins were used. Control eggs and eggs cultured with BAP bind similar levels of MBP-βDCE and show similar levels of fertilization when inseminated (data not shown), indicating that BAP does not adversely affect the egg surface.

A synthetic peptide corresponding to the disintegrin loop of fertilin β (AQDECDVT), identical to that used by Yuan et al. (6), was synthesized by Alpha Diagnostics; the N terminus was acetylated, and the C terminus was amidated. The peptide was purified by high-pressure liquid chromatography to >95% purity.

Egg Collection, Zona Pellucida (ZP) Removal, and In Vitro Fertilization—Cumulus cell-free and ZP-free metaphase II-arrested eggs were obtained from superovulated 6–8-week-old CF-1 mice (Harlan, Indianapolis, IN) as described previously (4). Following ZP removal, the eggs were allowed to recover for 60 min. All gamete cultures were performed in Whitten’s medium (29) containing 22 mM NaCO3 and 15 mg/ml bovine serum albumin (Albimax I; Life Technologies, Inc.) at 37 °C in a 5% CO2 atmosphere, 5% O2, and 90% air in a 5% CO2 atmosphere, 5% O2, and 90% air in a 5% CO2 atmosphere, 5% O2, and 90% air. In vitro fertilization of ZP-free eggs in the presence of MBP fusion proteins was performed essentially as described previously (4, 28) with the following modifications. ZP-free eggs were incubated in medium containing 0.5 mg/ml MBP, MBP-βDCE, MBP-βD, or MBP-βD for 60 min prior to insemination. (The concentration of 0.5 mg/ml was based on our previous studies (7, 28) and corresponded to 10.0 μg MBB, 6.4 μg MBB-βDCE, 9.0 μg MBB-βD, and 10.0 μg MBB-βD.) Following this incubation, eggs were inseminated for 15 min with...
RESULTS

Activity of Fusion Protein-presented Fertilin β Disintegrin Loop Peptides—We first wanted to define a minimal number of amino acids that could be used from the fertilin β disintegrin domain in these studies. To do this, we compared three recombinant fertilin β MBP fusion proteins (MBP-βDCE, corresponding to the complete extracellular domain of mature mouse fertilin β (297 amino acids); MBP-βD, corresponding to the disintegrin domain (89 amino acids); and MBP-βDL, the eight amino acids of the predicted disintegrin loop) for their abilities to bind to the egg plasma membrane and to inhibit sperm binding to the plasma membranes of ZP-free eggs during in vitro fertilization. These recombinant fertilin β proteins were generated as MBP fusion proteins for two reasons: (a) the activity of MBP-βDCE had already been characterized (7, 28), and (b) the MBP expression system worked well for expressing βDCE and βD, whereas the BAP expression system did not work for these proteins (data not shown). These experiments showed that all three recombinant fertilin β proteins (a) bound the egg membrane and (b) reduced the levels of sperm-egg binding to ~40% of the levels observed with the control protein MBP (data not shown). These studies of MBP-βDL confirmed that the presentation of these eight amino acids at the terminus of a bacterial fusion protein is effective; specifically, the addition of these eight amino acids (AQDECDVT, corresponding to the predicted disintegrin loop of fertilin β) to the C terminus of MBP confers on MBP the ability to bind to eggs and to perturb sperm-egg interactions.

We then generated a fusion protein with this fertilin β disintegrin loop sequence presented at N terminus of MBP. There were two reasons for this. First, the BAP expression system for fusion protein-presented peptides had been effective in studies of in vitro protein-protein interactions (27, 31). Second, by presenting fertilin β disintegrin loop sequences with a fusion protein other than MBP, we could test the effects of these peptides on the binding of MBP-βDCE to eggs, which is detected with an anti-MBP antibody. MBP-βDCE was used at 0.5 mg/ml (6.4 μM) based on our previous studies on the use of this luminometric immunoassay, which indicated that optimal signal-to-noise ratios were obtained from eggs cultured in this concentration of recombinant fertilin proteins (data not shown; see Refs. 7 and 28). In these experiments, BAP-βDL inhibited the binding of MBP-βDCE in a concentration-dependent manner, with ~50% inhibition observed with 10 μM BAP-βDL and maximal inhibition (~20% of binding levels observed for MBP-βDCE with no BAP protein added) observed with 30–40 μM BAP-βDL (Fig. 1). In contrast, BAP-βDLs had virtually no effect on the binding of MBP-βDCE at either 10 μM or 40 μM (Fig. 1). Interestingly, BAP-βDL was more effective at inhibiting MBP-βDCE binding to eggs than was a synthetic peptide version of the fertilin β disintegrin loop sequence. This synthetic peptide at 250 μM reduced MBP-βDCE binding to ~80% of control (no protein or peptide) levels (data not shown). (Higher concentrations of this synthetic peptide were not tested in
these experiments, since 500 μM had significant toxic effects on the eggs (data not shown).

Identification of Critical Amino Acids in the Fertilin β Disintegrin Loop—Regarding the pentapeptide sequence of the fertilin β disintegrin loop (QDECD in the mouse; Table I), there is controversy over whether the QDE tripeptide, which aligns with the RGD tripeptide in snake venom disintegrins, or the ECD tripeptide, which is highly conserved in fertilin β homologs (Table I), mediates the interaction of fertilin β with the egg surface. To determine which amino acid(s) is critical for the functioning of the fertilin β disintegrin loop, we tested nine different point-mutated versions of BAP-DL (Table II) in two different bioassays. The rationale for the design of the mutant forms was as follows. Four mutants (mutants 1, 9, 7, and 8; Table II) focused on one or both residues of the highly conserved diacidic sequence, the DE of the QDECD sequence. One mutant (mutant 2; Table II) introduced an alanine residue in place of the cysteine residue in the QDECD sequence. Three mutants (mutants 4–6) focused on the terminal aspartic acid residue of the QDECD sequence, and one mutant (mutant 3) introduced alanines for both acidic residues of the ECD tripeptide in the QDECD sequence.

The BAP-presented disintegrin loop mutants were assayed for their abilities to perturb the binding of MBP-βDCE to eggs (Fig. 2) and to bind to eggs (Fig. 3). In the assays of the BAP-presented peptides' effects on MBP-βDCE binding, three different concentrations of BAP-presented peptides were tested: 5 μM, the concentration at which we observed ~35% inhibition of MBP-βDCE binding with wild-type BAP-βDL; 10 μM, the concentration at which we observed ~50% inhibition of MBP-βDCE binding with wild-type BAP-βDL; and 30 μM, the concentration at which we observed ~80% inhibition of MBP-βDCE binding with wild-type BAP-βDL (Fig. 1). The BAP-presented βDCE peptides that showed dramatically reduced activity at 10 μM were tested at 30 μM to determine if the mutants that had no effect at 10 μM would have an effect at the higher concentration of 30 μM. The BAP-presented βDCE peptides that showed activities similar to that of wild-type BAP-βDL at 10 μM were tested at 5 μM to determine if the mutants that were effective at 10 μM would show reduced activity (as compared with wild-type BAP-βDL) at the lower concentration of 5 μM. The results from the inhibition assay and the binding assay agreed with each other, since the respective abilities of the mutant BAP-presented peptides to inhibit MBP-βDCE binding to eggs (Fig. 2) are mirrored by their respective abilities to bind to eggs (Fig. 3). Assays of the inhibition of MBP-βDCE binding to eggs indicated that there were three general groupings of mutant peptide activities: (a) those which functioned similarly to the wild type disintegrin loop sequence (Fig. 2A); (b) those that showed a moderate loss of function as compared with the wild type sequence (Fig. 2A); and (c) those that showed a dramatic loss of function (Fig. 2B; these subgroups are shown in separate panels of Fig. 2 for easier presentation).

Alanine substitution for the first asparatic acid of the QDECD sequence had almost no effects, since BAP-QAECD had activity in these two assays virtually identical to that of the BAP-βDL wild type (Figs. 2 and 3), with the only statistically significant difference (p < 0.05) being in its ability to inhibit MBP-βDCE binding at 30 μM as compared with the BAP-βDL control (Fig. 2B).

Alanine substitution for the glutamic acid of the cysteine had subtle effects on the activities of the BAP-presented disintegrin loop peptides. The differences between the activities of BAP-βDL and BAP-QADCD or BAP-QDEAD in both assays (Figs. 2 and 3) were statistically significant. A lysine substitution for the glutamic acid (BAP-QDKCD) resulted in a somewhat greater disruption in the activities then the alanine substitution (BAP-QADCD) did; BAP-QDKCD showed statistically significant differences in its abilities to bind to eggs (Fig. 3) and to perturb MBP-βDCE binding (Fig. 2A) as compared with BAP-QADCD. Comparisons of BAP-QADCD (single alanine substitution) with BAP-QQACD (double alanine substitution) revealed that there were no statistically significant differences in how these two proteins functioned in the two assays (Figs. 2A and 3).

In contrast, substitutions for the terminal aspartic acid of the QDECD sequence resulted in dramatic losses of function of the BAP-presented peptide in the two assays. BAP-QDECA and BAP-QDECK were significantly less able to inhibit the binding of MBP-βDCE to eggs (Fig. 2B) and to bind to eggs
**Analysis of the Fertilin β Disintegrin Domain**

### Table II

| Fusion protein-presented peptide | Oligonucleotide and deduced amino acid sequences |
|---------------------------------|-------------------------------------------------|
| MBP-βDL (wild type)             | CTAGTGCC CAA GAT GAG TGT GAT GTC ACA TAAC       |
|                                 | A Q D E D C D V T stop                          |
| BAP-βDL (wild type)             | CTAGTGCC CAA GAT GAG TGT GAT GTC ACAG          |
|                                 | A Q D E D C D V T                               |
| BAP-βDLΔs (scrambled control)   | CTAGTGCC CAA GAT GAG GCA GAT GTC ACAG          |
|                                 | ATGT CTA ACA GAG TCT CTC CGA CTA CGAG TCCTAG   |
|                                 | T D C Q E A D V                                 |
| BAP-QAECΔD (mutant 9)           | CTAGTGCC CAA GCA GAG TGT GAT GTC ACAG          |
|                                 | A Q A E C D V T                                |
| BAP-QAACΔD (mutant 1)           | CTAGTGCC CAA GCC GCC TGT GAT GTC ACAG          |
|                                 | A Q A E C D V T                                |
| BAP-QDΔCΔD (mutant 7)           | CTAGTGCC CAA GAT GAG TGT GAT GTC ACAG          |
|                                 | A Q D A E C D V T                               |
| BAP-QDKΔCΔD (mutant 8)          | CTAGTGCC CAA GAT GAG TGT GAT GTC ACAG          |
|                                 | A Q D A E C D V T                               |
| BAP-QDEADΔ (mutant 2)           | CTAGTGCC CAA GAT GAG GCA GAT GTC ACAG          |
|                                 | A Q D A E C D V T                               |
| BAP-QDECAΔ (mutant 4)           | CTAGTGCC CAA GAT GAG TGT GAT GTC ACAG          |
|                                 | A Q D A E C D V T                               |
| BAP-QDECEΔ (mutant 5)           | CTAGTGCC CAA GAT GAG TGT GAT GTC ACAG          |
|                                 | A Q D A E C D V T                               |
| BAP-QDECKΔ (mutant 6)           | CTAGTGCC CAA GAT GAG TGT GAT GTC ACAG          |
|                                 | A Q D A E C D V T                               |
| BAP-QDΔCAΔ (mutant 3)           | CTAGTGCC CAA GAT GAG TGT GAT GTC ACAG          |
|                                 | A Q D A E C D V T                               |

**DISCUSSION**

In this paper, we present two key findings. First, the addition of eight amino acids from the mouse fertilin β disintegrin loop (AQDECDVT) to a bacterial fusion protein (MBP or BAP) conveys to the protein the abilities to (a) bind to eggs and (b) to perturb the binding of sperm or of recombinant fertilin β (MBP-βDCE) to eggs. Second, we have identified the amino acids that are important for the fertilin β disintegrin loop (as a BAP-presented peptide) to be able to perturb the binding of MBP-βDCE (Fig. 2) and to be able to bind to eggs (Fig. 3). We find that the second aspartic acid in the predicted disintegrin loop pentapeptide sequence QDECD is critical for the biological activity of BAP-βDL. When this aspartic acid is replaced with an alanine (QDECA) or lysine (QDECK), the BAP-presented peptide shows a dramatic loss of activity in the two functional assays (perturbation of MBP-βDCE binding (Fig. 2B) or binding of the BAP-presented peptides themselves (Fig. 3)). The BAP-presented peptide with the conservative substitution QDECE has partial activity as compared with the wild-type sequence QDECD. A subset of the point-mutated BAP-presented disintegrin loop peptides, BAP-QDΔCΔD, BAP-QDKΔCΔD, and BAP-QDEADΔ, had subtle losses of activities (on average ~66% of the activity levels of control BAP-βDL). In contrast, substitution of alanine for the first aspartic acid in the predicted disintegrin loop pentapeptide sequence resulted in almost no loss of activity; BAP-QAECΔD and wild type BAP-βDL are similarly capable of perturbing MBP-βDCE binding to eggs and of binding to eggs themselves. The double-substituted BAP-QAAΔCΔD had similar activity levels to BAP-QDΔCΔD, suggesting that the loss of activity of the BAP-QAAECΔD is primarily due to the alanine substitution for the glutamic acid residue and not the first aspartic acid residue, in agreement with the observation that BAP-QAECΔD retains virtually wild-type levels of activity in the two functional assays. The double-substituted BAP-QQΔCΔCΔD shows the most extensive losses of activity of all of the point-mutated peptides tested as compared with control BAP-βDL. Taken together, these data indicate that the conserved ECD sequence of the mouse fertilin β disintegrin loop, especially the terminal Asp residue, contributes more to the protein’s activity than does the QDE sequence that aligns with the RGD tripeptide in other disintegrins (Table I).

BAP-presented peptides have been used previously in enzyme-linked immunosorbent assay-based and protein blot overlay assays of protein-protein interactions (27, 31). In this study, we show that fusion protein-presented peptides (at either the N terminus of BAP or the C terminus of MBP) are a viable and economical alternative to synthetic peptides for studies of cell adhesion. Moreover, in parallel studies with the fertilin βDL synthetic peptide, a greater extent of inhibition of MBP-βDCE binding was observed with lower molar concentrations of BAP-presented fertilin βDL peptides than with the synthetic peptide with the same amino acid sequence (data not...
The principal purpose of this study was to determine which amino acids of the fertilin \( \beta \) disintegrin loop are critical for sperm-egg binding, similar to the way BAP-\( \beta \text{DL} \) was more effective than BAP-\( \beta \text{DL} \) control (defined as 100%). Statistical analysis (analysis of variance with Fisher’s protected least significant difference post hoc testing) revealed that BAP-\( \beta \text{DL} \) and BAP-\( \beta \text{AECD} \) bound at similar levels; that BAP-\( \beta \text{AQACD} \), BAP-\( \beta \text{QDACD} \), BAP-\( \beta \text{QAFAD} \), and BAP-\( \beta \text{QDECE} \) bound at similar levels; and that the levels of binding of BAP-\( \beta \text{DECK} \) were similar to those of BAP-\( \beta \text{DECA} \) and BAP-\( \beta \text{DECE} \). All other comparisons showed statistically significant differences (\( p < 0.05 \)).

This could be due to increased stability and/or improved presentation/conformation of the \( \beta \text{DL} \) amino acid sequence at the N terminus of BAP as compared with its synthetic peptide version.

The principal purpose of this study was to determine which amino acids of the fertilin \( \beta \) disintegrin loop are critical for function, focusing on the pentapeptide sequence QDECD, which aligns with RGD sequences in snake disintegrins and includes the highly conserved ECD sequence (Table I). Slight losses of function are observed with the substitutions QDACE and QDEAD as compared with the wild type sequence. In agreement with our results with BAP-\( \beta \text{DEAD} \), a trimer peptide with the cysteine residue of the ECD replaced with a serine residue (ESD) does not reduce the incidence of sperm-egg fusion, while a trimer peptide with the wild type-ECD sequence does have an inhibitory effect (26). Thus, the cysteine residue in the fertilin \( \beta \) disintegrin loop appears to contribute to the interaction of this sperm protein with the egg surface. (It should be noted that a longer synthetic peptide with the cysteine constraint in a disulfide bond inhibit platelet aggregation (16, 20), although catrocollastatin, atrolysin A and catrocollastatin, suggest the cysteine in the fertilin ECD could have been more effective at perturbing the cysteine residue (ESD) does not reduce the incidence of sperm-egg fusion. However, one might hypothesize that the activity of this 14-mer is due to the presence of other key residues identified in this study (i.e. the Glu and the Asp of the QDECD pentapeptide) and that a peptide with a normal ECD could have been more effective at perturbing sperm-egg binding, similar to the way BAP-\( \beta \text{DL} \) was more effective than BAP-\( \beta \text{DEAD} \). An interesting question remaining is whether this cysteine residue forms a disulfide bond. Based on data in a number of studies, this may vary between different ECD-containing disintegrin proteins. Studies of two snake disintegrins, atrolysin A and catrocollastatin, suggest the cysteine in the ECD could be disulfide-bonded, since synthetic peptides with the cysteine constrained in a disulfide bond inhibit platelet aggregation (16, 20), although catrocol-

![Graph](http://www.jbc.org/)
several of the snake disintegrin-like domains (Table I). ECD peptides do, suggesting that the cysteine in the ECD do not perturb mouse sperm-egg fusion, whereas monomeric dimerized through a disulfide bond between cysteine residues remains to be determined, although this may be reflective of a new feature of aββ that has not been previously identified through studies of its interaction with laminins.

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