

Correct Proteolytic Cleavage Is Required for the Cell Adhesive Function of Uvomorulin

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Abstract. All Ca^{2+} -dependent cell adhesion molecules are synthesized as precursor polypeptides followed by a series of posttranslational modifications including proteolytic cleavage. The mature proteins are formed intracellularly and transported to the cell surface. For uvomorulin the precursor segment is composed of 129-amino acid residues which are cleaved off to generate the 120-kD mature protein. To elucidate the role of proteolytic processing, we constructed cDNAs encoding mutant uvomorulin that could no longer be processed by endogenous proteolytic enzymes and expressed the mutant polypeptides in L cells. Instead of the recognition sites for endogenous proteases, these mutants contained either a recognition site of serum coagulation factor Xa or a new trypsin cleavage site. The intracellular proteolytic processing of mutant polypeptides was inhibited in both cases. The un-

processed polypeptides were efficiently expressed on the cell surface and had other features in common with mature uvomorulin, such as complex formation with catenins and Ca^{2+} -dependent resistance to proteolytic degradation. However, cells expressing unprocessed polypeptides showed no uvomorulin-mediated adhesive function. Treatment of the mutant proteins with the respective proteases results in cleavage of the precursor region and the activation of uvomorulin function. However, other proteases although removing the precursor segment were ineffective in activating the adhesive function. These results indicate that correct processing is required for uvomorulin function and emphasize the importance of the amino-terminal region of mature uvomorulin polypeptide in the molecular mechanism of adhesion.

THE cell adhesion molecule (CAM)¹ uvomorulin is synthesized as a precursor polypeptide of 135 kD followed by a series of posttranslational modifications including proteolytic processing, glycosylation, and phosphorylation (Peyri ras et al., 1983; Vestweber and Kemler, 1984). The mature form of uvomorulin is formed intracellularly and transported to the cell surface. During these processes the cytoplasmic domain of uvomorulin associates with three proteins (102, 88, and 80 kD) named catenin α , β , and γ , respectively (Ozawa et al., 1989). The uvomorulin-catenin complex formation has recently been found to play an important role for uvomorulin function and the connection of the protein to actin (Ozawa et al., 1990).

Ca^{2+} -dependent CAMs consist of a gene family of structurally and functionally related molecules which exhibit a remarkable similar topology as revealed by secondary structure predictions (Takeichi, 1988; Hatta et al., 1988; Kemler et al., 1989). Members of this group express their adhesive properties only in the presence of Ca^{2+} , and Ca^{2+} protects these proteins from proteolytic degradation (Takeichi, 1977; Hyafil et al., 1981; Gallin et al., 1983). The extracellular parts of the proteins are largely composed of repeating domains, each domain with two putative Ca^{2+} -binding motifs

which are well conserved and at analogous positions in all proteins. All Ca^{2+} -dependent CAMs are synthesized as precursor polypeptides, but in contrast to the extended homology between the mature proteins the respective precursor segments show no structural similarity. Each precursor segment varies in length and sequence comparison reveals a rather weak homology, of 28–40% at the nucleotide level. However, precursor segments of mouse uvomorulin, chicken L-CAM (Gallin et al., 1986; 1987), mouse P- (Nose et al., 1987), and chicken N-cadherins (Hatta et al., 1988) all terminate in the sequence Leu-(Arg/Lys)-Arg-(Gln/Arg)-Lys-Arg and the respective mature proteins all have the amino-terminal sequence (Asp/Glu)-Trp-Val-(Ile/Met)-Pro-Pro-Ile in common, suggesting that proteases of similar specificities are involved in processing.

Proteolytic processing is a common and effective mechanism of physiological regulation. In general, proteolytic processing releases a conformational restraint on an inactive precursor to generate a biologically active protein (for review see Neurath, 1989). The role of proteolytic cleavage in the activation of viral glycoproteins has been defined for some viral glycoproteins such as the F (fusion) protein of Sendai or the hemagglutinin (HA) protein of influenza virus (Homma and Ohuchi, 1973; for review see Wiley and Skehel, 1987). In each case proteolytic cleavage of the precursor

1. Abbreviation used in this paper: CAM, cell adhesion molecule.

protein creates on the transmembrane subunit a new hydrophobic amino-terminal domain (25–30 amino acid residues), “the fusion peptide,” which is implicated in the fusion activity of the protein (Gething et al., 1978; for review see Wiley and Skehel, 1987).

We have been interested in the role proteolytic processing might have on the assembly of the uvomorulin–catenin complex, cell surface expression, and adhesive function. For this we have constructed cDNA in which the endoproteolytic cleavage site (Arg-Arg-Gln-Lys-Arg) was replaced by a cleavage site for serum coagulation factor Xa (Arg-Ile-Glu-Gly-Arg). In a second cDNA construct we replaced the endogenous cleavage site by amino acid residues Arg-Thr-Gln-Thr-Arg following a similar substitution strategy, which blocked the processing of hemagglutinin and which could be cleaved by trypsin (Kawaoka and Webster, 1988). We show that unprocessed uvomorulin has most characteristics in common with the mature protein but exhibits no adhesive function. Protease treatment converts the unprocessed form into mature protein which has adhesive properties. However, exact cleavage is necessary for the activation of the adhesive function.

Materials and Methods

cDNA Construction

Plasmid pSUM-1 with full-length uvomorulin cDNA has been described (Ozawa et al., 1989). cDNAs encoding uvomorulin polypeptides with substitutions in the putative endoproteolytic cleavage site were constructed as schematically outlined in Fig. 1. A 30-bp Rsa I–Tth III I fragment coding for 10 amino acid residues with the endoproteolytic cleavage site was replaced by one of the following synthetic oligonucleotides. An oligonucleotide with the sequence of ATCCAGGCTTAAGAACACAGACGAGACT and its complementary oligonucleotide were used to substitute Arg and Lys residues in the cleavage site to Thr residue (see Fig. 1). An oligonucleotide ATCCAGGCTTAAGAATCGAGGGTCGAGACT and its complementary oligonucleotide were used to change Arg-Gln-Lys sequence to Ile-Glu-Gly creating the recognition sequence of coagulation factor Xa (Ile-Glu-Gly-Arg) (see Fig. 1). The sequence of the substituted cDNAs was confirmed by direct DNA sequencing (Hattori and Sakaki, 1986) and the cDNAs were subcloned into the expression vector pSVtkneo β (Nicolas and Berg, 1983) as described (Ozawa et al., 1989).

DNA Transfection

Purified plasmid DNAs were introduced into mouse L-tk⁻ cells by calcium phosphate precipitation together with pSVtkneo β at a 10:1 molar ratio as described (Ozawa et al., 1989). After 2 wk of G418 (1 mg/ml) selection, cells expressing uvomorulin were isolated by FACS and cloned.

Antibodies

Antibodies against the extracellular part of mature uvomorulin (anti-gp84) were purified as described (Vestweber and Kemler, 1985). Antibodies against the precursor segment of uvomorulin (antiprecursor) were prepared as follows. A 400-bp Pst I–Pvu II fragment of uvomorulin cDNA that encodes the carboxy-terminal 127 amino acid residues of the precursor segment and the amino-terminal 7 amino acid residues of the mature protein was subcloned into the Pst I–Sma I site of Bluescript KS(+) vector (Stratagene Corp., La Jolla, CA) to adjust the reading frame of the fragment to that of the cloning site of protein A expression vector pRIT2T (Pharmacia Fine Chemicals, Uppsala, Sweden). After digestion with Eco RI and Bam HI, the cDNA fragment was subcloned into Eco RI–Bam HI site of pRIT2T. The protein A–fusion protein was purified on an IgG Sepharose 6 Fast Flow (Pharmacia Fine Chemicals) column. The fusion protein was injected into rabbit as described (Ringwald et al., 1987). For affinity purification of antibodies protein A or the fusion protein (1 mg) each coupled to 1 ml of IgG Sepharose 6 Fast Flow (5 mg/ml of dimethyl-suberimidate in 0.1 M sodium borate buffer, pH 8.0 for 1 h at room temperature) were used sequentially

to obtain antibodies specific for the precursor segment. The purified antibodies (antiprecursor) reacted with the precursor polypeptide but not with mature uvomorulin in immunoblot, immunoprecipitation, and immunofluorescence experiments.

Immunoprecipitation and Immunoblot

Cells (1×10^6) were labeled with 50 μ Ci/ml of [³⁵S]methionine (Amersham Corp., Arlington Heights, IL) for 16 h and lysed with PBS containing 1% Triton X-100, 1% NP-40, 2 mM CaCl₂, and 1 mM PMSF. After centrifugation supernatants were incubated with affinity-purified antibodies and immune complexes were collected by protein A–Sepharose CL4B (Pharmacia Fine Chemicals) as described (Vestweber and Kemler, 1985).

To analyze Ca²⁺ sensitivity of uvomorulin polypeptides, cells (5×10^5) were washed with PBS and incubated with 1 ml of 0.01% (wt/vol) of trypsin (type XI; Sigma Chemical Co., St. Louis, MO) in HEPES-buffered saline (HBS) containing 2 mM CaCl₂ or 1 mM EGTA for 10 min at 37°C. After addition of 40 μ l of soybean trypsin inhibitor (0.5% in HBS; Boehringer, Mannheim, FRG), cells were collected and boiled in 500 μ l of SDS-PAGE sample buffer (Laemmli, 1970). Aliquots (100 μ l) were subjected to SDS-PAGE and immunoblot analysis was done as described (Ringwald et al., 1987). Treatment of cells with 0.01% (wt/vol) chymotrypsin (type VII; Sigma Chemical Co.) was done in a similar way. The reaction was terminated by adding PMSF at a final concentration of 1 mM.

Cell Aggregation

Cells were dissociated by trypsin in the presence of 2 mM CaCl₂ as described above or by incubation with PBS containing 2 mM EDTA and 2% chicken serum for 10 min at room temperature. After washing with the 1:1 mixture of HBS and DMEM containing 4% FCS, cells were resuspended in the same medium containing 5 μ g/ml of DNase I (Boehringer). Cells were allowed to aggregate for 45 min at 37°C with a constant rotation of 70 rpm. In some experiments serum coagulation factor Xa (Boehringer) was added in the aggregation medium. The extent of cell aggregation was calculated according to Nagafuchi and Takeichi (1988) by the index (No-Nt)/No where Nt is the total particle number after the incubation (45 min) and No is the total particle number at the initiation of incubation.

Results

Cell Surface Expression of Mutant Uvomorulin Polypeptides with the Precursor Segment

The putative endoproteolytic cleavage site of uvomorulin precursor polypeptide contains two pairs of a basic amino acid doublet (Arg-Arg-Gln-Lys-Arg), which are conserved in mouse uvomorulin and chicken L-CAM. To express mutant uvomorulin polypeptide containing the precursor seg-

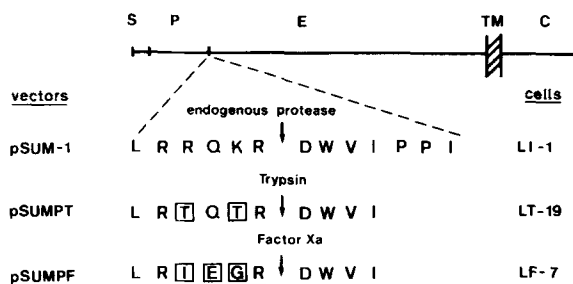


Figure 1. Scheme of uvomorulin polypeptide and the amino acid sequence of wild-type and mutant polypeptides at the endoproteolytic cleavage site. pSUM-1 is an expression vector with cDNA encoding wild-type uvomorulin. pSUMPT and pSUMPF are vectors with cDNAs that encode uvomorulin polypeptides with substitutions shown in the figure. The one-letter amino acid code is used. The substituted amino acids are boxed and the recognition sequence of blood coagulation factor Xa is underlined.

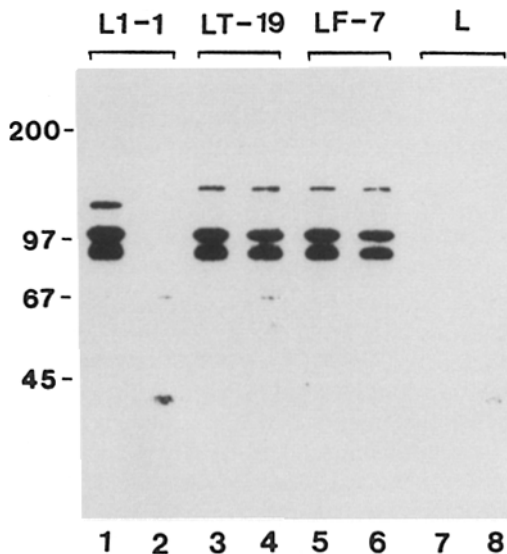


Figure 2. Immunoprecipitation analysis of normal and mutant uvomorulin polypeptides. L cells expressing normal (*L1-1*) or mutant uvomorulin (*LT-19* and *LF-7*) or untransfected L cells (*L*) were labeled with [³⁵S]methionine. Lysates were immunoprecipitated with antibodies against the extracellular part of mature uvomorulin (*anti-gp84*, lanes 1, 3, 5, and 7) or antibodies against the precursor segment (*anti-precursor*, lanes 2, 4, 6, and 8). Immunoprecipitates were analyzed by 8% PAGE.

ment on the cell surface, we replaced this sequence by the recognition site (Ile-Glu-Gly-Arg) of a highly specific serum protease, coagulation factor Xa (Nagai and Thøgersen, 1987), which is not expected to be used by endogenous proteases. The second construct contained the substitution Arg-Thr-Gln-Thr-Arg. A similar substitution was shown to block efficiently the processing of the precursor protein of influenza virus hemagglutinin glycoprotein and the precursor segment could subsequently be removed by trypsin treatment (Kawaoka and Webster, 1988). For convenience this substitution is subsequently referred to as “trypsin recognition site” to distinguish from the “factor Xa recognition site” mentioned above. The respective substitutions were carried out by replacing a 30-bp *Rsa* I-Tth III I fragment of uvomorulin cDNA with synthetic oligonucleotides.

The mutant uvomorulin cDNAs were introduced into L cells together with the neomycin-resistant gene (neo-gene) and G-418-resistant cells were selected for cell surface expression of mutant uvomorulin by FACS with antibodies against the precursor segment. Transfectants were subjected to immunoprecipitation and immunoblot analysis using either affinity-purified antibodies against the precursor segment (antiprecursor), or antibodies against the extracellular part of mature uvomorulin (*anti-gp84*).

Both anti-precursor and anti-gp84 antibodies recognized a 140-kD precursor protein in cell lysates from *LT-19* (trypsin recognition site) and *LF-7* (factor Xa recognition site). As shown in Fig. 2, lanes 3–6, both mutant uvomorulins complex with catenin α , β , and γ , which have the known molecular weights of 102, 88, and 80 kD, respectively. In immunoblots antiprecursor and anti-gp84 antibodies reacted exclusively with the respective 140-kD mutant uvomorulin from cell lysates of *LT-19* and *LF-7* cells (Fig. 3, lanes 4, 7, and 10). The 140-kD mutant proteins differ in their molecu-

lar weight from the endogenous uvomorulin precursor (135 kD), which is most likely due to differences in glycosylation since the 140-kD proteins should contain the complex carbohydrate moieties of mature uvomorulin. In pulse-chase experiments mutant uvomorulin is also first synthesized as a 135-kD polypeptide which is processed to the 140-kD protein (not shown).

Immunofluorescence tests with antiprecursor antibodies revealed specific membrane staining of *LT-19* and *LF-7* cells with virtually no intracellular reaction, indicating that the precursor segment had no inhibitory effect on the transport of uvomorulin to the cell surface (not shown, but see below). These results clearly demonstrate that proteolytic processing of uvomorulin precursor polypeptides is not required for the assembly of the uvomorulin-catenin complex and cell surface expression of uvomorulin.

Although proteolytic processing is efficiently inhibited in both mutant polypeptides it was important to demonstrate that the newly introduced recognition sites could indeed be used by the respective enzymes. *LT-19* cells (trypsin recognition site) and *LF-7* cells (factor Xa recognition site) were treated with Ca²⁺/trypsin (0.01%) or with 2 U/ml factor Xa, respectively. In both cases the enzymatic treatment converted the mutant polypeptide into 120-kD proteins (Fig. 3, lanes 5 and 11). The respective 120-kD proteins were indistinguishable by their electrophoretic mobility from the 120-kD mature uvomorulin (compare Fig. 3, lanes 2, 5, and 11). From these results it is concluded that both mutant polypeptides can be converted into mature proteins by exogenous treatment with the respective enzyme. Assuming that the enzymes do not penetrate into cells, these results suggest that all of the mutant polypeptides are expressed on the cell surface.

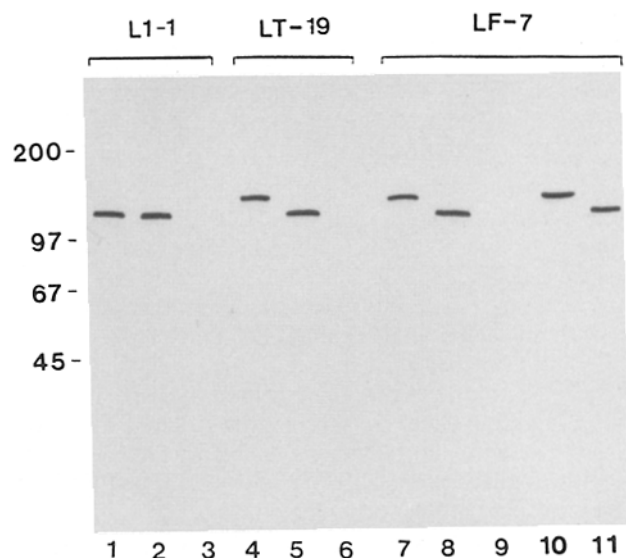


Figure 3. Trypsin and factor Xa digestion and Ca²⁺ sensitivity of normal and mutant uvomorulin polypeptides. Cells expressing normal (*L1-1*) and mutant (*LT-19* and *LF-7*) uvomorulin polypeptides were lysed with SDS-PAGE sample buffer without trypsinization (lanes 1, 4, and 7) or after trypsinization in the presence (lanes 2, 5, and 8) or in the absence of Ca²⁺ (lanes 3, 6, and 9) subjected to immunoblot analysis using anti-gp 84 antibodies. *LF-7* cells dissociated with PBS containing 2 mM EDTA and 2% chicken serum were incubated for 1 h in the presence (lane 11) or in the absence (lane 10) of factor Xa (2 U/ml) and analyzed.

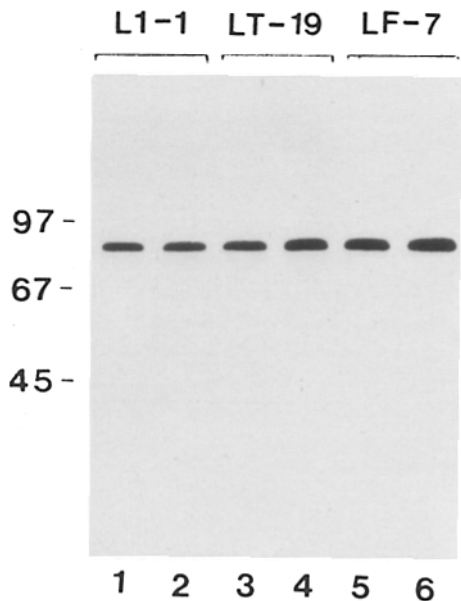


Figure 4. Immunoprecipitation with anti-gp84 of metabolically labeled L1-1, LT-19, and LF-7 cells. Cells were treated with 0.1% trypsin/2 mM Ca^{2+} for 30 min (lanes 1, 3, and 5) or 60 min (lanes 2, 4, and 6) at 37°C and soluble material was subjected to immunoprecipitations. An 84-kD trypsin/ Ca^{2+} -resistant fragment is generated from wild-type and mutant ovomorulin.

Properties of the Extracellular Domain of Mutant Uvomorulin Polypeptides

It is well known that Ca^{2+} -dependent CAMs are resistant to trypsin digestion in the presence of Ca^{2+} but are degraded if Ca^{2+} is omitted. By treatment of cells with trypsin in presence or absence of Ca^{2+} it can be tested whether the respective protein parts of the mutant ovomorulins exhibit these Ca^{2+} -dependent characteristics. Cells were treated with 0.01% trypsin, 2 mM Ca^{2+} which are the standard conditions for dissociating cells in Ca^{2+} -dependent cell aggregation assays (see below). Immunoblot analysis from cell lysates of L1-1, LT-19 and LF-7 cells digested with Ca^{2+} /trypsin or EGTA/trypsin gave identical results as that in the presence of Ca^{2+} , a 120-kD protein was generated in each case (Fig. 3, lanes 2, 5, and 8) and that in the absence of Ca^{2+} the proteins were degraded (Fig. 3, lanes 3, 6, and 9).

Interestingly, LF-7 cells expressing the mutant polypeptide with the factor Xa recognition site were also converted by Ca^{2+} /trypsin treatment into a 120-kD protein with an identical electrophoretic mobility (Fig. 3, lane 8). This can best be explained either by trypsin cleavage in the factor Xa recognition site or in the vicinity of this site where in fact several potential trypsin cleavage sites are located. One of these sites either in the precursor region or in the mature protein part could be used by trypsin to generate a 120-kD protein from the LF-7 mutant polypeptide.

One striking characteristic of all Ca^{2+} -dependent CAMs is that the extracellular part of the proteins are even resistant to extensive tryptic digestion when Ca^{2+} is present. For ovomorulin, an 84-kD soluble trypsin/ Ca^{2+} fragment can thus be generated that harbors the functional site(s) for cell adhesion (Wheellock et al., 1987).

To test whether mutant polypeptides exhibit also this feature, L1-1, LT-19 and LF-7 cells were digested with 0.1% tryp-

sin/2 mM Ca^{2+} at 37°C for 30 or 60 min and solubilized material was subjected to immunoprecipitations with anti-gp84 (Fig. 4). An 84-kD fragment was precipitated in each case indicating that the respective mature parts of the mutant proteins exhibit this structural similarity to wild-type ovomorulin.

Cell Aggregation Assays of Cells with Mutant Uvomorulin Polypeptides

Both mutant ovomorulin polypeptides were recognized by monoclonal antibody DECMA-1 that is known to inhibit cell aggregation (Vestweber and Kemler, 1985). To analyze the adhesive properties of mutant polypeptides cells were dissociated with PBS containing 2 mM EDTA and 2% chicken serum. Under these conditions, the 140-kD mutant ovomorulin polypeptides remained intact on the cell surface as judged by immunoblot analysis (Fig. 3). When cells were allowed to aggregate, L1-1 cells expressing mature ovomorulin showed strong Ca^{2+} -dependent adhesiveness, which can be inhibited by monoclonal antibody DECMA-1. However, LT-19 and LF-7 cells expressing mutant ovomorulin polypeptides or control untransfected L cells showed almost no cell adhesion (Fig. 5, *open bars*). Thus mutant polypeptides exhibit no adhesive function in spite of having other properties in common with normal ovomorulin. In cell mixing experiments between L1-1 and LT-19 or LF-7 cells mutant polypeptides were unable to interact with mature ovomorulin (not shown). This suggests that the remaining precursor segment in the mutant ovomorulin might interfere with adhesiveness. Consequently, cells were dissociated by Ca^{2+} /trypsin treatment under conditions which were shown to convert the 140-kD mutant polypeptides into 120 kD proteins (Fig. 3). In subsequent cell aggregation assays only L1-1 and LT-19 (trypsin recognition site) showed ovomorulin-dependent cell adhesion (Fig. 5, *closed bars*). In contrast, LF-7 cells (factor Xa recognition site) still showed no adhesiveness although the mutant polypeptide was converted into a protein with the same electrophoretic mobility as the mature ovomorulin (Fig. 3, lane 8). These results clearly demonstrate that the removal of the precursor segment in LT-19 cells activates the adhesive function of the mutant ovomorulin polypeptide.

The results with LF-7 cells were rather unexpected as that a similar conversion of the mutant polypeptide to a 120-kD protein did not activate the adhesive properties. The most likely explanation is that the trypsin cleavage sites in LT-19 and LF-7 mutant polypeptides were not identical but trypsin converted both mutant proteins to a similar molecular weight. Thus, as stated above trypsin could either cleave in

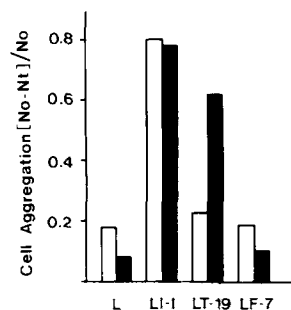


Figure 5. Aggregation of cells expressing normal (L1-1) or mutant (LT-19 and LF-7) ovomorulin polypeptides. Cells dissociated by PBS containing 2 mM EDTA and 2% chicken serum (*open bars*), or by 0.01% trypsin and 2 mM CaCl_2 (*closed bars*) were allowed to aggregate for 45 min in the presence of 2 mM CaCl_2 .

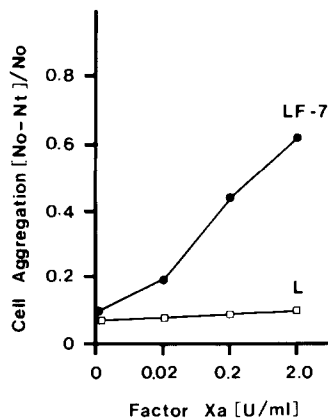


Figure 6. Cell aggregation in the presence of factor Xa. LF-7 cells expressing mutant uvomorulin with the factor Xa recognition site or untransfected L cells were dissociated with 0.01% trypsin in the presence of 2 mM CaCl₂ and allowed to aggregate in the presence of factor Xa as indicated. Increasing amount of factor Xa-induced aggregation of LF-7 cells.

the precursor region or in the mature protein part of LF-7 mutant polypeptides. To distinguish between both possibilities LF-7 cells were dissociated by Ca²⁺/trypsin and cell aggregation assays were performed in the presence of factor Xa. As shown in Fig. 6, the additional treatment of LF-7 cells with factor Xa led to an increased cell aggregation. This aggregation was uvomorulin-dependent since it could be inhibited by mAb DECMA-1 and a similar treatment of untransfected L-cells had no effect (Fig. 6). From these results we conclude that trypsin cleaves in the precursor segment near the factor Xa recognition site and that additional treatment with factor Xa removes residual precursor sequences. These results suggest that correct proteolytic cleavage of the precursor polypeptides is necessary for the acquisition of the cell adhesion activity of uvomorulin. Even few remaining amino acid residues as in the case of Ca²⁺/trypsin digestion of LF-7 cells could be inhibitory, indicating that the amino-terminal part of the mature uvomorulin has an important role in its adhesive properties. Concordant with this view are experiments in which chymotrypsin treatment of LT-19 and LF-7 cells generated in both cases a 120-kD protein with a similar electrophoretic mobility as mature uvomorulin, but the cell adhesive properties were not activated (data not shown).

Discussion

From the present work we conclude that a major biological function of endogenous proteolytic processing is to create an active adhesive form of uvomorulin. We show that substitutions in the putative recognition site effectively inhibit the intracellular proteolytic processing of the precursor polypeptides. Unprocessed polypeptides can be found in roughly similar amounts to normal uvomorulin on the surface, demonstrating that intracellular transport and cell surface expression of uvomorulin can occur in the absence of proteolytic processing. Unprocessed uvomorulin also associates with catenins and the mature part of the proteins show the typical Ca²⁺-dependent resistance to trypsin digestion.

However, cells expressing the unprocessed form exhibit no uvomorulin-dependent cell aggregation. Proteolytic removal of precursor segments results in the activation of adhesive properties, demonstrating that the presence of the precursor segment suppresses the cell adhesion function. More important, correct proteolytic cleavage is necessary for this activation, since cleavage at the wrong position(s) does not activate adhesive properties. Remarkably, incorrect proteolytic cleav-

age can generate a 120-kD protein similar in molecular size to mature uvomorulin but does not initiate adhesiveness. Most likely residual small peptides exhibit inhibitory effects. This suggests that the amino-terminal region of mature uvomorulin is of critical importance for adhesiveness.

The molecular mechanisms of cell adhesion mediated by uvomorulin, or Ca²⁺-dependent CAMs in general, are not known, although a homophilic interaction is generally accepted. We have previously shown that the extracellular part of uvomorulin is largely composed of three domains with two putative Ca²⁺-binding sites in each domain at precise positions (Ringwald et al., 1987). The domain structure is most likely essential for the generation of correct protein conformation and the Ca²⁺-dependent resistance to proteolytic degradation. The amino-terminal 27 amino acid residues of uvomorulin are not part of this domain structure but exhibit a 74% identity to chicken L-CAM and a 63% identity to chicken N- and mouse P-cadherin suggesting some functional significance of this region (Kemler et al., 1989). Our results strongly support this notion which led us to suggest that the amino-terminal region contains an active site for the inter-molecular interaction. The presence of even small fragments of the precursor segment may prevent successful interaction either by steric hindrance or by masking a recognition site.

These results have to be seen in the light of recent attempts to localize the specificity determining sites of P- and E-cadherin with the use of chimeric molecules between both CAMs (Nose et al., 1990). This work demonstrated clearly that the binding specificities of cadherins are located in the amino-terminal 113 amino acid residues. However, the results did not allow the determination of a single site within that region that could mediate binding specificity. The authors discuss their results by suggesting either multiple binding sites or that the binding specificity is determined by the conformation of the amino terminal region unique for each CAM. The latter interpretation is in good agreement with the observations presented here.

In the light of our work, the following sequence of events can be considered: Uvomorulin is synthesized as a precursor polypeptide, whose major function might be to prevent intracellular homophilic interaction. Proteolytic processing creates the active form of mature uvomorulin by conformational changes leading to alterations in and/or the exposure of active binding sites. Alternatively, proteolytic processing could induce self-association of uvomorulin polypeptides into oligomeric structures with improved binding properties.

During this study it became apparent that different proteases can generate 120-kD proteins that by their electrophoretic mobility are indistinguishable from normal uvomorulin but which are not functional. This observation might have broader biological implications. Uvomorulin is the major adhesive component in the formation and the maintenance of epithelial cell interaction and has been thought to act as a kind of a glue to preserve the integrity of an epithelial sheet (Boller et al., 1985; Gumbiner et al., 1988; Kemler et al., 1989). Changes in uvomorulin-mediated adhesion might be one prerequisite in the multistep mechanism of carcinogenesis where epithelial cells acquire invasive and metastatic properties and examples of highly metastatic cells with a low amount of uvomorulin/E-cadherin have been reported (Hashimoto et al., 1989).

However, increased metastatic ability is not always accompanied by a decrease of uvomorulin/E-cadherin (Shimoyama et al., 1989). Our results have shown that incorrect proteolytic cleavage of uvomorulin precursor polypeptides give rise to inactive proteins that have the same apparent molecular weight as the normal active protein. Such a mechanism could also occur during carcinogenesis. Highly metastatic cell lines have been shown to have elevated levels of proteases including chymotrypsin like proteases (Lowe and Isaacs, 1984; for review see Nicolson, 1989). It may be possible that malproteolytic cleavage of uvomorulin precursor polypeptides could generate an inactive form that stably expressed on the cell surface allows cells to escape from their adhesive environment.

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