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Multimerisation of A disintegrin and metalloprotease protein-17 (ADAM17) is mediated by its EGF-like domain

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ABSTRACT

A disintegrin and metalloprotease protein 17 (ADAM17) is a transmembrane zinc dependent metalloprotease. The catalytic activity of the enzyme results in the shedding of a broad range of membrane proteins. The release of the corresponding ectodomains induces a switch in various physiological and pathophysiological processes. So far there is not much information about the molecular mechanism of ADAM17 activation available. As for other transmembrane proteases, multimerisation may play a critical role in the activation and function of ADAM17. The present work demonstrates that ADAM17 indeed exists as a multimer in the cell membrane and that this multimerisation is mediated by its EGF-like domain.

1. Introduction

The zinc dependent transmembrane membrane metalloprotease ADAM17 (a disintegrin and metalloproteinase 17) is responsible for the shedding of a large number of substrates, so far more than 75 are described [1]. Among these proteins are growth factors, like TGF-α and adhesion molecules, like ICAM-1 as well as cytokines and cytokine receptors, like Tumor Necrosis Factor-α (TNF-α) nd the interleukin-6 receptor (IL-6R) [1]. Shedding of these proteins results not only in the release of their ectodomains from the cell surface, but also in the generation of soluble mediators, like cytokines and agonistic receptors like TNF-α [2,3] and the IL-6R, respectively [4]. The broad substrate spectrum reflects the importance of ADAM17 as a key regulator in various physiological and pathophysiological processes, like development, inflammation and cancer progression. The importance of this enzyme is underlined by the fact that ADAM17 knockout mice are embryonic lethal [5].

The knowledge about the activation and function of ADAM17 is still incomplete. Members of the ADAM-family are multi-domain type-I transmembrane proteins with an extracellular part consisting of up to five extracellular domains; a pro-, a catalytic-, a disintegrin-, a cysteine rich- and an Epidermal Growth Factor- (EGF-)like-domain [6]. It is well known that the pro-domain is needed for the proper folding and inhibition of the catalytic domain. Disintegrin domains are involved in cell-adhesion [7], whereby the disintegrin domain of ADAM17 is able to interact with α5β1 integrin [8]. Furthermore, disintegrin domains are supposed to act as a scaffold for its C-terminal neighboring domain [6]. ADAM17 and ADAM10 are atypical members of the ADAM-family due to their lack of a cysteine-rich domain whose function is probably taken over by their EGF-like domains [6,9]. It has been supposed that the cysteine-rich-, or in case of ADAM10 and ADAM17 the EGF-like-domain, are participating in activation, thereby supporting the removal of the pro-peptide from the catalytic domain [10] and in the specific recognition of substrates [9,11]. Also the transmembrane region has been described to participate in the interaction with substrates [12]; whereas the cytoplasmic region is known to be involved in regulatory events [1,13,14]. Members of the meprin- and membrane-type matrix metalloproteases (MT-MMPs) are, like the ADAMs, multi-domain transmembrane zinc depended metalloproteases and members of the metzincins superfamily. All members of this superfamily contain a conserved (HEXXHXXG/NXXH/D) consensus sequence in the active site of the catalytic domain which coordinates the zinc ion [15]. Members of the Meprins and MT-MMPs exist as multimers. Meprins are known to generate disulfide linked homo- or heterodimers. The mature meprin-α subunit is not membrane anchored and forms high aggregated protease complexes in solution [15–17]. The dimerisation of the meprin-α subunits with the meprin-β subunit leads to the generation of disulfide-linked membrane anchored heterodimers of meprin A. This heterodimer tends to further non-covalent aggregation leading to membrane anchored tetramers. In contrast, meprin B consistent of two meprin-β subunits and exist

Abbreviations: ADAM17, a disintegrin and metalloprotease 17; TNFα, tumor necrosis factor α; IL-6R, interleukin-6 receptor; DN-ADAM17, dominate-negative ADAM17; GPI, glycosylphosphatidylinositol; Mef, mouse embryonic fibroblasts.

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Table 1
Oligonucleotides used for cloning of ADAM17 variants.

Construct	Origine	Tamplate	Tag	Primer 5′	Primer 3′	Tag Primer 5′	Tag Primer 3′
flulllength ADAM 17	murine	ADAM17	Pc	GGGGTACCATGAGGCGGCGTCTCC	CGGGATCCGCACTCTGTCTCTTTGCTGTCAACTCG	GATCCGATTACAAGGATGACGACGA TAAGTGAGC	GGCCGCTCACTTGCCATCGATCAGCCGTG GATCGACCTGGTCTTCG
flulllength ADAM 17	murine	ADAM17	FLAG	GGGGTACCATCGAGGCGGCGTCTCC	CGGGATCCGCACTCTGTCTCTTTGCTGTCAACTCG	GGCCGCTCACTTGCCATCGATC AGCCGTGGATCGACCTGGTCTTCG	GGCCGCTCACTTATCGTCGTCATCCTTGTAATCG
ADAM17 GPI	murine	ADAM17	PC	GGGGTACCATGAGGCGGCGTCTCC	GCCGTGGATCGACCTGGTCTTCGGATCCGTC AATGAAATCCCAAAATCGC	CGGAATTCGCTCAGCTCCTTGCCAT CGATCAGC CGTGGATCGACCTGG	
DN-ADAM17 Δ cyto	humane	DN-ADAM17	HA	GGGGTACCGCCACCATGAACTC CTTCTCC	ATAGTTTAGCGGCCGCTTTATCCAATTTCTTA TCCACACAATGG	GGCCGCTCAAGCGTAATCCGG AACATCGTA TGGCTAG	GATCCTACCCATACGATGTTCCGGATTACGCT TGAGC
DN-ADAM17 GPI	humane	DN-ADAM17	HA	GGGGTACCGCCACCATGAACTC CTTCTCC	CCGCGATCCCTCGTCAATGAAATCCCAAAATCG		
DN-ADAM17 Δ Dis	humane	DN-ADAM17	Myc	TCCGCTCGAGTTCTGCGACAGG GAACAGC	CCCAAGCTTTCAGCACTCTGTTTCTTTGCTGTC AACACG	GATCCTACCCATACGATGTTCCG GATTACGCTGAGC	TCAGCTCAGCGTAATCCGGAACATCGTAT GGGTAG
DN-ADAM17 Δ Dis-GPI	humane	DN-ADAM17	HA	TCCGCGGCCCTTCTGCGACAGG GAACAGC	CGCGGATCCCTCGTCAATGAAATCCCAAAATCG	GATCCTACCCATACGATGTTCCG GATTACGCTGAGC	TCAGCTCAGCGTAATCCGGAACATCGT ATGGCTAG

as a homodimer in the plasma membrane [16,17]. Also MT1-MMP is a multimer as the formation of dimers is mandatory for its biological activities, like proMMP2 activation and collagen degradation [18]. The close relation of those multi-domain proteases to ADAM17 and the fact that ADAM17, lacking the catalytic domain, the so called dominant-negative (DN−) ADAM17, acts as inhibitor of the wild-type molecule [19], implicate that ADAM-proteases might also exist as dimers or multimers. In order to study the multimerisation of ADAM17 differently tagged molecules were generated and used for co-immunoprecipitation experiments. The resulting data show that ADAM17 indeed exists as multimer. By using different deletion variants of ADAM17, we identified the EGF-like domain as the minimal requirement for multimerisation.

2. Material and methods

2.1. Generation of C-terminal tagged full-length ADAM17 and deletion variants

The murine full-length ADAM17 cDNA was amplified by PCR, flanked 5′-end by a KpnI and 3′-end by a BamHI restriction site. Oligonucleotides encoding the PC- or FLAG-tag (Table 1) containing a 5′-end BamHI- and a 3′-end NotI restriction site were phosphorylated and hybridized (Eurofins MWG Operon, Germany). Both fragments were ligated into pcDNA3.1 neo (+) digested with KpnI and NotI (Fermentas, St. Leon-Rot, Germany). The resulting vectors were used to generate the C-terminal tagged deletion variants.

In order to generate HA-tagged variants the C-terminal tag was removed using BamHI and NotI restriction enzymes, and oligonucleotides coding for the HA-tag and the respective restriction sites were ligated into these vectors (Table 1).

DN-ADAM17 was used as template for constructs lacking the metalloprotease domain [20]. The DN-ADAM17ΔDis construct containing a C-terminal myc-tag (Table 1) was cloned behind the IL-6R signal peptide into pcDNA3.1 zeo(−) using XhoI and HindIII restriction enzymes (Fermentas, St. Leon-Rot, Germany).

Glycosylphosphatidylinositol (GPI) anchored variants were cloned into pcDNA3.1 (+), which comprises the coding sequence for a myc-tag and the GPI-signal sequence of TRAIL behind a KpnI and a BlpI restriction sites. To introduce an additional tag in front of the GPI anchor, the sequence of a PC-tag flanked 5′-end by a BamHI- and 3′-end by a BlpI site was attached to the extracellular domain of ADAM17 by two successive PCR reactions (Table 1), and cloned into the GPI-vector using KpnI and BlpI sites. The resulting plasmid was used for cloning GPI deletion variants between KpnI and BamHI sites, and for introduction of the HA-tag between BamHI and BlpI sites.

2.2. Co-immunoprecipitation

HEK-293 cells and Mef$^{ADAM17ex/ex}$ [20] were cultured in DMEM high-glucose containing 10% fetal calf serum (FCS), penicillin (60 mg/l) and streptomycin (100 mg/l) (PAA Laboratories, Marburg, Germany). Transfection was performed using 15 µl Turbofect (Fermentas, St. Leon-Rot, Germany) and 5 or 6 µg DNA (HEK296 or Mef$^{ADAM17ex/ex}$) according to manufacturer's instructions. For co-transfection experiments 2.5 µg or 3 µg (HEK296 or Mef$^{ADAM17ex/ex}$) of each construct was used. After transfection the cells were cultured for 2 days in DMEM high-glucose containing 5 % FCS, harvested, lysed in 250 µl lysis buffer containing 20 mM Tris/HCl pH 7.6, 150 mM NaCl, 2 mM EDTA and "complete" protease inhibitor mixture without EDTA (Roche applied science, Mannheim, Germany) and afterwards centrifuged for 15 min at 14.000 rpm. For co-immunoprecipitation experiments the lysates were divided into three parts. Two times 100 µl were mixed with