ROMANIAN ACADEMY
INSTITUTE OF BIOCHEMISTRY

Ph.D. THESIS SUMMARY

- STRUCTURAL DOMAINS OF EDEM3 AND THEIR ROLE IN ERAD -

SCIENTIFIC COORDINATOR:
DR. ŞTEFANA MARIA PETRESCU

Ph.D. CANDIDATE:
LAURA-GEORGIANA MĂNICA

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TABLE OF CONTENTS (Ph.D. thesis in extenso)

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Firstly, I would like to acknowledge my supervisor, Dr. Ștefana Petrescu, for all her support. I really appreciate the opportunity of conducting my doctoral thesis in the Institute of Biochemistry of the Romanian Academy. I am thankful for the trust, professionalism and patience that I was granted along these years.

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AIMS OF THESE STUDIES

This paper aims to unravel the role of EDEM3 (**E**R **D**egradation-**E**nhancing α-**M**annosidase-like protein **3**) in the endoplasmic reticulum associated degradation (ERAD), by understanding the function of its structural domains.

Being described most of the times through its mannosidase activity, EDEM3 was considered a redundant protein of the glycosyl-hydrolase 47 family (GH47). However, recent studies indicate that EDEM3 has some physiological roles, like androgen cellular signaling or modulation of plasma triglycerides levels. At the base of these observations may lay precisely its unique structure that is composed of three functional domains: a GH47 mannosidase-like domain, a protease associated domain and an intrinsically disordered domain.

In order to verify the relationship between structure and function, I used a series of truncated EDEM3 proteins, obtained previously by deletion of the functional domains. My main two objectives were to investigate the effect of this constructs over the degradation of some well-known ERAD substrates and to discover their involvement in establishing molecular complexes in ERAD. The third objective was to investigate EDEM3 role in other cellular processes. My final objective was to develop a high-performance anion exchange-pulsed amperometric detection method in order to identify and quantitate N-glycans. As a result, I employed a series of experimental approaches such as cell culture techniques, molecular biology, mass spectrometry and anion exchange chromatography.

The paper contains four different chapters. In **INTRODUCTION** I described the mechanisms composing endoplasmic reticulum quality control of protein folding: hydrophobicity evaluation and classical chaperones, the activity of oxidoreductases and the impact of N-glycans conformation. Further, I presented the key steps of ERAD, starting with recognition of misfolded proteins and finishing with their proteasomal destruction. I also detailed the role of mannosidases in ERAD, especially the one of EDEM3.

The second chapter, **MATERIALS AND METHODS** contains two distinct parts, as its name denotes. In Materials, I listed all the equipment, laboratory consumables and chemical substances that were used. In Methods I described the methodology that was applied for each experimental approach. All this research was in compliance with good laboratory practice guides.

I structured the chapter **RESULTS** according to the main objectives of this thesis. In order to understand the relationship between EDEM3 conformational domains and its function, firstly I determined the properties of the EDEM3 constructs in an EDEM3-KO cell line. Secondly, I addressed their effect over the mannosidase processing and degradation of three ERAD substrates, namely ST, NHK and pCiL. I also investigated the association between EDEM3 mutants and these substrates trying to gain insight into the role of each functional domain. Next, I analyzed the EDEM3 constructs in the larger context of ERAD, looking both at EDEM3 cooperation with other mannosidases and at EDEM3 interactions with other members of ERAD. Moreover, I described EDEM3 implications in cellular functions like cell migration and invasion. Finally, I developed a high-performance anionic exchange-pulsed amperometric detection method which helped me to identify and quantitate N-glycans. This method can be used in glycosylation evaluation both in *in vivo* and *in vitro* systems.

The last chapter, **CONCLUSIONS**, resumes the principle observations made in this study and positions EDEM3 among the essential members of ERAD.

INTRODUCTION

Endoplasmic reticulum (ER) quality control (ERQC) of protein folding may be divided into three categories, depending on the property that is evaluated: (1) assessment of the hydrophobic character of the protein chain; (2) assessment of oxidoreductases activity; and (3) assessment of N-glycans conformation. Although they have different characteristics, these three processes act together in order to maintain the efficiency of protein folding and trafficking, starting from the moment of their insertion into the ER lumen.

N-glycosylation is the most common form of glycosylation and occurs in the ER. This gives the protein molecule increased solubility and increased folding kinetics [42,43]. N-glycans can also determine the function of the protein [44-46]. Moreover, as folding progresses, the attached glycans are processed by various enzymes and interact with ER resident lectins. The evaluation of the newly acquired conformation at each stage plays an important role in glycoprotein quality control.

When the protein is inserted into ER lumen at the level of the Sec61 translocon, the oligosaccharide-transferase complex (OST) recognizes the asparagine-X-serine/threonine sequence, where X can be any amino acid, except proline. Here, the OST transfers *en bloc* the preformed N-glycan [49]. The process can take place both co- and post-translationally through the catalytic subunits STT3A and STT3B, respectively.

Short after completion of N-glycosylation, the newly attached glycan undergoes a series of enzymatic reaction that dictate his fate in the secretory pathway (Figure 1). The triglucosylated form has a half-life of a few seconds, glucosidase I (GI) removing a terminal glucose residue. The resulting diglucosylated form (G2M9) is the ligand for malectin [50], a lectin with an increased cellular level in ER stress. The second carbohydrate residue is processed more slowly by glucosidase II (GII). The monoglucosylated form (G1M9) is subsequently recognized by the lectin couple calnexin-calreticulin (CNX-CRT) [53,54]. This association increases the folding efficiency by preventing protein aggregation, aberrant oligomerization or premature degradation of folding intermediates.

The removal of the last glucose residue by GII leads to the dissociation of the substrate from the CNX-CRT complex, as they have low affinities for the non-glucosylated form (M9). The folding status is questioned by UDP-glucose glycoprotein-glucosyl transferase (UGGT) [57,58]: if the protein is correctly folded, it can be transported to the Golgi apparatus; if the protein is misfolfed or unfolded, UGGT reattached a α1-3Glc residue to the A branch of the glycan.

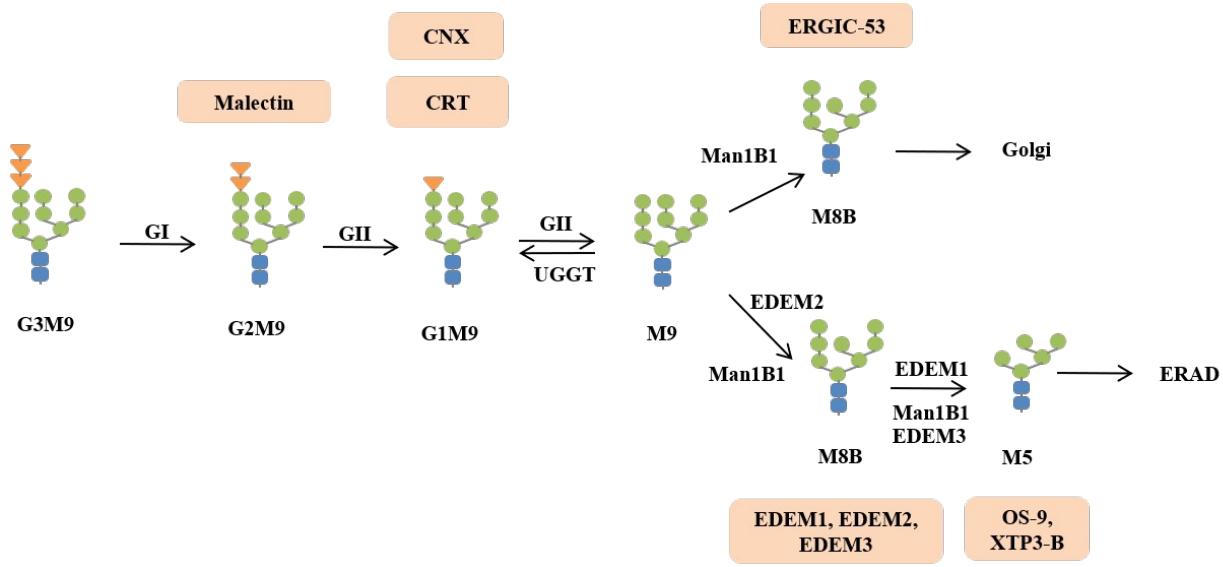


Figure 1. ER lectins and their role in ERQC. Glucosidase I (GI) removes the first glucose residue a few seconds after the transfer of the N-glycan to the surface of the protein. The diglucosylated form may interact with malectin. Glucosidase II (GII) removes the second glucose residue and the monoglucosylated form enters the calnexin-calreticulin (CNX-CRT) cycle. GII also removes the last residue of glucose, which leads to the dissociation of the

CNX-CRT substrate and the UGGT can now interrogate the folding status. If the protein is properly folded, Man1B1 processes branch B by trimming a mannose residue. The protein in question can interact with ERGIC-53 and be transported to the Golgi apparatus. If the protein in misfolded or unfolded, UGGT adds a molecule of glucose and the CNX-CRT cycle may resume. When the substrate is definitively marked as incorrectly folded, EDEM proteins process the polysaccharide and deliver the substrate to other lectins, OS-9 and XTP3-B. The latter send the protein to degradation by ERAD. For simplicity, NAcGlc residues were omitted in the names of the glycans.

Once re-glucosylated, the substrate can re-enter the CNX-CRT cycle, the process being repeated until the protein folds or is marked for degradation [61]. However, it is unclear how this works. It is considered that there is a time frame in which a given glycoprotein can fold. This hypothesis is also supported by the slow action of mannosidases, compared to that of glucosidases.

If the protein is correctly folded, the ER mannosidase I (ERManI/Man1B1) removes a mannose residue located on the B branch, giving rise to the M8B form. The substrate can now interact with a lection protein that is also a sorting receptor at the ER-Golgi interface, ERGIC-53 [62,63]. Finally, the proteins are packed into transport vesicles using the COPII machinery and sent to the Golgi apparatus [64]. In contrast, misfolded proteins generate stress and trigger the unfolded protein response (UPR), which leads to ERAD.

Degradation of ERAD substrates begins with their recognition and targeting as misfolded proteins [68]. Initially, the removal of a single mannose residue from the N-glycan structure was thought to be sufficient for degradation [69]. However, it was shown that a substrate has to expose an α-1,6 mannose on the C branch [70] to be recognized by the ERAD machinery. The enzymatic process can continue until the removal of four mannose residues [71]. A series of other proteins are involved in this mechanism, namely chaperones (BiP), lectins (OS9, XTP3-B), mannosidases (Man1B1, EDEM), transmembrane protein complexes (Derlin1) and ubiquitin ligases (HRD1).

The central dogma has changed over time, today EDEM2 (ER degradation-enhancing α-mannosidase-like protein 2) being considered the initiator of ERAD. Ninagawa et al. [117] demonstrated that EDEM2 removes the first mannose residue from branch B and EDEM3 and EDEM1 process the N-glycan from M8B to M5. The authors name this two stages as rate-limiting reactions, the onset of ERAD being dependent on EDEM3 activity. It is known that the EDEM proteins are active both *in vivo* and *in vitro* [118, 119].

Described for the first time in 2006, EDEM3 has long been regarded as the classical mannosidase. It consists of 931 amino acids (Figure 2), preserving the GH47 mannosidase-like domain and showing a KDEL domain for ER retention. EDEM 3 overexpression accelerates the degradation of NHK and TCRα. Yu et al. [118] have identified ERp46 as the determinant of EDEM3 action. The two proteins interact through a disulfide bond, which leads to changes in the conformation of the mannosidase-like domain of EDEM3 and to the onset of its activity.

Some unexpected physiological implications have also been described for EDEM3. In its structure there is a protease associated domain (PA) located between amino acids 674-779 (Figure 2). In general, PA domains are involved in establishing protein-protein interactions [141]. However, Xu et al. [142] assigned for this domain a role in lipoprotein regulation. A genetic mutation in the P746S position leads to decreased blood triglyceride levels due to increased expression of the LRP1 receptor (Low density lipoprotein Receptor-related Protein 1).

Figure 2. Comparative structures of EDEMs. The MLD denotes the mannosidase-like domain, the PA stands for the protease associated domain. The yellow rectangle represents the transmembrane domain of EDEM1, the blue triangles are the intrinsically disordered domains of EDEM1, while the black rectangles are the signal peptide of EDEM2 and EDEM3.

In prostate adenocarcinoma cells LNCaP, regulation of EDEM3 expression is modulated by androgen hormone levels [143]. Androgen treatment increases both the total amount of glycosylated proteins and the expression of EDEM3. This observation has direct implication in the progression of prostate cancer, admitting that cell adhesion, migration, signaling and metabolism of cancer cells are dependent on protein glycosylation. In turn, EDEM3 silencing affects the viability of this cells.

Kofanova et al. [144] propose another unique use for the EDEM3 gene, namely that of biomarker of peripheral mononuclear blood (PBMC) cell quality assessment. These cells are

used in the diagnosis of inflammatory and infectious diseases. In order to provide a correct answer to the investigator, they must be maintained in optimal conditions before experimental manipulation. Thus, the authors indicate the EDEM3-interleukin 8 system as a possible biomarker in addressing the quality of PBMC maintained for a long time at room temperature in various anticoagulant systems.

It is therefore understood that EDEM3 is a key player in ERAD, being both an active mannosidase and an ER stress modulator. EDEM3 structure guides the degradation of misfolded proteins and contributes to the regulation of physiological processes. As a result, I investigated in this paper the role of EDEM3's structural domains in ERAD.

RESULTS

The main objective of this study was to decipher the relationship between the functional domains of EDEM3 and its physiological role in the context of ERAD. To this end, I studied a series of truncated EDEM3 proteins obtained previously in the laboratory. By sequential deletion of the structural domains of EDEM3, the following proteins were obtained: ΔPA, lacking the PA domain; ΔIDD, lacking the IDD domain; ΔMAN, lacking the mannosidase-like domain and MAN, selectively expressing the mannosidase-like domain. For all, the signal sequence and the KDEL domain were maintained. In addition, a tag (HA) was introduced in order to facilitate the identification of the proteins by various biochemical methods.

EDEM3 CONSTRUCTS CHARACTERIZATION

Firstly, I described EDEM3 mutants as stably expressed proteins in two cell lines, at the predicted molecular mass. They also showed post-translational modifications specific of the ER, such as oligomannosidic N-glycans and disulfide bridge formation. Furthermore, all EDEM3 constructs colocalize with ER resident proteins, calnexin and SeL1L.

EDEM3 CONSTRUCTS AND THEIR EFFECT UPON ERAD SUBSTRATES

Next, I studied the effect of EDEM3 constructs over the degradation of three ERAD substrates. The first one investigated was soluble tyrosinase (ST), a truncated mutant of tyrosinase. I transfected the corresponding plasmids in a EDEM3-KO cell line for 48h. Following Western blot or pulse chase analysis (Figure 3), I identified the mannosidase-like domain as the main responsible for ST mannose processing and degradation. Suprisingly, deletion of PA or IDD domains was not vital for protein function. However, there is an increase in the half-life of ST in the presence of ΔPA compared to WT EDEM3. The singular expression of MAN led to an unexpected phenomenon: a mutant capable of degrading an ERAD substrate even in the absence of obvious mannosidase activity.

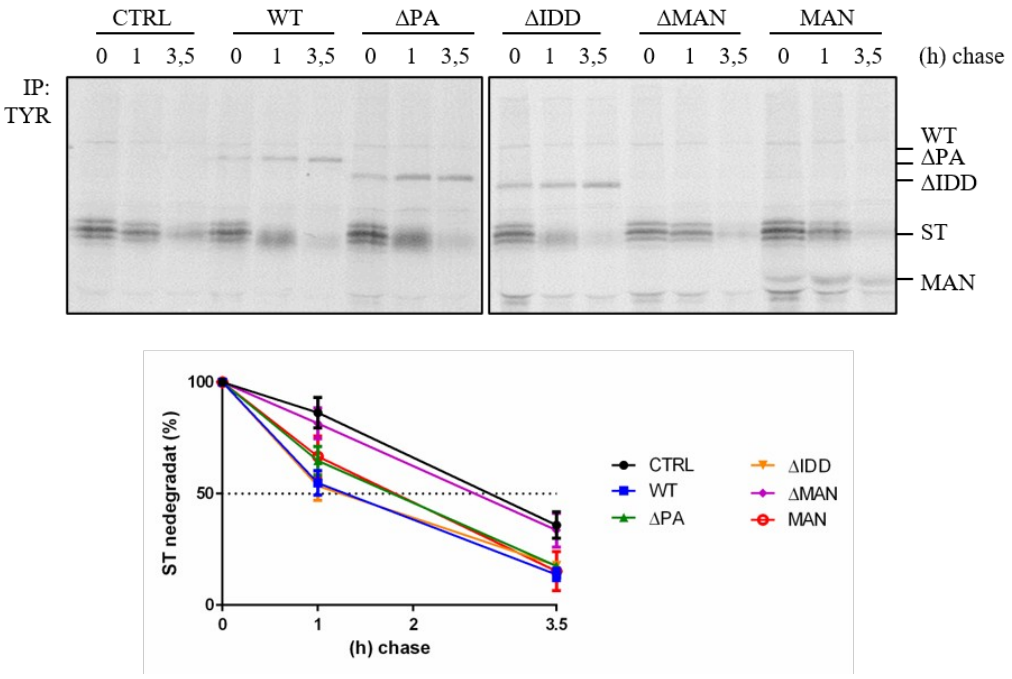
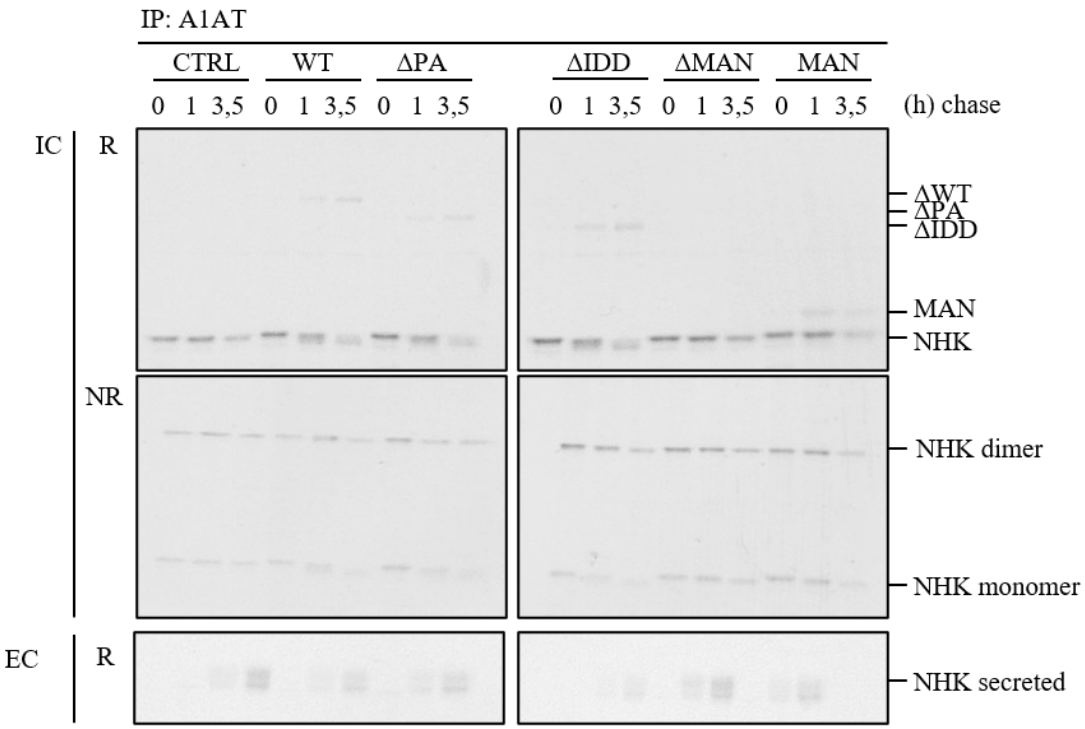


Figure 3. ST degradation in the presence of EDEM3 constructs. EDEM3-KO cells were co-transfected to express ST and EDEM3 constructs. They were starved, pulsed and chased for the indicated time points. Cellular lysates were incubated over-night with anti-TYR antibodies and the resulting immunocomplexes were separated by SDS-PAGE. Results were visualized by autoradiography. ST degradation rate (%) is represented as (mean ± SD), where n=3.

The second investigated substrate was the truncated variant of alpha-1-antitrypsin, Null Hong Kong (NHK) (Figure 4). The extracellular level reached by the ERAD substrate is consistent with the activity of EDEM3 constructs. It appears that the IDD domain plays an important role in NHK degradation and secretion, providing a negative feedback on the processing of this substrate. In its absence, NHK is efficiently retained in ER, only a small being amount available for secretion. Moreover, the secretion rate decreases significantly compared to control sample. Similarly, the WT, ΔPA and MAN forms decrease NHK secretion, supporting their degradative activity and ability to retain the substrate in the ER. On the other hand, ΔMAN construct allows the secretion of NHK, an expected phenomenon given the inactivity of this protein. NHK dimers are formed regardless of the enzymatic activity of EDEM3. They are present from the start of the experiment and remain stable during the investigated time interval.



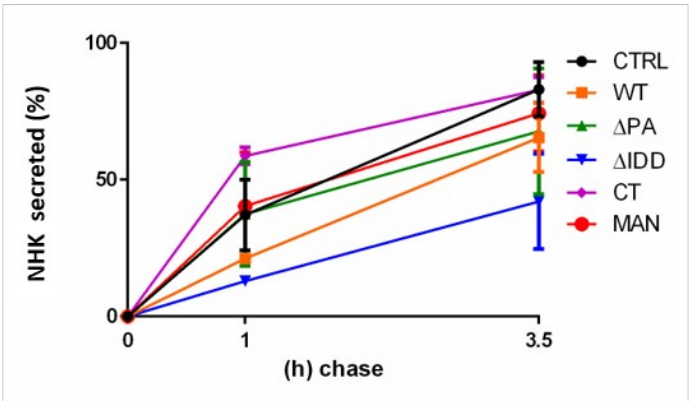


Figure 4. NHK degradation in the presence of EDEM3 constructs. EDEM3-KO cells were co-transfected with EDEM3 constructs and NHK plasmids for 48h. Cells were starved, pulsed and chased for the indicated time points. The cellular media was also collected. Both cell lysates (IC, intracellular) and cell media (EC, extracellular) were incubated over-night with anti-A1AT antibodies and the resulting immunocomplexes were separated in reducing (R) and non-reducing (NR) conditions. NHK secretion rate (%) was quantified and represented as (mean ± SD), n=3.

EDEM3 CONSTRUCTS AND THEIR ASSOCIATION WITH ERAD SUBSTRATES

By analyzing Figures 3 and 4, I detected an early association of EDEM3 mutants with ST and NHK, except for the ΔMAN mutant. The seems to be an upward trend in the association between the two substrates and WT, ΔPA and ΔIDD. The interaction between the enzyme and the substrate increases in intensity over time. The same observation has been made in the past by other authors for EDEM1. In contrast, the MAN mutant possesses a different pattern of association, the interaction reaching a maximum at 1h chase and then decreasing in magnitude. Consequently, I studied this association profile by immunoprecipitation and Western blot (Figure 5).

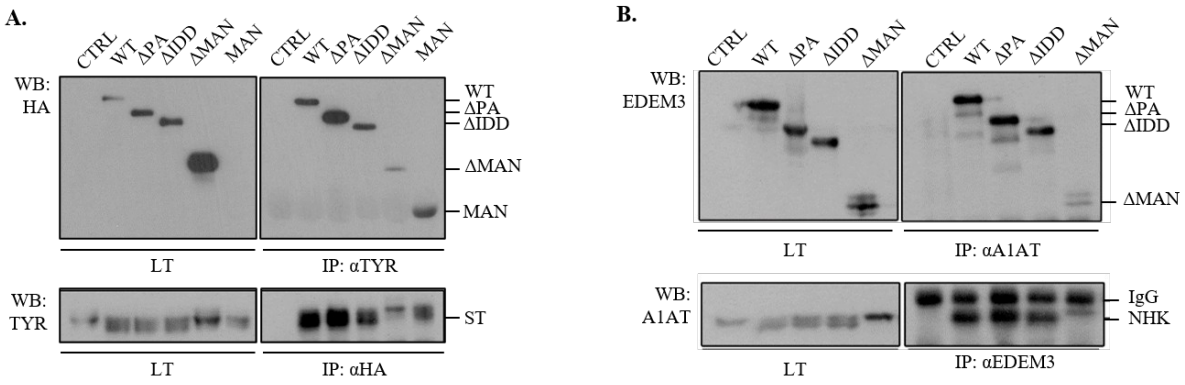


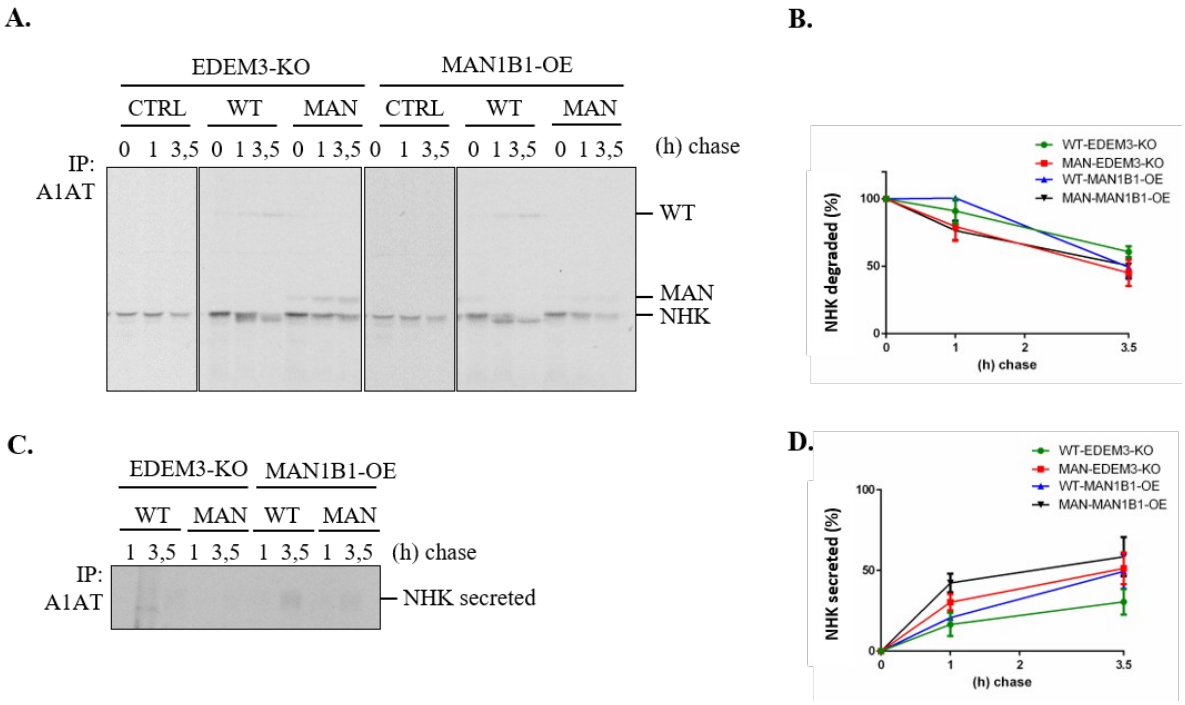
Figure 5. EDEM3 constructs association with ST and NHK. EDEM3-KO cells were co-transfected with corresponding plasmids for 48h. Cells were lysed and processed for immunoprecipitation with anti-HA and anti-TYR antibodies (**A**) or with anti-EDEM3 and anti-A1AT antibodies (**B**). Results were visualized by chemiluminescence. LT stands for total lysates.

Summarizing the observations of all these experiments, I identified the mannosidase-like domain as the main responsible for the association of EDEM3 with misfolded proteins. Sequential deletion of PA and IDD does not influence the ability of EDEM3 to interact with ERAD substrates. Moreover, mannosidase-like domain itself can engage in the establishment of protein-protein interactions. However, there is a difference in the dynamics of this process, supporting a lectin type association mediated by EDEM3. The advanced processing of mannose residues leads to an increase in the intensity of interactions, suggesting the importance of the enzymatic activity of EDEM3 on the establishment of stable complexes within ERAD.

ROLES OF EDEM3 CONSTRUCTS IN ERAD

In this chapter I set out to study the involvement of EDEM3 in the broader context of ERAD. I investigated both its collaboration with other mannosidases of the GH47 family and its association with key ERAD proteins using mass spectrometry.

Unexpectedly, co-expression of EDEM3 and Man1B1 did not lead to accelerated degradation of the ERAD substrates (Figure 6) compared to the single expression of EDEM3. The ΔMAN mutant does not cooperate with Man1B1, suggesting that the enzymatic activity of EDEM3 contributes to the functionality of the system. Rather, an improved secretion of NHK



was observed.

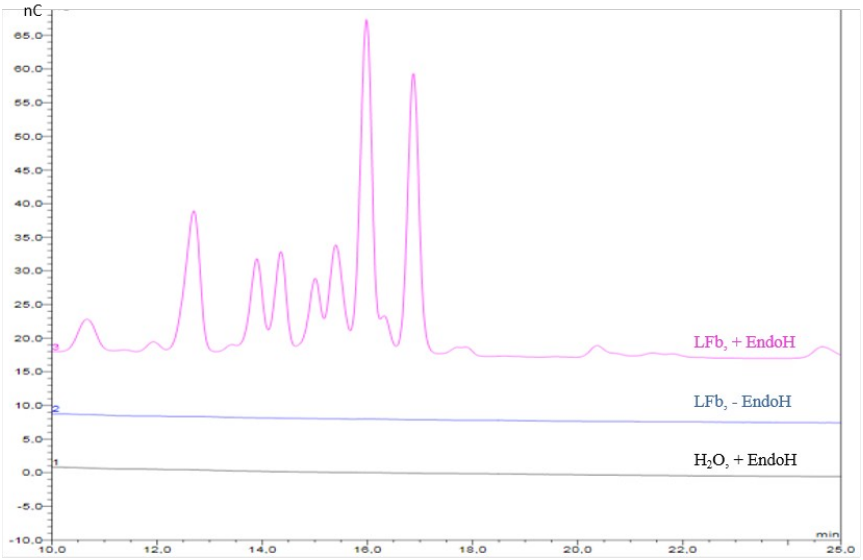
Figure 6. Pulse chase analysis of NHK processing by EDEM3 and Man1B1. (**A**) EDEM3-KO and MAN1B1-OE cells were co-transfected with the corresponding plasmids. Cells were starved, pulsed and chased for the indicated time points. Cell lysates were incubated over-night with anti-A1AT antibodies and the results were visualized by autoradiography. (**B**) The intracellular level of NHK from (A) was quantified and represented as (mean ± SD), n=3. (**C**) Same as in (A), except harvested cell media was used. (**D**) The extracellular level of NHK from (C) was quantified and represented as (mean ± SD), n=3.

EDEM3 has an unusual profile of interactors. First, the used experimental design influenced the quantity of extracted proteins from cell lysates. Additionally, the strength of the lysis buffer led to the maintenance or loss of proteins interactions. Using mass spectrometry, I identified EDEM3 interactors involved rather in protein folding. The association with SeL1L or calnexin remains unclear, EDEM3 having probably a specific role in ERAD. Moreover, supplementary co-immunoprecipitation experiments have strengthened previous observations regarding the unpredictability of the observed associations.

DEVELOPMENT OF A HPAE-PAD METHOD FOR N-GLYCANS IDENTIFICATION AND QUANTIFICATION

The last objective of this paper was to develop a technique that allows rapid analysis of EDEM3 mannosidase activity by identification and quantification of high-mannose N-glycans. Here, I laid the foundation of a high-performance anion-exchange chromatography method, coupled with pulsed amperometric detection (HPAE-PAD).

With the help of the developed method I managed to separate the high-mannose N-glycans of a purified protein, namely bovine lactoferrin (Figure 7). I tested the same methodology on a cell lysate, in an attempt to determine its glycosylation profile. However, a



Column: Dionex CarboPac PA100, ⌀2 mm. **Eluent**: 100 mM NaOH + 250 mM NaOAc in 100 mM NaOH, 20 °C, 0.3 mL/min. **Injection volume**: 10 µL. **Detection:** Disposable gold working electrode, Au-PTFE, coupled with reference electrode Ag/AgCl. **Chromatographic system**: ICS-5000 HPIC, software Chromeleon 6.8.

number of optimization steps are needed regarding the extraction protocol of N-glycans from cellular lysates.

Figure 7. High-mannose N-glycans profile of bovine lactoferrin identified by HPAE-PAD.

CONCLUSIONS (Ph.D. thesis in extenso)

Acquiring a native conformation is indispensable for the functionality of proteins. Despite sustained cellular effort, many proteins that passes through the ER fail in attaining a native form. In order to avoid the accumulation or aggregation of incorrectly folded proteins, they are eliminated by ERAD, following a process of selection, transport and dislocation to the cytosol.

In this paper I aimed to elucidate the contribution of EDEM3 in ERAD. Although it has been described as a redundant protein, it has some peculiarities compared to the other members of the GH47 family. Previous work in my laboratory lead to the identification of three distinct functional domains in its structure: a mannosidase-like domain, a protease associated domain, and an intrinsically disordered domain. Moreover, it is the only GH47 mannosidase that possesses a KDEL domain.

Thus, to achieve the purpose of this work, I used a series of truncated EDEM3 proteins obtained by sequential deletion of its structural domains. The resulting constructs were the following: ΔPA, which does not express the PA domain; ΔIDD, which does not express the IDD domain; ΔMAN, which does not express the MAN domain, and MAN, which does not possess PA and IDD, expressing solely the mannosidase-like domain. In all cases, the signal sequence and the KDEL domain were maintained and a molecular tag (HA) was additionally introduced.

The first step of the study was the characterization of the EDEM3 mutants. In order to be able to distinguish between the effects of the wild type protein, expressed endogenously by the cellular system, and the effects of mutant proteins, expresses exogenously by transient transfection, I generated an EDEM3-KO cell line. Here, I observed that EDEM3 constructs are stably expressed at the predicted molecular masses. They present similar expression levels, except for ΔMAN and MAN. However, the observed differences can be attributed to a faulty recognition pattern of the used antibody, as the folding of this constructs proceeds with the same efficiency. Moreover, I provided evidence to support the ability of the mannosidase-like domain to form disulfide bridges.

I also demonstrated that all EDEM3 mutants are degraded by the 26S proteasome, being in the same time glycosylated proteins. They are retained in the ER, being sensitive to EndoH trimming action and being co-localized with two ER resident proteins, calnexin and SeL1L.

Next, to attain one of the main objectives of the study, I investigated the effect of EDEM3 mutants over the degradation of three ERAD substrates. WT, ΔPA and ΔIDD proteins have similar mannose and degradative activity, while ΔMAN has a markedly diminished action. Surprisingly, the MAN protein, although it loses its enzymatic capacity, it retains the degradative one. Moreover, it seems that there is a substrate-dependent action of EDEM3 that highlights the subtle role of structural domains in the degradation of misfolded proteins. Specifically, the mannosidase-like domain becomes the main responsible for the activity of EDEM3 in ERAD. Its deletion leads to the inactivity of the molecule. The PA domain appears to be necessary for ST degradation, as its removal increases the half-life of the substrate for 30 minutes. In contrast, the IDD domain provides negative feedback on NHK degradation, its deletion accelerating both the degradation and the secretion of the substrate.

The mannosidase-like domain is also the main responsible for EDEM3 association with misfolded or unfolded proteins, the ΔMAN construct losing any ability to engage in a complex with them. Sequential deletion of PA or IDD does not influence the property of corresponding mutants to interact with NHK or ST. The mannosidase-like domain by itself can engage in establishing transient protein-protein interactions but with a different dynamic when compared with WT. The MAN construct reaches a maximum of association, after which the intensity decreases, suggesting the preference of EDEM3 for enzymatically processed ERAD substrates.

My second main objective was to highlight the relationship between EDEM3 mutants and other ERAD members. Regarding its cooperation with Man1B1, I observed that overexpression of these two mannosidases does not improve the accelerated degradation of ERAD substrates. This phenomenon may result from the blockage of the retrograde transport to the ER, taking into consideration the Golgi location of Man1B1. Furthermore, I have shown that EDEM3 is not redundant when compared to EDEM1 and EDEM2, the mannosidases collaborating in the degradation of NHK.

However, EDEM3 has an unusual profile of interactors. The experimental conditions influenced protein extraction efficiency, the strength of the lysis buffer leading to the maintenance or loss of protein interactions. Using mass spectrometry, I identified unexpected

proteins as possible interactors of EDEM3, such as BiP, ERdj6 or riboforin 1. In contrast, SeL1L or calnexin showed unstable association profiles.

The third objective, namely the involvement of EDEM3 in other cellular processes was achieved using the amelanotic melanoma cell line A375. EDEM3 expression decreases in hypoxia, contrary to EDEM2, influencing at the same time the level of HIF1α. Also, overexpression of EDEM3 improves cell migration, which points to the ability of EDEM3 in modulating the cancer cells signaling.

The last objective was to develop a HPAE-PAD method for the study of N-glycans conformation. I managed to determine the glycosylation of a purified protein. Nevertheless, this method may be used to characterize *in vivo* activity of a given mannosidase.

In conclusion, the results presented in this paper make a new contribution to the role of EDEM3 in ERAD. The structural domains of the protein have different properties, each regulating EDEM3 activity accordingly. The main original observations made by this study are:

- EDEM3 constructs are stably expressed in an EDEM3-KO cell line, being located in the ER;
- The mannosidase-like domain of EDEM3 can engage into disulfide bridges, its folding being sensitive to reducing agents;
- The mannosidase-like domain of EDEM3 is the main responsible for protein activity;
- The mannosidase-like domain of EDEM3 is able to degrade misfolded proteins even in the absence of its enzymatic activity;
- The PA and IDD domains are not vital for EDEM3 function but they confer substrate specificity, acting as positive or negative regulators;
- EDEM3 associates with ERAD substrates only through its mannosidase-like domain;
- EDEM3 association with ERAD substrates is a lectin-type association, the interaction intensity increasing with the trimming of the substrate;
- EDEM3 and Man1B1 do not collaborate in NHK degradation, but rather in NHK extracellular secretion;
- EDEM3 has a different response to hypoxia when compared to EDEM2;
- EDEM3 modulates cell migration and viability;
- The developed HPAE-PAD method may be successfully used in N-glycosylation studies.

LIST OF PUBLICATIONS

SCIENTIFIC ARTICLES

1. **Manica, G.**; Ghenea, S.; Munteanu, C.V.A.; Martin, E.C.; Butnaru, C.; Surleac, M.; Chiritoiu, G.N.; Alexandru, P.R.; Petrescu, A.-J.; Petrescu, S.M. EDEM3 Domains Cooperate to Perform Its Overall Cell Functioning. *Int. J. Mol. Sci*. **2021**, *22*, 2172. https://doi.org/10.3390/ijms22042172. **IF: 4,556**.

COMMUNICATIONS

1. **Manica, G.**; Butnaru, C.; Ghenea, S.; Chiritoiu, M.; Marin, I.; Petrescu, S.M. New mechanistic insights from studies of EDEM3 conformational domains. Presented at *RSBMB Annual International Meeting*, **2019**, Iasi, Romania.
2. **Manica, G.**; Ghenea, S.; Butnaru, C.; Munteanu, C.V.A.; Petrescu, A.-J.; Petrescu, S.M. Investigation of the multi-domain structure of EDEM3. Presented at *RSBMB Annual International Meeting*, **2018**, Bucharest, Romania.
3. **Manica, G.**; Chiritoiu, G.N.; Butnaru, C.; Ghenea, S.; Petrescu, S.M. EDEM3 and its function in ERAD. Presented at *RSBMB Annual International Meeting*, **2017**, Timisoara, Romania.

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