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EDEM1 regulates ER-associated degradation by accelerating de-mannosylation of folding-defective polypeptides and by inhibiting their covalent aggregation

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Abstract

Proteins expressed in the endoplasmic reticulum (ER) are covalently modified by co-translational addition of pre-assembled core glycans ($glucose_3$-$mannose_9$-*N*-$acetylglucosamine_2$) to asparagines in Asn-X-Ser/Thr motifs. *N*-Glycan processing is essential for protein quality control in the ER. Cleavages and re-additions of the innermost glucose residue prolong folding attempts in the calnexin cycle. Progressive loss of mannoses is a symptom of long retention in the ER and elicits preparation of terminally misfolded polypeptides for dislocation into the cytosol and proteasome-mediated degradation. The ER stress-induced protein EDEM1 regulates disposal of folding-defective glycoproteins and has been described as a mannose-binding lectin. Here we show that elevation of the intralumenal concentration of EDEM1 accelerates ER-associated degradation (ERAD) by accelerating de-mannosylation of terminally misfolded glycoproteins and by inhibiting formation of covalent aggregates upon release of terminally misfolded ERAD candidates from calnexin. Acceleration of Man_9 or Man_5 *N*-glycans dismantling upon overexpression was fully blocked by substitution in EDEM1 of one catalytic residue conserved amongst α1,2-mannosidases, thus suggesting that EDEM1 is an active mannosidase. This mutation did not affect the chaperone function of EDEM1.

Keywords: Endoplasmic reticulum; ERAD; EDEM1; Calnexin; Quality control; ER α-mannosidase; *N*-Glycans; Glycoprotein

Folding-defective polypeptides must rapidly be removed from the ER lumen to avoid interference with the maturation of newly synthesized proteins. This is a crucial part of the protein quality control operating in the ER and requires recognition of terminally misfolded chains, their extraction from cycles of futile folding attempts, unfolding, and dislocation into the cytosol for proteasome-mediated degradation [1,2]. As originally shown by Su and co-workers, the activity of ER-resident mannosidases is required for degradation of folding-defective glycopolypeptides expressed in the ER lumen [3]. Genetic evidence in *Saccharomyces cerevisiae* where the Man_8 isomer ***B*** (Fig. 1A) generated by the ER α-mannosidase I (ERMan I) is a strong degradation signal [4,5] and numerous studies with inhibitors in mammalian cells revealed the active involvement of kifunensine-sensitive α1,2-mannosidase(s) in ERAD regulation [6–10]. In the mammalian ER extensive removal of α1,2-mannoses generating Man_{5-6} configurations has been reported to be required to target folding-incompetent polypeptides for disposal [11–15]. The ERMan I can only very inefficiently remove more than one mannose residue from *N*-glycans *in vitro* [16] and *in vivo* [17]. Thus, it has been proposed that other *N*-glycanases might cooperate in the extensive de-mannosylation of ERAD candidates [18].

EDEM1 (for ER degradation enhancing α-mannosidase-like protein [19]), EDEM2 and EDEM3 [20,21] are

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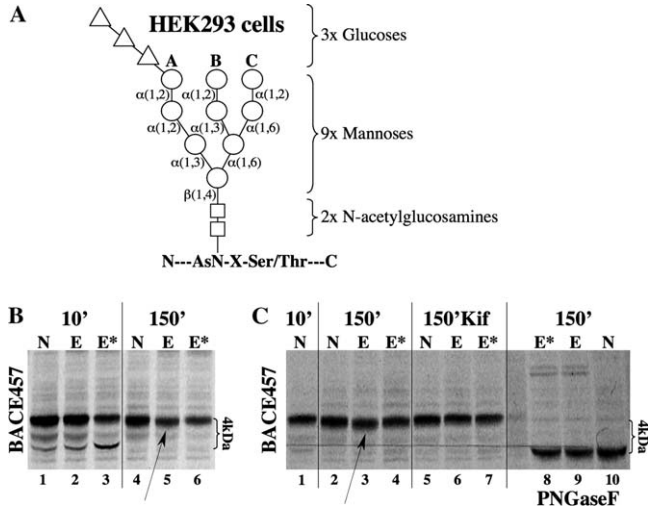

Fig. 1. EDEM1 accelerates removal of mannoses from BACE457 *N*-glycans. (A) The structure of the *N*-glycan transferred on nascent polypeptide chains. (B) Labeled BACE457 was immunoisolated from detergent extracts 10 and 150 min after synthesis in cells expressing normal (N) or high level of EDEM1 (E) or E220Q EDEM1 (E*). The 10 min (lanes 1–3) and the 150 min chase times (lanes 4–6) are separated by a vertical line, samples are loaded in the same gel. (C) BACE457 was immunoisolated from cells after a 10 min chase or after a 150 min chase without (lanes 2–4) or with kifunensine (lanes 5–7). Samples shown in lanes 2–4 were treated with PNGaseF to remove the two BACE457-bound *N*-glycans. This caused a Mr loss of about 4 kDa. After removal of *N*-glycans, the BACE457 isolated from cells with elevated level of E220Q (lane 8), elevated level of EDEM1 (lane 9), or with normal level of EDEM1 (lane 10) have the same electrophoretic mobility. In panels B and C the shift of 4 kDa caused by removal of the two BACE457 *N*-glycans is shown as a reference. Vertical lines are depicted to separate the various chase times and conditions loaded in the same 10% SDS–polyacrylamide gel.

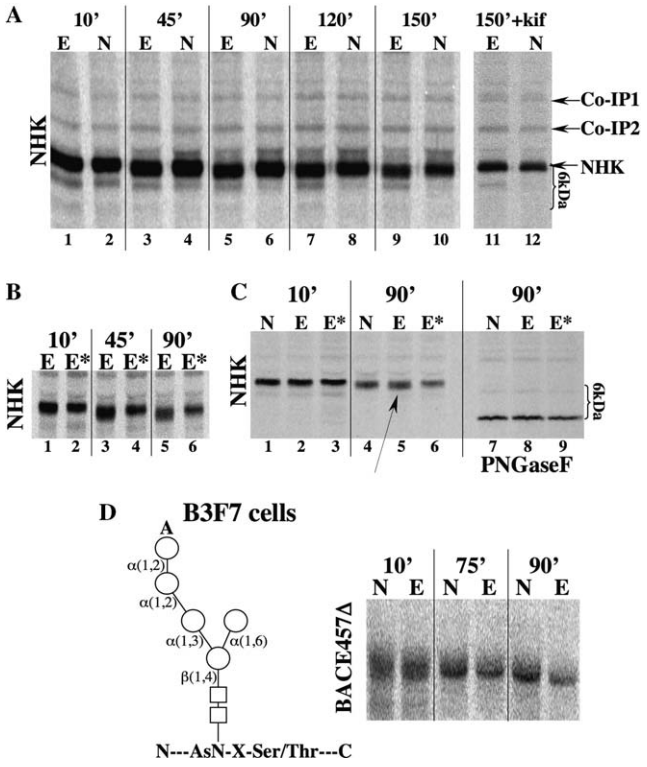

Fig. 2. EDEM1 accelerates removal of mannoses from NHK *N*-glycans and can remove mannoses from *N*-glycans branch ***A***. (A) EDEM1 overexpression accelerated *N*-glycan processing in NHK. Please note that EDEM1 overexpression specifically caused a mobility shift of NHK, without affecting mobility of Co-IP1 and Co-IP2, two unidentified polypeptides cross reacting with the specific NHK antibody. The 10 min (lanes 1–2), 45 min (lanes 3–4), 90 min (lanes 5–6), 120 min (lanes 7–8), and 150 min chase times (lanes 9–10) are separated by a vertical line; samples are loaded in the same gel. (B) Overexpression of the inactive E220Q EDEM1 (E*) abolished the mobility shift caused by overexpression of EDEM1. The 10 min (lanes 1–2), 45 min (lanes 3–4), and 90 min chase times (lanes 5–6) are separated by a vertical line; samples are loaded in the same gel. (C) After a 10 min chase, NHK immunoisolated from N, E, and E* cells had the same mobility (lanes 1–3). After a 90 min, the protein expressed in cells with high EDEM1 content in the ER (lane 5) showed faster mobility. Removal of the three NHK *N*-linked glycans abolished the differences in electrophoretic mobility (lanes 7–9). The 10 min (lanes 1–3), 90 min (lanes 4–6), and the samples treated with PNGaseF (lanes 7–9) are separated by a vertical line; samples are loaded in the same gel. (D) The mannose structure of the ($glucose_3$-$mannose_5$-*N*-$acetylglucosamine_2$-) *N*-glycan transferred on nascent chains in B3F7 cells is shown. Labeled BACE457Δ was immunoisolated from B3F7 cells expressing a normal (N) and an elevated level of EDEM1 (E), 10, 75, and 90 min after synthesis. The 10, 75, and 90 min chase times, are separated by a vertical line; samples are loaded in the same gel. In panels A and C the shift of 6 kDa caused by removal of the three NHK *N*-glycans is shown as a reference.

stress-inducible members of the third subfamily of the glycosylhydrolase 47 enzymes (GH47), also including ERMan I (subfamily 1) and a collection of Golgi α-mannosidases (subfamily 2). Studies on yeast EDEM (Htm1/Mnl1) [22,23], and two of the three mammalian orthologues (EDEM1–3) [15,20] initially failed to reveal hydrolytic activity despite the conservation of the catalytic residues employed by ERMan I and the Golgi hydrolases [19,24–26]. More recent data indicated that EDEM3 is an active *N*-glycanase [27], suggesting a potential role for this subfamily of GH47 in glycan trimming during ERAD.

Here we show that EDEM1 up-regulation as induced under ER stress conditions accelerates de-mannosylation of ERAD candidates in cell lines characterized by normal (Glc_3-Man_9-$GlcNAc_2$, Fig. 1A) or aberrant (Glc_3-Man_5-$GlcNAc_2$, Fig. 2D) core glycosylation. Acceleration of *N*-glycans dismantling upon overexpression was fully blocked by substitution in EDEM1 of one catalytic residue conserved amongst α1,2-mannosidases and was sensitive to cell exposure to kifunensine. Overexpression of EDEM1, and of the EDEM1 mutant that did not affect the rate of core glycan processing, efficiently prevented the formation of disulfide-bonded aggregates containing terminally misfolded glycoproteins released from calnexin, thus accelerating protein disposal. This showed that the chaperone activity of EDEM1 is independent of induction of accelerated substrate de-mannosylation. Our data identify EDEM1 as a multi-functional, stress-induced regulator of the mammalian ERAD.

Materials and methods

Cell lines, transient transfections. Human embryonic kidney cells (HEK293) and B3F7 cells (mutant Chinese hamster ovary cell line deficient in Dol-P-Man synthase that transfers to glycoproteins a truncated