Insulin-Degrading Enzyme: Structure-Function Relationship and its Possible Roles in Health and Disease

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Abstract: Insulin-degrading enzyme (IDE) or insulysin is a highly conserved Zn^{2+} -dependent endopeptidase with an "inverted" HxxEH motif. *In vivo*, IDE contributes to regulate the steady state levels of peripheral insulin and cerebral amyloid β peptide (Aβ) of Alzheimer's disease. *In vitro*, substrates of IDE include a broad spectrum of peptides with relevant physiological functions such as atrial natriuretic factor, insulin-like growth factor-II, transforming growth factor-α, β-endorphin, amylin or glucagon. The recently solved crystal structures of an inactive IDE mutant bound to four different substrates indicate, in accordance with previous compelling biochemical data, that peptide backbone conformation and size are major determinants of IDE recognition and substrate selectivity. IDE-N and IDE-C halves contribute to substrate binding and may rotate away from each other leading to open and closed conformers that permit or preclude the entry of substrates. Noteworthy, stabilization of substrate β strands in their IDE-bound form may explain the preference of IDE for peptides with a high tendency to self-assembly as amyloid fibrils. These structural requirements may underlie the capability of some amyloid peptides of forming extremely stable complexes with IDE and raise the possibility of a dead-end chaperone-like function of IDE independent of catalysis. Furthermore, the recent recognition of IDE as a varicella zoster virus receptor and its putative involvement in muscle cell differentiation, steroid receptor signaling or proteasome modulation suggest that IDE is a multi-functional protein with broad and relevant roles in several basic cellular processes. Accordingly, IDE functions, regulation or trafficking may partake in the molecular pathogenesis of major human diseases and become potential targets for therapeutic intervention.

Keywords: Insulin degrading enzyme, insulysin, metallopeptidases, amyloid β, peptides, Alzheimer, diabetes mellitus type 2, varicella zoster virus.

I. INTRODUCTION

Since the early studies by Mirsky and Broh-Kahn, who detected in 1949 an insulin-degrading activity in tissue homogenates [1], a specific protease -now known as insulin-degrading enzyme (IDE) or insulysin (EC 3.4.34.56)- has been identified and extensively characterized [reviewed in 2]. The initial interest on IDE as a protease involved in the turn over of insulin has broadened exponentially in the past decade due to its several unique, sometimes puzzling features in terms of structure, function, subcellular localization and multiple biological roles. These include the capability of IDE of degrading many peptides prone to amyloid formation, including the amyloid β (Aβ) of Alzheimer's disease (AD), its remarkable conformation-dependent substrate recognition mechanism, its possible participation in basic cellular processes related to growth and differentiation and finally, the increasingly growing evidence supporting that IDE may partake in the pathogenesis of human diseases such as AD or diabetes mellitus type 2 (DM2). These topics will be orderly reviewed here, with emphasis on the most recent advances on IDE structure-function relationship, the importance of its diverse subcellular localizations, and its possible biological significance in normal and pathological processes.

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*The one-letter amino acid code is used in this review.

II. IDE EXPRESSION, BIOCHEMISTRY AND ENZYMATIC ACTIVITY

IDE is an extremely conserved Zn^{2+}metallo-endopeptidase. It is currently classified as belonging to the M16A family of metallopeptidases defined by an "inverted" sequence at the catalytic site (HxxEH)* as compared to the classical sequence (HExxH) found in thermolysin, neprilysin (NEP) (M13 family) and other members of the clan [2]. Yet, despite the inversion of the catalytic site and connectivity, and the lack of sequence homology, some of the clan members share a striking similarity in the fold of their catalytic region, a fact that clearly supports convergent evolution [3] Fig. (**1**). Human IDE is synthesized as a single polypeptide with predicted 1019 residues encoded by a gene located in chromosome 10 [4, 5]. IDE cDNA predicts two possible initiation sites (M1 and M42), with the latter favored by an upstream Kozak consensus sequence. Yet, the amino-termini of the actual protein products remain to be experimentally defined. This is not a minor point regarding subcellular sorting and distribution, since the region comprised between M1 and M42 may contain a mitochondrial targeting sequence [6] or a signal peptide albeit with a rather low probability score, as analyzed with SignalP 3.0 [7]. As it will be discussed below, IDE is a ubiquitous protein and the

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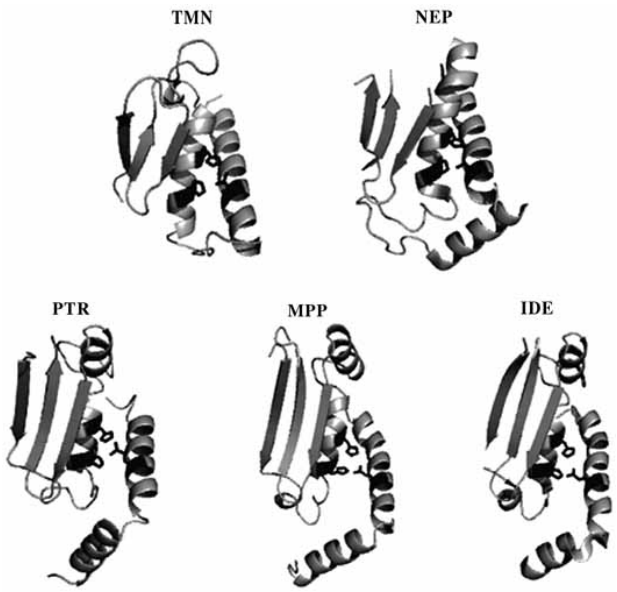

Fig. (1). Comparison of the overall fold of the catalytic regions of thermolysin (TMN) and neprilysin (NEP) (M13 family), with pitrilysin (PTR), mitochondrial processing peptidase (MPP), and insulin-degrading enzyme (IDE) (M16 family). The following segments are depicted in ribbon diagrams: TMN, residues 98-181 (PDB 4TMN); NEP, residues 473-481, 541-559, 570-620 and 642-664 (PDB 1DMT); MPP, residues 45-118, 136-173 (PDB 1HR6); PTR, residues 64-134 and 155-190 (PDB 1Q2L); IDE, residues 83-157 and 173-195 (PDB 2G54). The H-H-E zinc-coordinating residues are shown in black. Figures were constructed from the indicated PDB ID codes by using PyMOL v1.02.

signals that govern its targeting to cellular compartments remain obscure. IDE is ubiquitous not only with regard to subcellular location but also to its tissue distribution. IDE has been found in almost every tissue and cell type examined, with a relatively high expression in both insulin-sensitive and insulin-insensitive cells [8]. Compelling evidences gathered from *in vitro* studies, cell transfection, inhibition with monoclonal antibodies and, more recently, from IDE-deficient mice, clearly point at IDE as a key protease in insulin metabolism, as discussed below [9-11 reviewed in 12,13]. Despite its recognized importance in insulin clearance, little is known about how IDE expression is regulated. Recent work suggests that IDE has a CG-rich, "slippery" promoter typical of housekeeping genes with several transcription initiation sites [14 and Leal MC, unpublished observations]. No mechanisms have yet been described to explain how IDE is regulated as a down-stream target of insulin signaling, mediated by PI3K and Akt in neuronal cells both *in vitro* and in transgenic mice over-expressing human Aβ [15]. In addition, an increased IDE expression can be seen in astrocytes in response to aggregated Aβ through the activation of ERK signaling [16]. Several IDE mRNA species have been detected in most tissues that can be grouped into those with a long 3'UTR (~2 to 6 kb) and those with a very short 3'UTR (~300 b) [14,17]. The significance of these mRNA species is not known, although it is tempting to speculate that long 3'UTRs containing adenine-uracyl rich elements (ARE) may be used to regulate mRNA stability. Mutagenesis of key residues together with cell transfection of IDE mutants has confirmed the predictions that H108, E111, H112 and E189 are crucial for enzymatic activity, with the triad H108-H112-E189 coordinating a zinc atom and E111 activating a water molecule for the nucleophilic attack of the substrate amide bond [18, reviewed in 2,13]. IDE biochemical features have been extensively characterized using purified enzyme from diverse sources including tissues, red blood cells or recombinant human or rat (which share 95% identity) IDEs expressed in *E.coli* and insect cells [2,13,19,20]. Size-exclusion chromatography, light scattering, sedimentation equilibrium centrifugation, limited proteolysis and crystallography have established that IDE assembles as a stable homodimer, a quaternary structure that may account for the cooperative behavior seen with some of the studied peptide substrates [21]. Although insulin shows the highest affinity as inferred from the apparent Km (low nanomolar range), IDE can degrade a large number of peptides of unrelated sequence including IGF-II, TGF-α, glucagon, amylin, atrial natriuretic peptide, calcitonin, β endorphin, Aβ, amyloid Bri (ABri), amyloid Dan (ADan) and the C-terminal domain of Aβ precursor protein (APP-AICD), just to mention some with physiological or potential pathological significance [22, 23 reviewed in 24]. Despite this lack of sequence specificity, IDE is not a general peptidase since peptides related to insulin, like proinsulin or IGF-I, although capable of binding to IDE are degraded at a very slow rate and may act as competitive inhibitors [25]. The analysis of cleavage sites has shown that IDE has a preference for hydrophobic or basic bulky residues at the P1' site and a limitation imposed by the substrate size, with insulin (~5.8 kDa) at the upper limit. Likewise, IDE is uncapable of degrading small oligomeric forms of amyloid peptides [26]. In addition, peptides positively charged at their C-termini, such as IGF-1 are poor substrates. Thus, all these evidences obtained from biochemical work have correctly predicted many of the features related to recognition and mechanism of activity later revealed or confirmed by the three-dimensional structures.

Regulation of IDE Activity

Some important aspects of IDE function as a peptidase that may be highly relevant to its metabolic regulation remain to be clarified. Several putative allosteric effectors have been proposed including fatty acids, polyphosphates and ATP. Long chain fatty acids and their acetyl-coenzyme A thioesters, at physiological concentrations, may inhibit IDE activity in a non-competitive manner through their binding to a consensus region mapped to positions 565-582 (human IDE numbering) [27]. In addition, a direct modulation of activity by ATP has been suggested, with studies showing a stimulatory effect of ATP upon short substrates (8-13 residues long) and inhibitory or no effect upon insulin [28-30]. The postulated mechanism for the stimulatory effect of ATP involves a major intra-molecular conformational change that may result in an "open" state of IDE (see below) as assessed by dynamic light scattering, native electrophoresis and circular dichroism [31]. Moreover, both endogenous IDE purified from rat muscle and highly purified rat recombinant IDE have shown to have

intrinsic ATPase activity [32]. Yet, no ATP binding site has been identified and these issues await further biochemical and structural studies. Another allosteric behavior of IDE (more related to classical cooperativity) is revealed by the sigmoidal initial velocity vs susbtrate concentration plots, particularly when assayed with short substrates [33]. The complete loss of homotropic or heterotropic allosteric property by its isolated N-terminal half (that retains some residual proteolytic activity) suggests that the C-terminal half, which mediates dimerization, is necessary to propagate conformational or dynamic changes that modulate the affinity of the second binding site in the IDE dimer [34]. The interaction with small, non-substrate proteins may also modulate IDE activity. A group of yet unidentified heat-stable proteins of ~10-14 kDa has been shown to co-purify with IDE from liver and spleen homogenates and may behave *in vitro* as competitive inhibitors [35-37]. In one of these studies, ubiquitin was found to bind reversibly to IDE, displace insulin binding and inhibit its degradation [37]. However, a recent detailed mass spectrometry study suggested that ubiquitin did not inhibit insulin degradation by IDE but it changed the cleavage preferences on the insulin B-chain [38]. Exploring in depth these allosteric properties may yield important information about the long-range intra or inter-molecular interactions that modulate IDE activity. An obvious limitation at present is IDE size, which hampers the use of common strategies that have contributed for the understanding of allostery, such as multiple mutational analysis, NMR relaxation measurements or the rate of hydrogen/deuterium exchange [reviewed in 39]. The availability in a near future of a large number of amino acid sequences of the IDE family along species may allow sequence-based statistical analysis of the co-evolution of functionally coupled amino acids to tackle this important issue relevant to drug design [40, 41].

III. IDE AS A PROTEASE: STRUCTURAL AND FUNCTIONAL CORRELATIONS

A major advance in our understanding of IDE structure and function was recently attained from the crystallographic studies of Y. Shen, W. Tang and colleagues, who obtained crystals of a recombinant human IDE inactive mutant (E111Q) bound to four different substrates: insulin B chain, amylin, amyloid β1-40 and glucagon [42]. The 3-D structures show that each IDE monomer is made of two halves (IDE-N, residues 43-515 and IDE-C, residues 542-1016) linked by an extended loop (residues 516-542). Each half is composed of two α/β roll domains (N1, 43-285; N2, 286-515; C1, 542-768 and C2, 769-1016) with an overall ~22 % sequence similarity. IDE-N contains the catalytic site with the Zn^{2+}-coordinating triad (H108, H112 and E189) and the water-activating E111 side chains. IDE-N and IDE-C have extensive interactions and form a chamber that is just large enough to accommodate a substrate with the volume of a natively folded insulin molecule. The comparison of substrate-bound IDE with *E.coli* substrate-free protease III or pitrilysin (PDB accession code 1Q2L) (which share ~25% sequence identity), suggested that IDE may sway between open and closed conformations Fig. (**2**). In substrate-free pitrilysin, the N and C-terminal halves are rotated away and there are no contacts between N1 and C2 as in IDE. In the "closed" state, substrate is entrapped within the internal IDE chamber, which needs to switch to the "open" state to allow the exit of products and to start a new round of substrate binding. Although there is no direct structural evidence for this mechanism, analysis of the activity of IDE double mutants in which key interacting residues from IDE-N and IDE-C were replaced by cysteines (D426C/K899C, N184C/Q828C and S132C/E817C) support the concept of the open and close IDE conformers. In effect, in the presence of reducing agents, IDE cysteine mutants had a 30-40 fold

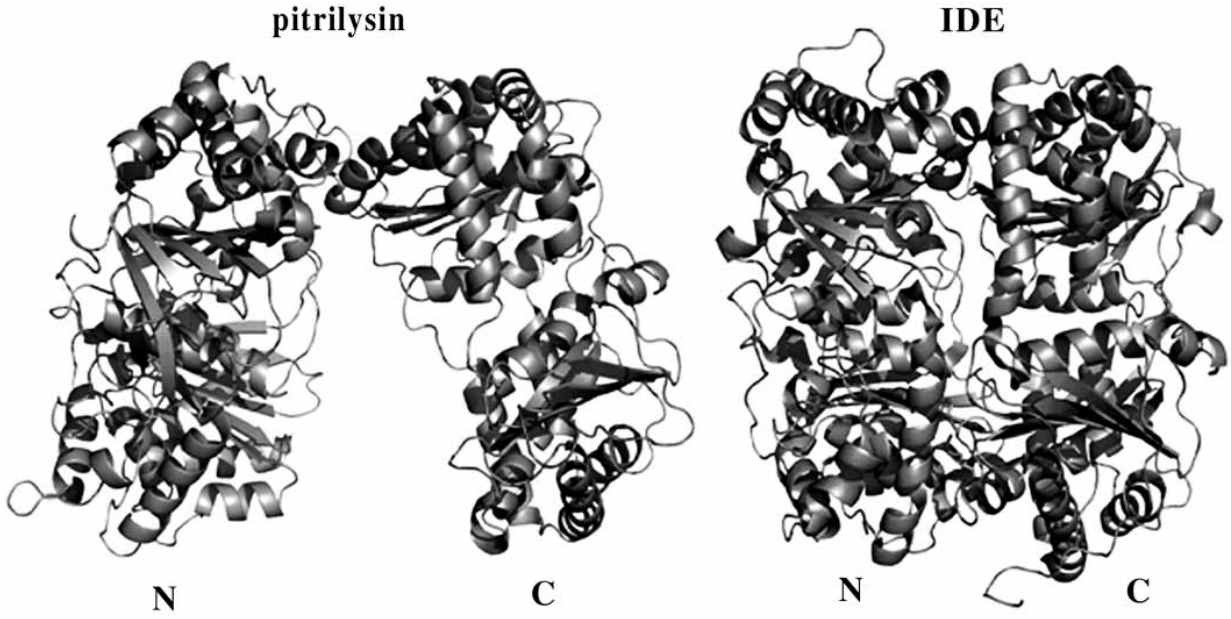

Fig. (2). Comparison between substrate-free pitrilysin (left) and substrate-bound IDE (right) to show the "open" and "closed" conformations. N and C, amino and carboxyl-terminal halves, respectively. Ribbon representations were constructed from PDB ID codes1Q2L (Pitrilysin) and 2G54 (IDE) by using PyMOL v1.02.

increase in catalytic activity while upon oxidation and disulfide binding, activity was abolished. The crystal structure of a substrate-free IDE [31] also shows the "closed" state, indicating that this is a more stable conformer and suggesting that a displacement of the "open-closed" equilibrium to the open state by yet undetermined factors may be needed to switch on or to increase IDE activity as a major regulatory mechanism. From each of the four substrates bound to IDE-E111Q, two segments were solved. A short N-terminal 3-5 residue strand and a downstream cleavage site region of 7-13 residues that form β-sheets with IDE N1 strands β12 and β6, respectively. Despite gross differences in the conformations adopted by each of the free substrates in solution, these two resolved segments appear remarkably similar, with an extended conformation in the four IDE-bound substrates. The bulky hydrophobic residues at the Pl sites interact with Fl41 at the Sl of IDE N1, while the hydrophobic Pl' sites are buried in a hydrophobic patch that surrounds Sl' in IDE-N1. IDE-C2 residues R824 and Y831 form hydrogen bonds with the backbone of Pl and Pl'. These findings fully concur with previous biochemical experiments indicating that IDE-N contained the catalytic residues while IDE-C contributed to the binding of substrates. While the surface of IDE-N facing the substrate is neutral or negatively charged, that from IDE-C is positively charged. Taken together, these structural evidences indicate that IDE "specificity" is dictated by several substrate requirements (and limitations) including: 1) size (up to ~6 kDa), 2) stabilization of a β conformation in the cleavage region upon binding to IDE, 3) preferred residue types at P1 and P1' as mentioned above, and 4) the lack of positive charges at the C-terminus. Yet, the plasticity and extended nature of the binding subsites in IDE may account for notable exceptions, such as the major cleavage sites in Aβ between H13-H14 and H14-Q15 [25]. The sensitivity of IDE activity to thiol-modifying agents such as *p*-chloro-mercuriphenyl-sulphonic acid (PCMPS), N-ethylmaleimide (NEM) or iodoacetamide has been recently tackled by a comprehensive two-way cysteine mutagenesis strategy [43]. Single C>S mutants revealed that the IC_{50} of NEM was increased by changing C178 or C819, while the inverse experiment by replacing each C in a C-free IDE (all 13 Cs mutated to Ss) showed restoration of the effect of NEM with C812 and C819. Finally, complete resistance to NEM was only attained when the three residues C178, C812 and C819 were changed. However, the magnitude of the effects of C replacements was highly dependent of the type of substrate used, adding complexity to the interpretation of the possible mechanisms involved. As a remarkable example, alkylation of C819 (in a C-free IDE background) eliminated hydrolysis of a 10-residue fluorogenic peptide while this effect was greatly attenuated when insulin was used as substrate. Alkylation of wild-type IDE, in contrast, resulted in complete inhibition for both substrates. It is possible that the underlying mechanism of IDE thiol-sensitivity through C178, C812 and C819 involves allosteric changes that affect substrate binding. Due to their proximity, it is tempting to speculate that C812 and C819 side chains alkylation may disrupt the hydrogen bonds of R824 and/or Y831 with the substrate backbone, being this effect less dramatic for longer peptides still capable of binding other IDE subsites, Fig. (**3**). In the case of C178, it resides on the same α-helix that contains E189 (Zn^{2+}-binding residue) and very close to L116 (C178 sulphur- L116 carbonyl distance =3.94 Å) which lies on the HxxEH-containing helix. Alternatively, C178 may disrupt the apposition of IDE-N and IDE-C due to its proximity to T825 [43]. These interpretations are consistent with the complete inhibition attained for all substrates after alkylation of the three key cysteines due to separate allosteric "hits" affecting multiple binding sites. Analysis of the C mutants also contributed to strengthen the notion of the remarkable plasticity of IDE. In effect, the treatment with NEM of an IDE mutant containing C590 in a C-free background activated by 40% the degradation of Aβ while there was no effect upon insulin hydrolysis, suggesting that C590 may differentially affect substrate binding [43]. With regard to its quaternary structure, IDE forms stable homodimers through its IDE-C domains and it has been proposed that assembly into tetramers may regulate IDE activity by stabilizing the closed conformer [29, 42].

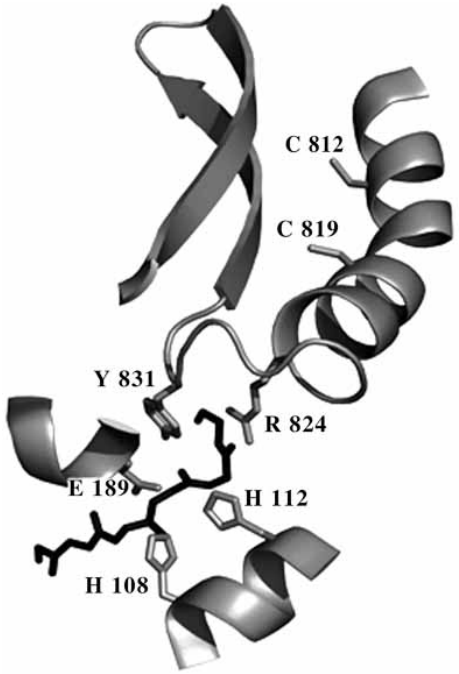

Fig. (3). Diagram showing the proximity of C812 and C819 to the binding site of IDE (PDB ID code 2G54). Side chains are indicated with the one-letter-code. Note that R824 and Y831 make hydrogen bonds with the backbone of insulin B chain (residues 13-18), depicted in black. The model was constructed by using PyMOL v1.02.

IV. IDE SUBCELLULAR LOCALIZATIONS

Although IDE is now regarded as ubiquitous, Fig. (**4**); the relationship between its subcellular localization and functions and the signals that determine its sorting are far from being understood. Early studies using ^{125}I-insulin binding and degradation together with sucrose gradient fractionation of different tissues and cell lines showed that 50-95 % of IDE activity was found in the cytosol, while the rest was localized in the particulate region of the gradients [reviewed in 12,13]. Yet, a cytosolic localization of IDE

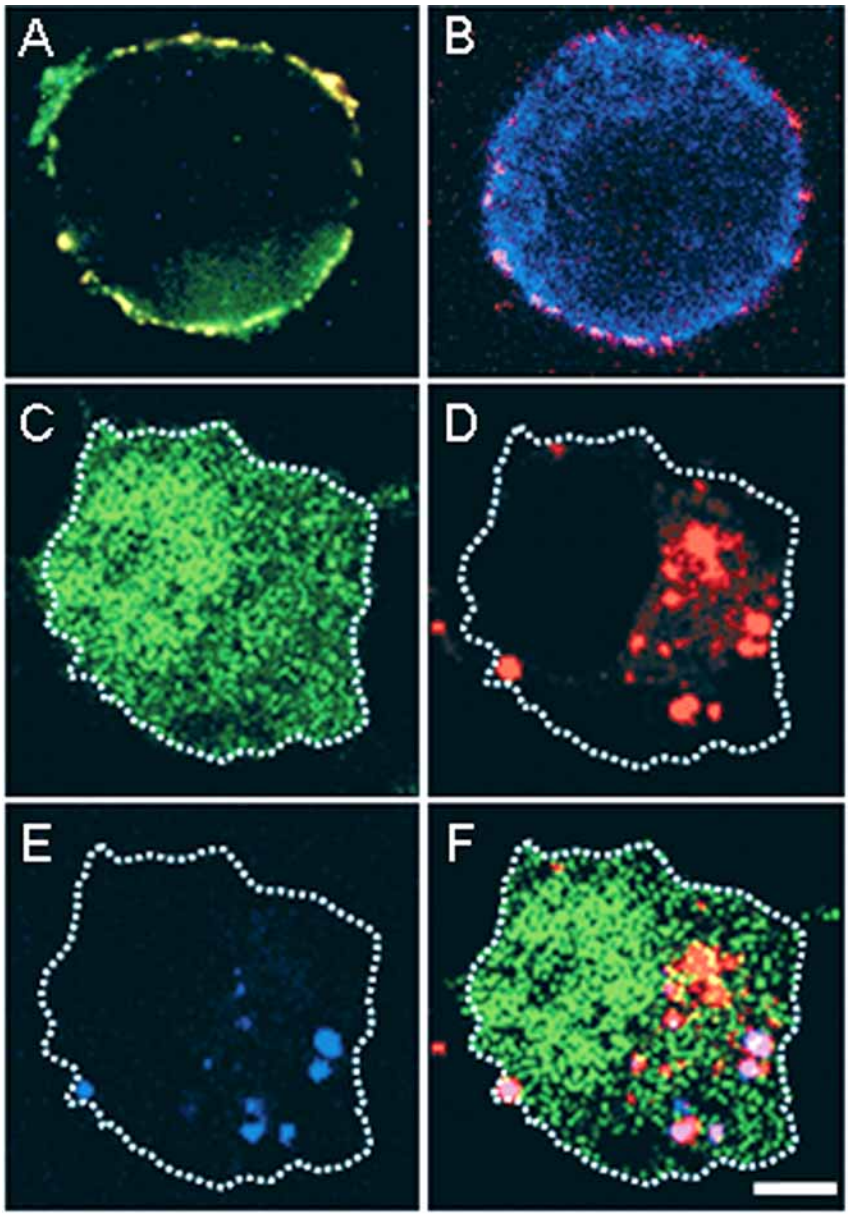

Fig. (4). Subcellular distribution of IDE. **A,** immunofluorescence of unpermeabilized neuroblastoma N2a cells with anti-IDE 1C1/3A2 monoclonal antibodies (green) and colera toxin subunit B (red), showing the co-localization of both proteins on the plasma membrane (yellow). **B**, immunolocalization on live, unpermeabilized N2a cells of membrane IDE (red) followed by permeabilization, fixation and labeling to show cytosolic IDE (blue). **C**, N2a cells transfected with pEGFP showing the cytosolic distribution of EGFP, **D**, multivesicular bodies specifically labeled with the fluorescent lipid analog N-Rh-PE (red). **E,** immunofluorescence with anti-IDE (blue). **F**, merging of the three labels in **C**, **D** and **E** shows co-localization of IDE with MVBs. Bar size = 5 μm

does not fully support a physiological role in the proposed processing of insulin or Aβ, two of the major disease-associated IDE substrates. IDE has been also described by immunocytochemistry, degradation assays and cell-surface biotinylation on the plasma membrane (PM) of muscle cells, pancreatic acini, CHO cells and neurons [44-47]. Insulin degradation has been postulated to take place in an endosomal compartment, in which IDE has been localized, after receptor binding and internalization [48-50]. Regarding Aβ, the most likely sites of encounter with IDE are the extracellular space, the outer side of the PM and the lumen of the endosomal pathway, a major subcellular location of Aβ generation by the activity of β and γ-secretases upon APP [51-53]. The primary structures of human, drosophila, and rat IDE lack a typical signal peptide to enter the classical secretory pathway or membrane-spanning sequences [4,17, 54]. Moreover, no post-translational modifications of IDE have been described to account for these localizations and additional work is required to clarify how IDE becomes attached to membranes and translocates to the luminal and extracellular spaces. Degradation of substrates such as insulin or Aβ may be dependent of a regulated or limiting mechanism by which IDE is targeted to membranes and translocated, regardless of its relatively high levels in the

cytosolic compartment. We have recently characterized a population of active IDE molecules tightly associated with lipid "rafts" or detergent-resistant membranes in neurons and brain tissue [55]. These membrane domains are involved in various aspects of Aβ metabolism such as generation, turn over or aggregation. [56, reviewed in 57]. IDE may represent an example of a growing number of proteins with an "eclipsed distribution" [58]. Within this concept, the highly uneven IDE distribution, in which cytosolic isoforms largely predominate, may mask minute amounts of the protease in specific subcellular domains essential for the degradation of relevant peptide substrates such as insulin or Aβ. The presence of functional IDE in cell culture conditioned media and extracellular fluid in tissues is well documented [59-61]. This localization of IDE occurs without cell damage and therefore it reflects a non-classical type of protein secretion. Since extracellular IDE seems to be involved in a highly active apolipoprotein E-assisted degradation of Aβ, elucidating the mechanism of IDE secretion may be relevant to the process of Aβ accumulation in the human brain [62]. Our group has recently demonstrated that a significant population of IDE molecules is routed to the extracellular space via exosomes derived from multivesicular bodies (MVB) Fig. (**4**), a process that was Ca^{2+} and Rab11-GTPase-dependent [Bulloj A and Morelli L, unpublished observations]. It is interesting to note that a dysfunction of the late endosomal pathway has been shown in patients with Down syndrome and AD [63], raising the possibility that in these disorders, the release of IDE to the extracellular space may be impaired. IDE contains a canonical peroxisomal targeting motif, A/SKL, situated at the extreme carboxyl terminus. This A/SKL motif is both necessary for peroxisomal sorting and sufficient for directing normally cytosolic or secretory passenger proteins to the peroxisomes [64,65] In rat liver, immunoprecipitation and immuno-electron microscopy showed that approximately 10-20 % of total IDE is associated with the matrix of peroxisomes [66]. This localization is not necessary for insulin degradation, as assessed by mutations of the target sequence [67], and it was suggested that the function of IDE in peroxisomes is related to the degradation of leader peptides of peroxisomal proteins [68]. Alternatively, IDE may be involved in the proteolysis of oxidized proteins [66]. Recently, a mitochondrial location of IDE has been reported [6]. In marked contrast to M42-IDE, cell transfection studies showed that M1-IDE was localized within subcellular organelles consistent with mitochondria. This longer isoform contains an N-terminal mitochondrial targeting sequence and its physiological role may be related, as in peroxisomes, with the proteolytic disposal of cleaved mitochondrial leader peptides.

V. IDE IS A MULTIFUNCTIONAL PROTEIN

The ubiquitous distribution in insulin-sensitive and insulin-insensitive tissues together with the broad subcellular localization indicates that IDE is a highly conserved multi-functional protein. It seems likely that some of its functions are directly or indirectly related to its proteolytic activity upon peptide substrates, while other functions appear to be independent of proteolysis.

Insulin Signaling and Proteasome Regulation

As mentioned above, among the well supported functions of IDE as a peptidase is the regulation of insulin homeostasis, in which IDE participates through hydrolysis in the termination of insulin action. This has been supported by injection of radiolabeled insulin in rats, IDE overexpression by cell transfection, IDE neutralization with antibodies and more recently, with IDE silencing using siRNA [reviewed in 12, 13, 69]. Furthermore, hyperinsulinemia and glucose intolerance shown by IDE knock out mice are concurrent with this function [11]. Through proteolysis, IDE may be also involved in the inhibition of proteasomal activity elicited by insulin and other IDE substrates such as relaxin and atrial natriuretic peptide [70, 71]. This concept has been underscored by recent experiments showing that insulin-induced inhibition of proteasomal degradation of long-lived proteins was reduced after IDE partial silencing with siRNA in HepG2 cells [69]. The insulin-mediated effect upon the proteasome was dependent on its degradation by IDE, suggesting that it was exerted by insulin fragments [72].

Muscle Cell Differentiation

A role for IDE in muscle cell differentiation was suggested by early studies on myoblasts in which 1, 10 phenanthroline and PCMS, but not phosphoramidon, inhibited creatine kinase expression and cell fusion [73, 74]. Moreover, in rat primary skeletal muscle cells, the reduction of serum in the medium promoted an increase in IDE activity prior to differentiation and the whole process paralleled the rate of insulin degradation [75]. More recently, IDE has been shown to interact with stem cell antigen-1 (Sca-1), a protein marker of myogenic precursor cells associated with cholesterol-rich plasma membrane microdomains. Interestingly, chemical inhibition or siRNA knock down of IDE resulted in a sustained proliferation and delayed myogenic differentiation, mimicking the phenotype of Sca-1 inhibition or its displacement from lipid rafts [76]. Although the effect of IDE in these experiments is likely related to the degradation of growth factors, its key role in the Sca-1 signaling pathway strengthens the concept that specific and regulated subcellular localizations of IDE may be essential to discriminate its diverse cellular functions.

Degradation of Aβ and Related Peptides

With regard to other peptide substrates, there is *in vivo* evidence for the relevance of IDE in maintaining the steady state levels of Aβ and the intracellular domain fragments of APP (AICDs) in the brain [11, 77]. IDE is capable of degrading wild-type rodent and human monomeric Aβ and all the genetic variants associated with hereditary stroke and dementia [26].While Aβ has been extensively involved in the pathogenesis of AD and related disorders, at least one AICD, the 50-residue product of the ε cleavage of γ secretase, has been postulated to participate in transcriptional regulation and apoptosis in concert with specific adapter and binding proteins [reviewed in 78]. Yet, the biological significance of AICD degradation by IDE awaits further clarification, since its effects would depend on which are the AICD relevant

transcriptional targets (an issue that is still under debate). We have recently shown that Aβ peptides, in addition to being substrates of IDE, can remain bound to the protease with an extremely high stability reminiscent of the self-assembly of amyloid oligomers. Similar results were obtained when two other amyloid peptides associated with hereditary dementia, ABri and ADan, were used Fig. (**5**) [23, 79, 80]. The co-immunoprecipitation of IDE-Aβ stable complexes (IDE-AβSCx) from rodent and human brain raises the possibility that these are formed under physiological conditions and may reflect a catalytically-independent novel function of IDE. The endocytic pathway has been proposed as a major subcellular site of Aβ generation and IDE has been detected in endosomes. However, IDE catalytic activity is maximal at pH 7 and falls sharply below pH 6.5 (Llovera RE and Castaño EM, unpublished observations). Thus, it is possible that within this acidic compartment, an interaction between Aβ and IDE will be displaced toward the formation of IDE-AβSCx. Our experiments using an inactive mutant of IDE indicate that IDE-AβSCx is readily formed in the pH range 5.5-7 (de Tullio M, unpublished observations). We have proposed that under conditions in which hydrolysis is disfavored (i.e. suboptimal pH, oxidative environment) IDE may trap peptide monomers irreversibly within its cavity, acting as a "dead-end" chaperone and preventing the formation of toxic oligomers and allowing a controlled disposal of amyloidogenic peptides by the cell. These complexes between IDE and amyloid peptides may be routed to lysosomal or proteasomal degradation or removed out of the cell via an exosomal pathway [80].

Steroid Signaling

In vitro studies have suggested that IDE binds to androgen and glucocorticoid receptor and enhanced their DNA binding capacity [81]. Co-incubation of IDE with a peptide corresponding to the binding region of the androgen receptor inhibited the degradation of insulin. Moreover, in adult rats, testosterone and estradiol promoted the expression of IDE in testis and uterus, respectively [82]. Although these results await more evidences *in vivo*, they strongly suggest that IDE may play a part in the cross-talk between

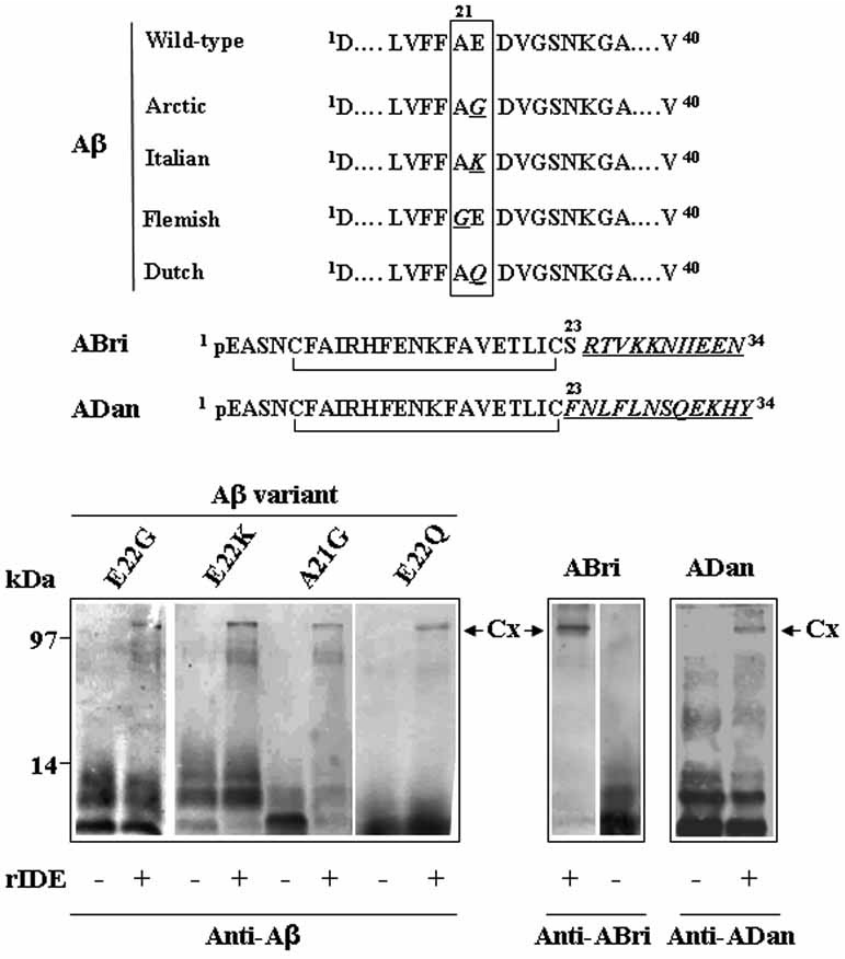

Fig. (5). Stable complexes formed between IDE and different amyloidogenic peptides. *Top panel*, schematic representation of Aβ peptides, in which substitutions associated with hereditary stroke or dementia are shown in italics and underlined. *Middle panel*, the sequence of the ABri and ADan peptides associated with autosomal dominant British and Danish dementia, respectively. In both cases, the stop codon is lost as the result of mutations and the novel sequence of the variant peptide is indicated in italics and underlined. pE denotes pyroglutamic acid, which is found in brain deposits. *Bottom panel*, Western blots of SDS-resistant complexes formed between IDE and Aβ peptides (labeled with anti-Aβ 6E10) or IDE-ABri and ADan (labeled with specific antibodies raised against the C-termini of each peptide). Complexes formed between IDE and amyloid peptides (Cx) are indicated by arrows. This figure is reproduced, with permission, from ref. 80.

insulin/insulin-like growth factors and the signal pathways of steroid hormones.

VI. IDE AND HUMAN DISEASES

Varicella Zoster Virus Infection

The recent identification of IDE as a cellular receptor for the varicella zoster virus (VZV) is a striking example of a catalytic-independent role of IDE related to human disease [83]. VZV is a ubiquitous neurotropic human herpes virus that causes chickenpox and often reactivates in adults causing zoster or, more rarely, cerebral vasculitis. Cell surface biotinylation followed by immunoprecipitation with immobilized glycoprotein E (gE), a protein that is indispensable for cell-to-cell spread and infectivity of VZV, indicated that the interaction of IDE with gE took place on the outer side of the plasma membrane. The significance of this interaction was further demonstrated by knocking down IDE with siRNAs, which significantly reduced infectivity and by IDE overexpression, that resulted in enhanced infection with both cell-free and cell-associated VZV. Both a catalytically active IDE and an inactive mutant (IDE-E111Q) were capable of binding a ~50-residue region within the N-terminal domain of gE to a similar extent. Pull-down assays indicated that the IDE-N half was sufficient for the interaction [84]. Although it is likely that VZV uses other receptors, such as the mannose-6-phophate receptor, these results strongly suggest that IDE may play a significant role in human VZV infection or reactivation.

Diabetes Mellitus

Several biochemical and genetic lines of evidence have raised the possibility that IDE is implicated in the pathogenesis of DM2. In the Goto-Kakizaki rat model of DM2, two mis-sense mutations resulting in amino acid replacements (H18R and A890V) were associated to hyperglycemia in congenic strains. Interestingly, cell transfection studies showed that both mutations were necessary to reduce IDE activity by 30%, a reduction that correlated with the lower IDE activity found in the muscle of these animals [85, 86]. In a different animal model, IDE-null mice showed a ~3-fold increase in insulin levels at 17-20 weeks of age and significant hyperglycemia at 60-120 min in a glucose tolerance test as compared to wild-type animals [11]. Regarding IDE levels in diabetic humans, a recent mRNA expression and proteomic study showed that IDE, among other components of the insulin pathway, was significantly reduced in T-cells and muscle tissue of DM2 patients as compared to controls [87]. Genetic studies in humans are still controversial about the possible association of polymorphisms or haplotypes in the IDE gene and the risk for DM2. In a large study on a Scandinavian and Canadian population, no associations were found in contrast to previous studies [88-91]. Yet, population background may play a part in these divergent results. Chinese women with polycystic ovary syndrome (PCOS) with the CC genotype of a single-nucleotide polymorphism (rs2209972) had higher fasting insulin level and homeostasis model assessment for insulin resistance (IR) as compared to women with PCOS with the TT genotype [92]. In addition, associations of IDE genetic polymorphisms with DM2 in Finnish and Korean populations have been reported [93, 94]. Regardless of the impact that variations in the IDE gene may have upon the susceptibility for DM2, it seems likely that the physiological role of IDE in insulin homeostasis is deranged in the context of IR. If, as it has been shown in neurons [15], IDE expression is under insulin signaling in peripheral tissues, IR may result in lower IDE levels and a slower insulin turn-over. It is known that persistent insulin stimulation may ultimately result in IR [95] that may sustain the reduction in IDE expression. Thus, IDE may be involved in a vicious cycle of self-promoting pathogenic events in DM2, typical of chronic metabolic disorders. Although the level of activity of IDE in pancreatic islet cells is not known, an indirect way by which IDE may affect the course of DM2 is through the degradation of amylin, an amyloidogenic peptide that accumulates in the islets and contributes to β-cell damage [96].

Alzheimer's Disease

There is compelling evidence for the participation of IDE and other Aβ-degrading metallopeptidases in the process of Aβ accumulation in the brain, a hallmark of AD. The expression and activity of IDE have been reported to be reduced in the hippocampus and cortex in sporadic AD [97-99]. In cortical microvessels from AD brains affected with extensive cerebrovascular Aβ deposition, IDE levels were increased, as determined by sandwich ELISA and yet, its activity was strongly reduced, indicating inhibition or inactivation of the protease [100]. It has been proposed that part of the loss of IDE activity in AD brain tissue may be due to post-translational modifications such as oxidative damage in vulnerable regions of the CNS, to which IDE is highly sensitive [101, 102]. The presence of IDE co-deposited with Aβ in senile plaques and microvessels in the cerebral cortex and leptomeninges of AD indicates a gross conformational change of the protease since its aggregation and deposition is not seen in normal brains Fig. (**6**) [103-105]. Whether this conformational change of IDE in AD brains is related to post-translational modifications or to the formation of irreversible complexes with Aβ [79] remain to be addressed. Yet, it is interesting to note that in three different transgenic mice models of familial AD, IDE increases its expression and activity in response to Aβ accumulation and in association with astroglial activation surrounding the plaques [16,106]. These results suggest that the abnormalities in IDE expression, processing or activity detected in sporadic AD are not the consequences of Aβ aggregation and deposition and may rather occur as part of upstream mechanisms in the pathogenesis of AD. The issue of the association of IDE genetic variants with the risk for AD is still controversial, with some studies reporting positive data and others showing no significant association. For a detailed review of the IDE-AD genetic association studies, the reader is referred to the AlzGene database (http: //www.alzgene.org) [107]. Although many factors may determine the divergent outcome of these studies, including a higher than expected heterogeneity in the association of AD with chromosome 10 [108], IDE remains as a candidate gene that may influence the development of AD. Interestingly, lymphoblastoid cells obtained from subjects of three chromosome 10-linked families showed a reduced IDE activity when insulin was used as substrate

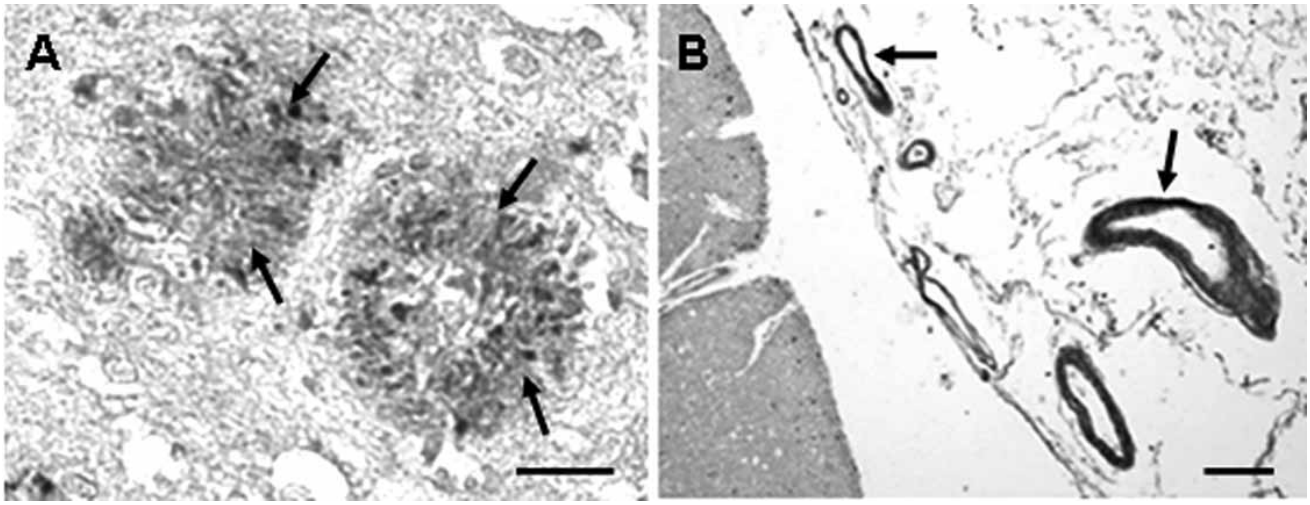

Fig. (6). IDE is abnormally deposited in AD brains. **A**, immunohistochemistry of a cortical section from AD brain showing IDE localized in senile plaques (arrows). The monoclonal anti-IDE 9B12 was used. Scale bar=25 μm. **B**, IDE immunoreactivity in the walls of amyloid-laden leptomeningeal vessels from AD brain. Scale bar=80 μm.

without changes in IDE mRNA or protein levels [109] The hypothesis that IR in the brain is related to AD pathogenesis [reviewed in 110] adds a possible involvement of IDE distinct from its role in degrading Aβ. A slow turn-over of insulin in the CNS due to reduced IDE activity may promote IR and dysregulation of neuroprotective pathways triggered in response to several agents, including aggregated Aβ [111].

VII. FUTURE PERSPECTIVE

The importance of IDE as a multi-functional protein with possible roles in the pathogenesis of human diseases warrants further efforts to answer some basic questions regarding its structure-function relationship, the factors and pathways that regulate its expression and the biochemical mechanisms that determine its subcellular localizations. As a protease, IDE shows the potential for the development or screening of compounds that increase its activity in a rather selective way. The preferential activation of IDE toward Aβ relative to insulin by dynorphin B-9 [21] or the unexpected increase in the hydrolysis of Aβ (but not of insulin) due to alkylation of C590 [43], are remarkable examples of substrate-selective effects related to IDE plasticity. In terms of expression and localization, more research is needed to explain why IDE activity is lower in the brains of AD patients as compared to non-demented controls and what may be the significance of its reduced levels in the association of IR with neurodegeneration. These few examples suffice to explain why IDE keeps attracting intense research aimed at developing novel strategies for the treatment of major human diseases in the near future.

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ABBREVIATIONS

Aβ	=	Amyloid β
ABri	=	Amyloid Bri
AD	=	Alzheimer's disease
ADan	=	Amyloid Dan
AICD	=	Amyloid intracellular C-terminal domain
APP	=	Aβ precursor protein
ARE	=	Adenine-uracyl rich element
CG	=	Cytosine guanine
CHO	=	Chinese hamster ovarian
CNS	=	Central nervous system
DM2	=	Diabetes mellitus type 2
EGFP	=	Enhanced green fluorescent protein
ERK	=	Extracellular signal-regulated protein kinase
gE	=	Glycoprotein E
IC50	=	Half maximal inhibitory concentration
IDE	=	Insulin degrading enzyme
IDE-AβSCx	=	IDE-Aβ stable complexes
IGF	=	Insulin-like growth factor
IR	=	Insulin resistance
Km	=	Michaelis-Menten constant
MPP	=	Mitochondrial processing peptidase
NEM	=	N-ethylmaleimide
NEP	=	Neprilysin
NMR	=	Nuclear magnetic resonance

N-Rh-PE	=	N-(lissamine rhodamine B sulfonyl)-phosphatidylethanolamine
PCMPS	=	p-chloro-mercuriphenyl-sulphonic acid
PCOS	=	Polycystic ovary syndrome
PI3K	=	Phosphatidylinositol 3- kinase
PTR	=	Pitrilysin
Sca	=	Stem cell antigen
siRNA	=	Small interfering ribonucleic acid
TGF	=	Transforming growth factor
TMN	=	Thermolysin
UTR	=	Untranslated region
VZV	=	Varicella zoster virus

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