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Targeting insulin-degrading enzyme to treat type 2 diabetes

Wei-Jen Tang*

Ben-May Department for Cancer Research, the University of Chicago

Abstract

Insulin degrading enzyme (IDE) selectively degrades peptides such as insulin, amylin, and amyloid β (A β) that form toxic aggregates, to maintain proteostasis. IDE defects are linked to the development of type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD). Structural and biochemical analyses revealed the molecular basis for IDE-mediated destruction of amyloidogenic peptides and this information has been exploited to develop promising inhibitors of IDE to improve glucose homeostasis. However, the inhibition of IDE can also lead to glucose intolerance. This review focuses on recent advances regarding our understanding of the structure and function of IDE and the discovery of IDE inhibitor, as well as challenges in developing IDE-based therapy for human diseases, particularly T2DM.

Keywords

Insulin degrading enzyme; Type 2 diabetes mellitus; Alzheimer's disease; small molecule inhibitors

Introduction

IDE (EC 3.4.24.56) is an evolutionarily conserved zinc metalloprotease that cleaves and inactivates several bioactive peptides with diverse sequences and structures, thus preventing the formation of peptide aggregates in many subcellular compartments (reviewed in [1–5]). IDE was initially discovered and named based on its ability to bind insulin (see glossary) with high affinity (~10 nM) and rapidly cleave it (K_{cat} =0.5-2/second) into fragments, causing its inactivation [6, 7]. IDE is subsequently found to degrade other bioactive peptides, e.g. glucagon, amylin, amyloid β . Thus, IDE has been implicated in diverse physiological and pathological functions.

Cellular regulation of IDE

IDE is expressed in all tissues and its levels can be modulated by many signals, including cellular stress, glucagon, and free fatty acids [4, 8, 9]. It is localized in the cytosol and growing evidence indicates that its proteolytic activity is subjected to complicated regulation

*Corresponding author - wtang@uchicago.edu.

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inside cells. IDE readily dimerizes [10, 11] and mutational analyses reveal that IDE dimerization allosterically regulates its catalytic activity [12, 13]. ATP can enhance the activity of IDE against short peptides, e.g. bradykinin, but not large substrates, e.g. insulin and A β [14]. IDE is composed of ~55 kDa homologous N- and C-domains (IDE-N and IDE-C, respectively) that are connected by a short linker to form the final 110 kDa protein (Figure 1A) [15]. The triphosphate moiety of ATP binds the highly positively charged surface of IDE-C to induce conformational changes in IDE [16, 17]. IDE also binds cellular proteins, including components of the cytoskeleton (vimentin, nestin) [18]. These interactions enhance its ability to degrade short peptides while suppress its ability to degrade insulin. Together, IDE dimerization and its binding with ATP and cytoskeletal proteins ensure that the enzyme preferentially degrades short peptides. Physical association of IDE with the 26S proteasome may also contribute to such preference [1, 19].

IDE exists in various subcellular compartments, including cytosol, intracellular vesicles, the plasma membrane, mitochondria, and the extracellular milieu [3, 20–22]. Its secretion can be regulated both by extracellular calcium levels via the calcium channel, calcium homeostasis modulator protein 1 (CALHM1), and by cholesterol-lowering drugs, e.g., statins [21, 22]. A sequence motif near its C-terminus has been shown to contribute to non-conventional translocation [23]. Similar to intracellular IDE, the catalytic activity of IDE in compartments outside the cytosol might also be regulated by its dimerization and surrounding cellular factors, but much less is known about such regulation.

IDE substrates and functions

Insulin, a biologically relevant IDE substrate, has pleiotropic functions including the regulation of metabolism of sugars, lipids, and amino acids; aberrant levels of insulin and improper responses to insulin and other hormones that control glucose levels are the primary causes of T2DM [24]. Insulin has a short half-life in circulation, presumably due to the action of high efficiency in the clearance mechanism, e.g. receptor-mediated internalization and degradation by IDE [25–27]. Insulin has two chains (A and B) held together by disulfide bonds (monomeric insulin). Upon synthesis and processing by pancreatic β cells, insulin oligomerizes to a hexamer and is secreted. As an oligomer, insulin is protected from degradation by IDE, as IDE only cleaves monomeric insulin [7]. IDE cuts both A and B chains once in a processive manner (without breaking the disulfide bonds) to generate non-functional insulin fragments [7]. Substantial *in vitro* and *in cyto* evidence supports the role of IDE in the clearance of insulin [1]. Furthermore, IDE null mutants, gene knockout, and pharmacological inhibition in rodents all result in elevated blood insulin levels (hyperinsulinemia) [5, 25, 26, 28, 29].

IDE also degrades and inactivates amylin and glucagon, additional peptides crucial to regulating blood glucose levels [5, 7, 15]. Amylin, also produced by pancreatic β cells, complements the action of insulin by slowing gastric emptying, regulating postprandial glucagon secretion, and reducing food intake [30]. Glucagon, secreted by pancreatic α cells, opposes the action of insulin, particularly in the liver [31]. Glucagon promotes the release of glucose from glycogen, stimulates gluconeogenesis, and triggers the release of fatty acids from stored triglycerides [31]. Glucagon can also enhance the response to stress [32].

Consistent with the role of IDE in the clearance of amylin and glucagon, administration of IDE inhibitors in mice leads to elevated levels of amylin and glucagon and modulates signaling by these hormones [5]. As glucose homeostasis is dependent on a complex interplay between many hormones such as insulin, amylin and glucagon, the effects of an IDE defect *in vivo* are expected to be complicated. Glucose intolerance is one of hallmarks for T2DM. IDE knockout (IDE-KO) mice exhibit age-dependent glucose intolerance, likely due to the hyperinsulinemia-associated onset of insulin resistance [25, 33]. However, the precise mechanism remains unresolved.

IDE also degrades A β , a ~4 kDa peptide derived from the cleavage of amyloid precursor protein and the primary component of plaques in the brains of AD patients [34]. A β has a high propensity to form various types of oligomers and amyloid fibrils that disrupt communications between neurons and cause cell death [35]. IDE degrades monomeric A β , thus preventing formation of oligomers and aggregates. Indeed, mice carrying an IDE inactivation mutation have increased A β accumulation and AD phenotypes [25, 28]. Conversely, overexpression of IDE in mouse brains leads to reduced A β accumulation and retarded progression of AD [36].

While rodent studies clearly show the role of IDE in the degradation of insulin, glucagon, amylin, and A β , the clear link of defect in IDE gene or activities with T2DM or AD in humans has been elusive. This is due to the complicated factors involved in the progression of these two chronic diseases and redundant mechanisms for the clearance of these peptides [3, 24, 34, 37]. For example, several proteases such as IDE, neprilysin, endothelin converting enzyme-1, and presequence protease, are involved in A β clearance [3, 38–40]. Nevertheless, several single nucleotide polymorphisms (SNPs) in non-coding regions of the human IDE gene on chromosome 10q are associated with T2DM [41–44]. Furthermore, non-sense and frameshift mutations have been found in the IDE gene in T2DM patients [45]. Several SNPs and genetic variants of IDE gene are also associated with late onset AD [46–49]. However such linkages are in dispute (reviewed in [50]). Recently, an IDE gene variant is found to link with increased IDE expression, reduced plasma A β , and reduced susceptibility to AD, which supports a role for IDE in A β clearance in humans [50].

In addition to the peptide substrates mentioned above, IDE degrades hormones, growth factors, and neurotransmitters including transforming growth factor- α , insulin-like growth factor-II, somatostatin and endorphins [1, 3, 5]. Recent studies have expanded the potential physiological roles for IDE. CC motif chemokines CCL3 and CCL4 play key roles in modulating inflammatory responses [51]. Structure-guided searches revealed that these chemokines can be effectively inactivated by IDE [52]. Furthermore, IDE is involved in the production of class 1 MHC restricted human tumor antigens [53]. A peptidomics approach found that IDE cleaves and inactivates calcitonin gene-related peptide, a neuropeptide associated with the control of blood pressure and many other biological processes [54, 55]. The list of IDE substrates will likely continue to expand. Challenges remain in identifying such peptides under physiological and pathological settings and elucidating how the altered IDE activities are linked to health and diseases in humans.

IDE may also prevent amyloid fibril formation by forming a stable complex with A β , leading to the hypothesis that IDE may serve as a “dead-end chaperone” [56]. α -synuclein, an amyloidogenic peptide involved in Parkinson’s disease, can form aggregates that are toxic to pancreatic β cells. Levels of α -synuclein are inversely correlated with IDE in β cells [57] and IDE can prevent α -synuclein from forming toxic aggregates non-catalytically [58]. Thus, IDE plays a role in proteostasis by catalytically and non-catalytically reducing monomer levels of amyloidogenic peptides [59, 60].

IDE biochemistry

Extensive biochemical and structural analyses have provided insight into the molecular basis of how IDE recognizes, unfolds, and degrades substrates with diverse primary sequences and tertiary structures. IDE dimerizes readily with high affinity (10 nM) [7]. Structures of dimeric IDE in the closed conformation reveal that IDE-N and IDE-C come together to form an enclosed catalytic chamber to engulf peptide substrates (Figure 1A) [7, 12, 15–17, 61–63]. Within the catalytic chamber, IDE has two key substrate binding sites: a catalytic cleft that coordinates a zinc ion, and an exosite that anchors the N-terminus of its substrates (Figure 1A, 1B). Residues from both IDE-N and IDE-C come together to form the catalytic cleft. Consequently, only in the fully closed conformation does the catalytic cleft form for efficient substrate destruction. The size of the catalytic chamber restricts IDE from engulfing peptides greater than ~80 amino acids in length. Moreover, the charge distribution of the catalytic chamber causes IDE to preferentially bind peptides that have a high dipole moment. Inside the catalytic chamber, IDE substrates are readily unfolded for degradation (Figure 1B). By anchoring the N-terminus of its substrates, the IDE exosite facilitates the unfolding of substrates and allows their stochastic cleavage at least nine amino acid residues away from their N-terminal ends (Figure 1B) [7].

At least two conformational switches exist for the catalytic cycle of IDE: the transition between the closed and open conformations, and motion at the catalytic cleft (Figure 2A). In the absence of substrate, IDE mostly exists in the open conformational state, which allows the enzyme to capture its substrates and release proteolytic products [12]. Interaction with substrates promotes a conformational switch from the open to the closed state. The conformational switch at the catalytic cleft involves a swinging motion of a door subdomain, which disorders the catalytic cleft of IDE [12] (Figure 1A, 2A). In case of amyloidogenic peptides (e.g. A β), they have high propensity to convert to β -stranded structures that self-assemble into intermolecular cross β -sheets, and thus form irreversible amyloid fibrils [64]. A β -bound IDE structures reveal that the β -strand of amyloidogenic peptides involved in cross β -sheets also forms an anti-parallel β -sheet with the door subdomain of IDE [15]. Since the door subdomain contains residues that are crucial for forming the catalytic cleft, the swinging motion of the door subdomain prevents IDE from hydrolyzing its substrates. Substrates within the catalytic chamber can only be degraded when binding of the substrate locks the IDE door subdomain in place to form a catalytic cleft. The propensity of amyloidogenic peptides to adopt a β -strand for amyloid fibril formation allows these peptides to stabilize the door subdomain of IDE. Thus, substrate-induced catalytic site stabilization allows IDE to preferentially degrade amyloidogenic peptides.

The current working hypothesis for the enzymatic reaction of IDE is as follows (Figure 2B). During the proteolytic cycle, both IDE and its substrates undergo conformational changes [7, 12]. Because IDE can exist in two states; an open, swinging-door, and fully closed conformational state, with the open state predominating [12], IDE substrates must interact favorably with the enzyme in all states. The closed conformation of IDE is stabilized by extensive contacts between IDE-N and IDE-C, rendering the catalytic chamber inaccessible to substrates. Only in the open state can the enzyme initiate binding with larger substrates, *e.g.*, insulin and A β . The dipolar charge distribution of IDE substrates permits good charge complementarity with the catalytic chamber of IDE in the open state. The anchoring of the N-terminal end of IDE substrates to the exosite also occurs when IDE is in the open state. Stabilized by these favorable contacts, IDE substrates then unfold and form a β -strand that interacts with and prevents the swinging motion of the door subdomain. This allows transient yet tight binding of the cleavage sites of the IDE substrates with the catalytic cleft of IDE so that proteolysis can occur. Such cleavage leads to further unfolding of IDE substrates. In most cases, this terminates the favorable interaction of the proteolytic products with IDE, leading to the product release as the enzyme transitions to the open state. However, the principles governing the closed-to-open transition are poorly understood. For some substrates, like insulin, two cleavages occur prior to the release of the degraded products [7]. Smaller peptide substrates, *e.g.* bradykinin, can also enter into the catalytic chamber of IDE via the gap created by the swinging motion of the door subdomain (Figure 2B). Such peptide substrates also need to stabilize the door subdomain in order to be cleaved by IDE. As smaller peptide substrates do not need to anchor to the exosite and be unfolded, IDE typically catalyzes the degradation of such substrates at a much higher rate, compensating for their lower binding affinity [3, 12].

Based on this catalytic cycle, one can envision that complex factors govern this dynamic process to determine how efficiently any given substrate is degraded by IDE. IDE undergoes a rapid open-closed transition, which determines whether the enzyme can capture the given peptide and whether the proper interaction of the IDE catalytic chamber with this peptide can occur to stabilize the closed state of IDE. This is, in part, controlled by the size and surface complementarity of the catalytic chamber with the given substrate. Furthermore, how fast a peptide can stabilize the catalytic cleft of IDE will determine whether such an interaction leads to productive degradation or a futile cycle. Together, such intricate IDE-substrate interaction allows IDE to selectively target peptides that have high propensity to form amyloid fibrils. Knowledge of the discrete steps in the catalytic cycle offers a rationale for selectively modulating the activity of IDE against disease-relevant subset of substrates such as insulin and A β .

IDE inhibitors: therapeutic potential and challenges

The role of IDE in the clearance of insulin suggests that IDE inhibitors could be used to modulate insulin levels in patients suffering from T2DM. The ideal IDE inhibitor to treat T2DM should preferentially reduce the clearance of insulin and amylin without affecting catabolism of other IDE substrates, *e.g.*, glucagon. Six decades ago, Mirsky and Perisutti reported that an endogenous inhibitor of IDE isolated from the liver could enhance the hypoglycemic action of insulin in rats and rabbits, highlighting the potential use of IDE

inhibitors to modulate the action of insulin [65]. Since then, steady progress has been made to use IDE inhibitors including bacitracin (an cyclic peptide antibiotic), N-ethylmaleimide (a thiol modifying agent), and 1,10-phenanthraline (a zinc metal chelating agent) to probe IDE activities *in vitro* [1, 66]. However, these inhibitors are rather non-specific and have low affinity to IDE [67].

With the advance in the structural analysis of IDE, at least five IDE inhibitors, including Ii1, BDM41367, 6bk, BDM44768, and NTE1, have been characterized structurally to elucidate how they bind IDE to inhibit its activity [5, 29, 67–70] (Figure 3). IDE inhibitor 1 (Ii1), the first reported high affinity IDE inhibitor (~2 nM), is a peptidomimetic hydroxamate that binds the catalytic site of IDE [67]. Ii1 can effectively reduce the clearance of insulin in cultured cells. However, the susceptibility of Ii1 to proteolytic inactivation and its high molecular weight pose significant challenges in further development. BDM41367 was found to be a partial antagonist of IDE that inhibits A β degradation but promotes insulin degradation [68]. The molecular basis for such complex regulation of the enzyme's catalytic activity is likely due to the binding of BDM41367 to both the N-terminus anchoring exosite and catalytic site of IDE (Figure 3). The A β -binding affinity of IDE is ~100-fold lower than its affinity for insulin. Such difference is postulated to contribute to the preferential inhibition of BDM41367 to suppress the degradation of A β . Thus, although not a promising drug candidate, BDM41367 highlighted the potential for identifying a substrate-selective inhibitor (or activator) of IDE. Three other IDE inhibitors (discussed below) have been analyzed in mouse models and their properties are summarized in Table 1 [5, 29, 70].

BDM44768 was initially identified using kinetic target-guided synthesis and its affinity for IDE and bioavailability were subsequently improved (Table 1) [29]. BDM44768 inhibits IDE activity by binding the catalytic cleft to directly compete with substrates, as well as by locking IDE in the closed conformation to prevent substrates from accessing the catalytic chamber (Figure 3). As expected, BDM44768 prevents insulin degradation by IDE and enhances insulin signaling *in vitro*. However, BDM44768 induces glucose intolerance in multiple mouse models regardless of the route of glucose administration [29]. These phenotypes are consistent with hyperinsulinemia and glucose intolerance observed in mice that have defective IDE [25]. This highlights the challenge in developing IDE inhibitors to better control blood glucose levels. It is worth noting that BDM44768 exhibits weaker inhibition to neprilysin and ECE (IC₅₀= 3 μ M and 7 μ M, respectively), which could contribute the off-target effects by BDM44768.

6bK is the first reported IDE inhibitor that shows promise for the treatment of T2DM [5, 71]. Based on its ability to bind IDE, 6bK was identified from a DNA-templated library of macrocycles. 6bK exhibits remarkable selectivity for IDE, likely due to the fact that 6bK binds a unique pocket outside both the catalytic cleft and N-terminal anchoring exosite (Figure 3) [5]. The ability of 6bK to improve oral glucose tolerance in high fat diet-induced obese (DIO) mice highlights its promise as anti-diabetic agent. However, the authors also reveal potential challenges of using 6bK to control blood glucose levels [5]. The administration of 6bK induces glucose intolerance when glucose is injected intraperitoneally. This complication may be due to the ability of 6bK to suppress the degradation of glucagon, which elevates blood glucose to induce hyperglycemia.

A research team at Eli Lilly has recently reported the characterization of IDE inhibitor, NTE-1 that has 4 nM IC₅₀ value (Table 1) [70]. NTE-1 is a fusion of two IDE inhibitors with low μ M affinities: a dipeptide aniline amide analog that binds the N-terminal substrate anchoring site and a quinoline-2 derivative that binds the 6bK-binding pocket (Figure 3, Table 1). Like 6bK, NTE-1 improves oral glucose tolerance in DIO mice. However, noticeable differences exist between the effects of these two inhibitors on the level of three glucose-modulating hormones in animals (Table 1). Together, studies from this set of IDE inhibitors strengthen the notion that IDE inhibitors can be used to better control blood glucose levels and highlight the potential to develop IDE inhibitors that can selectively reduce the clearance of certain IDE substrates for therapeutic benefits.

The observed differences between IDE inhibitors in animal models could be due to differences in pharmacokinetics, pharmacodynamics, and/or off-target effects of these compounds as well as the mouse models, housing environment, and experimental handling. Furthermore, these compounds may affect IDE activities differentially in different tissues or subcellular compartments. An attractive hypothesis is the contribution in mechanism of action of these compounds. Both 6bK and NTE-1, IDE inhibitors that elicit improved glucose tolerance, bind sites away from the catalytic cleft. These compounds may preferentially affect the degradation of certain IDE substrate(s) *in vivo* as they would alter the rate of the open-closed transition and/or reshape the properties of the catalytic chamber. Consequently, these inhibitors may alter the substrate selection of IDE *in vivo* instead of blocking the degradation of all IDE substrates. BDM44768, however, binds the catalytic cleft and directly competes with the binding of all IDE substrates. Consistent with this notion, the effect of 6bK to raise levels of insulin and amylin occurs much faster than that of glucagon while NTE-1 increased levels of insulin and amylin but not glucagon in mice (Table 1) [5, 70]. This raises the intriguing possibility that the desired outcome for IDE-based therapies could be generated by allosteric regulation of IDE inhibitors that bind away from the catalytic cleft and differentially affect the levels of selected subsets of IDE substrates.

The availability of small molecule inhibitors (6bK and NTE-1) that can improve glucose tolerance, offers the potential for further development of IDE-based therapies to treat T2DM. One major hurdle is addressing the potential side effects of IDE inhibition on other chronic diseases. For example, IDE inhibition, even if restricted from the central and peripheral nervous systems, might elevate A β in the circulation, which in turn could increase A β levels in the brain [49, 72, 73]. Thus, suppression of circulating A β clearance caused by IDE inhibitor could ultimately lead to A β -mediated cognitive impairment, as circulating A β is linked with brain A β . This hypothesis is based on the findings that the administration of agents that can sequester A β in the periphery can reduce A β levels in CNS [72, 73]. Consistent with attempts to reduce circulating A β to reduce A β levels in the brain, a recent clinical trial revealed that an A β -binding antibody holds promise in slowing the progression of dementia in patients with mild cognitive impairment [74].

Another potential complication of IDE inhibition-based treatment is the role of IDE in inflammation. Bone marrow transfer experiments reveal that IDE gene-knockout could promote inflammatory conditions such as atherosclerosis [75], which may be linked to the

role of IDE in the clearance of inflammatory chemokines [52]. Furthermore, elevated human amylin levels resulting from suppressing IDE activity could lead to the accumulation of misfolded amylin, leading to the formation of islet amyloid and β -cell loss. Amylin contributes significantly to islet amyloid depositions, which are found in the pancreas of most T2DM patients and are associated with the loss of pancreatic β -cell mass [59, 76, 77]. Such potential complications listed above may not be clinically relevant when IDE activity is acutely suppressed. However, efforts will likely be necessary to minimize unwanted side effects, as T2DM patients will likely take any IDE-inhibiting medicine for a prolonged period of time. This could be achieved by further modification of IDE inhibitors or the development of novel leads that can selectively suppress IDE activity against insulin.

Future efforts to develop small molecules that can modulate the specific aspects of IDE activities are vital to study IDE functions. Toward this goal, ML345 and its derivatives, a thiol-reacting IDE inhibitor that covalently links to IDE C819 with up to 50 nM affinity has also been developed recently [78, 79]. Its development stems from the notion that IDE is sensitive to thiol-mediated inactivation by oxidation and nitrosylation [62, 80, 81]. In addition, steady progress has been made to elucidate the structural basis of how ATP binds and activates IDE [17, 63, 82] and to develop small molecule chemicals that can enhance IDE activities *in vitro* [83]. However, substantial work will be required to improve such compounds before they can be used to probe IDE functions *in vivo*.

Concluding remarks

Protein misfolding and aggregation contribute significantly to the development and progression of many human illnesses [35]. IDE is a key component of the proteostasis machinery that curtails the aggregation of amyloidogenic peptides [59, 60]. While developing IDE inhibitors to treat T2DM is in its early stage of drug discovery and many challenges lie ahead, increased comprehension and appreciation of the structures and functions of IDE and continuing efforts in the development of small molecules that modulate IDE activity offer a path toward IDE-based therapeutic innovations to better maintain proteostasis, for the benefit of human health.

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Glossary

Amylin	a 37-residue peptide hormone co-secreted with insulin by pancreatic β cells. Amylin works together with insulin to lower blood glucose levels, thus amylin analogs have been developed to treat diabetes. Human amylin contributes to the formation of islet amyloid, which is associated with the loss of pancreatic β cells and type 2 diabetes
Amyloid β (Aβ)	the proteolytic product of amyloid precursor protein that is diverse in size and post-translational modifications. A β can readily form

	aggregates and amyloid fibrils, which are toxic to neurons. Extracellular plaque deposits of A β are one of two hallmarks of Alzheimer's disease (the other being intracellular neurofibrillary tangles of protein tau)
Amyloidogenic peptides	small proteins that typically exist as a soluble monomeric precursor but have a high propensity to undergo irreversible conformational changes to form nanometer size fibrils. Such amyloid fibrils and the intermediate aggregates are usually highly cytotoxic. Amyloid β (A β), insulin, and human amylin are well-known examples
Glucagon	a 29-residue peptide hormone that is secreted by pancreatic α cells to elevate blood glucose levels. Glucagon has been formulated for medical uses such as treating hypoglycemia, β -blocker overdose, and anaphylaxis
Insulin	a hormone produced by β cells. It is initially synthesized as preproinsulin. Upon entry into the endoplasmic reticulum, preproinsulin is further processed into A and B chains that are held together by disulfide bonds. Insulin lowers glucose levels in circulation and is thus administered to manage type 1 and late-stage type 2 diabetes mellitus, characterized by diminished insulin production
Proteostasis (protein homeostasis)	the process that cells use to control the abundance and folding of the proteome. The regulation of gene expression and various signaling pathways as well as the action of molecular chaperones and protein degradation systems are key mechanisms that maintain proteostasis
Type 2 diabetes mellitus (T2DM)	a metabolic disorder in which the human body cannot properly respond to insulin (insulin resistance), leading to hyperglycemia. T2DM is the most common form of diabetes mellitus

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Trends

Insulin degrading enzyme (IDE) is involved in the process of cellular protein homeostasis (proteostasis) by degrading monomeric form of many amyloidogenic peptides to prevent the formation of toxic aggregates and amyloid fibrils.

Consistent with the role of IDE in the clearance of insulin, amylin, and glucagon, three hormone vital for the glucose homeostasis, IDE defects lead to age-dependent glucose intolerance and are associated with T2DM.

IDE represents a promising therapeutic target for the treatment of T2DM as IDE inhibitors that do not bind the IDE catalytic cleft improve glucose tolerance.

Understanding the catalytic cycle of IDE provides a roadmap toward designing substrate-selective inhibitors for IDE.

Figure 1. IDE structure

(A) *Dimeric IDE structure*. The key features of IDE including IDE-N, IDE-C, catalytic zinc ion, and door subdomain are colored in cyan, green, grey, and red, respectively while the surface of catalytic chamber of IDE is in grey. (B) *Structural comparisons of IDE-bound and IDE-free substrates including insulin, Aβ, CCL3, TGF-α, IGF-II, and amylin*. Together, these structures reveal the partial unfolding of insulin, the importance for the anchoring of the N-terminal end of the substrate to the IDE exosite, and the requisite conformational switch of peptide substrates within the IDE catalytic chamber for the cleavages by IDE. IDE-bound substrates are colored red. IDE-free substrate structures are color grey with the cleavage sites by IDE indicated. The segments of substrates comparable to those revealed in IDE-bound substrate structure are colored transparent red.

Figure 2. IDE conformational switches and catalytic cycle

(A) Two key conformational switches for the IDE catalysis: open-closed transition (left) and swinging door motion (right). (B) A working model for IDE catalysis. IDE has two conformational states in the absence of substrate. The partially open state of IDE only allows the smaller peptides such as bradykinin to enter while the fully open state also allows larger substrate such as insulin to enter. The transition of IDE to the closed state induces the conformational switch of IDE substrate, leading to the progressive degradation. The reopening of IDE releases the products.

Figure 3. Structural bases for the binding of IDE inhibitors

IDE-N, door subdomain, and IDE-C are colored cyan, red, and green, respectively. The exosite that binds the N-terminus of IDE substrate, the catalytic cleft, and 6bK-binding site are marked. The pdb codes for IDE in complex with Ii1, BDM41367, 6bK, BDM44768, and NTE-1 are 3E4A, 4DTT, 4LTE, 4NXO, and 4PF9, respectively.

Table 1

Characterizations of IDE inhibitors that have been used in animal studies.

Chemical structure	IC ₅₀ (nM) (substrate)	Binding site(s)	Effects on IDE substrates <i>in vitro</i> or <i>in vivo</i>	Cellular studies	Glucose tolerance in mice	Other key observations in animal studies	REFS
<p>BDM 44768 (MW=448 Dalton)</p>	60 (Aβ ₁₋₄₂ ; ATTO655-CKLVFFAE DW)	Catalytic pocket formed by IDE-N and IDE-C	Increased the level of insulin and insulin-mediated signaling in mouse skeletal muscle and liver.	Increased secreted insulin in mouse islets and Aβ from SY5Y neuroblastoma cells.	Impaired oral glucose tolerance (oGTT) in B6 and NOD mice. Impaired i.p. injected glucose tolerance (ipGTT) in B6 mice. Had no effect on IDE ^{-/-} mice.	Enhanced insulin-induced hypoglycemia in mice.	[29]
<p>6bK (MW=745 Dalton)</p>	50 (model substrate, Mca-RPPGFSAF - K(Dnp))	Pocket 11 Å away from catalytic zinc ion	1. Increased levels of insulin and amylin 20 min after i.p. injection of 6bK in mice. 2. The increased glucagon level occurred only 135 min after i.p. injection.	N.A.	Improved oGTT but impaired ipGTT in both lean and DIO mice. Had no effect on IDE ^{-/-} mice.	Enhanced insulin-induced hypoglycemia in mice. Also enhanced amylin- or glucagon-induced hyperglycemia in mice.	[5]

Chemical structure	IC ₅₀ (nM) (substrate)	Binding site(s)	Effects on IDE substrates <i>in vitro</i> or <i>in vivo</i>	Cellular studies	Glucose tolerance in mice	Other key observations in animal studies	REFS
<p>NTE-1 (MW=733 Dalton)</p>	4 (insulin)	Exosite that binds the N-terminus of IDE substrate; 6bK binding pocket	<ol style="list-style-type: none"> 1. Inhibited the degradation of insulin and glucagon <i>in vitro</i>. 2. Increased the plasma level of amylin and insulin but not glucagon in mice. 3. Increased the plasma level of amylin but had no effect on half-life of insulin in Sprague Dawley rats. 	Decrease insulin clearance in HEK293 cells using NTE-2, a close analog of NTE-1.	Improved oGTT in DIO mice.	No effect on insulin-induced hypoglycemia in DIO mice.	[70]