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Degradation of Relaxin Family Peptides by Insulin-degrading Enzyme

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Abstract

Insulin-degrading enzyme (IDE) is a ubiquitously expressed metalloproteinase responsible for the intracellular degradation of insulin. IDE also interacts with other members of the insulin superfamily, including relaxin, but no studies have been reported regarding the interaction of other relaxin-like peptides with IDE. In this study, we determined that relaxin, relaxin-3 and InsL3 all competitively inhibited the degradation of insulin by IDE to different degrees, and all inhibited covalent cross-linking of insulin to IDE. Each of the peptides was degraded by IDE to various degrees (insulin>relaxin>InsL3=relaxin-3). In summary, relaxin, InsL3 and relaxin-3 all bound to IDE, competed with the binding and degradation of insulin, and were all substrates for the proteolytic activity of IDE. Therefore, it is possible that in addition to insulin, IDE may be important for the cellular proteolysis of relaxin, InsL3 and relaxin-3.

Keywords

Insulin-degrading enzyme; insulysin; relaxin; relaxin-3; InsL3; Relaxin-like factor

Introduction

The protease responsible for the intracellular degradation of insulin is insulin-degrading enzyme (IDE), also known as insulysin [1]. IDE is classified as a zinc endopeptidase (E.C. 3.4.24.56), and is a member of the M16 family of zinc metallopeptidases, which feature an inversion (H-X-X-E-H) of the traditional amino acid sequence at the zinc binding site [2]. In addition to insulin, IDE binds or degrades other members of the insulin family, including insulin-like growth factors I and II, relaxin, and bombyxin II [3,4]. However, IDE also degrades non insulin-like peptides such as atrial natriuretic peptide, adrenal corticotropic hormone, transforming growth factor alpha, β-endorphin, and glucagon [1]. There was no apparent consensus amino acid sequence for substrate recognition among these peptides, leading to speculation that specificity for IDE was determined by the structure of the substrate [5]. It was later learned that most of these substrates have the capability to form amyloid aggregates under certain conditions, and with the identification of Alzheimer's beta amyloid peptide and islet amyloid polypeptide (amylin) as IDE substrates [6,7], it is likely that IDE targets potentially amyloidogenic peptides.

Relaxin has considerable structural similarity to insulin, and can also form amyloid under certain conditions [8]. It has long been known that relaxin can bind to IDE, and can

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competitively inhibit the binding and degradation of insulin by IDE [3]. However, it was less clear whether relaxin is actually degraded by IDE. One report examined a relaxin-degrading enzyme that shared some, but not all, characteristics with IDE [9]. Therefore, the first goal of this study was to determine whether or not relaxin can be degraded by IDE. Because of the recent interest in additional relaxin family members, the second goal was to determine whether InsL3 (relaxin-like factor) and relaxin-3 are bound or degraded by IDE.

Materials and Methods

Porcine relaxin was kindly provided by Dr. O.D. Sherwood. Human relaxin-3 and InsL3 were purchased from Phoenix Pharmaceuticals. Monoiodinated ^{125}I-insulin was obtained from GE Biosciences. The homobifunctional amine-reactive covalent cross-linking reagent BS^3 was from Pierce.

For studies of competitive inhibition of insulin degradation, IDE was purified from rat liver by a series of chromatographic steps as described previously [6]. IDE and ^{125}I-insulin were incubated in the presence of unlabeled insulin, relaxin, relaxin-3 or InsL3 (1pM-1uM), and the degradation of ^{125}I-insulin was determined by the production of trichloroacetic acid-soluble products. Curve-fitting and IC_{50} calculations were performed using GraphPad Prism 5. For covalent cross-linking, IDE and ^{125}I-insulin were incubated on ice in the absence and presence of excess (1uM) insulin, relaxin, relaxin-3 or InsL3 for 40 minutes, and then exposed to BS^3 for 30 minutes. Samples were separated on SDS-PAGE, and radiolabeled bands were detected using a Storm phosphorimager (Molecular Dynamics). To determine degradation of native peptides, recombinant IDE was prepared using 293T cells transfected with a plasmid encoding human IDE fused to polyhistidine and FLAG tags at the N-terminus, and purified using cobalt affinity chromatography. Each peptide (2ug) was incubated alone or with IDE, in the absence or presence of the IDE inhibitor 1,10-phenanthroline (1mM). Samples were subject to 16% Tris/tricine gels and stained with Coomassie blue. The peptide bands were detected using near-infrared fluorescence on an Odyssey imager.

Results

The interaction of relaxin family peptides with IDE was examined by competitive inhibition of ^{125}I-insulin degradation. Consistent with previous results [3], unlabeled insulin reduced ^{125}I-insulin degradation with an IC_{50} of 36.3nM, while relaxin was less potent at 182.0nM. Interestingly, both relaxin-3 and InsL3 inhibited insulin degradation with potencies approaching that of insulin (53.7nM and 44.4nM, respectively). To determine if the relaxin family peptides were interacting with IDE at the same site as insulin, covalent cross-linking of ^{125}I-insulin to IDE was examined in the presence of excess concentrations of unlabeled insulin, relaxin, relaxin-3 or InsL3. In the absence of unlabeled competitor, a radiolabeled band migrating at approximately 110kDa was readily detectable, consistent with IDE cross-linked to ^{125}I-insulin. No radiolabeled bands were detected without the cross-linking reagent, or by cross-linking of ^{125}I-insulin without IDE. In the presence of excess unlabeled insulin, the band at 110kDa was undetectable. Similarly, cross-linking of insulin to IDE was completely blocked in the presence of relaxin, relaxin-3 or InsL3.

To determine if relaxin family peptides are substrates for the proteolytic activity of IDE, each peptide was exposed to IDE or buffer alone, and the disappearance of intact peptide was monitored using SDS-PAGE chromatography and staining. As shown previously [10], insulin was readily degraded by IDE, and this degradation was blocked in the presence of the zinc chelating agent 1,10-phenanthroline. Relaxin was also readily degraded, and this degradation was blocked with 1,10-phenanthroline. Both relaxin-3 and InsL3 were also

degraded, but to a much lesser extent than either insulin or relaxin. However, in both cases the degradation was blocked using 1,10-phenanthroline.

Discussion

Several members of the insulin family of hormones are substrates for IDE. For many years, it was known that relaxin would bind to IDE [3], but it was unknown whether relaxin could be degraded by IDE. One report described a “neutral thiol proteinase” (NTP) that degraded monotyrosyl porcine relaxin [9]. While this enzyme shared many characteristics with IDE, there were some inconsistencies that prevented absolute identification of this enzyme as IDE. In this study, we have verified that relaxin interacts with IDE, and inhibits the degradation and binding of insulin by IDE. Relaxin was less potent than insulin in inhibiting IDE, consistent with the lower affinity of relaxin reported for NTP [9]. We have demonstrated that IDE actively degrades relaxin. Taken together, these data suggest that NTP was most likely IDE.

The more recent identification of the relaxin-like peptides has provided additional potential IDE substrates for investigation. In this study, we provide evidence that relaxin-3 and InsL3 potently inhibit the degradation of insulin by IDE, and inhibit cross-linking of insulin to IDE. This suggests that insulin, relaxin, relaxin-3 and InsL3 all bind to the same site on IDE. In addition, both relaxin-3 and InsL3 were degraded by IDE, although to a much lesser extent than either relaxin or insulin. Therefore, the data suggest that relaxin binds IDE with relatively low affinity, but is degraded at a rapid rate. Conversely, InsL3 and relaxin-3 bind IDE with high affinity, but are slowly degraded.

The physiological relevance of IDE degradation of relaxin and related peptides remains to be determined. To date, in studies using rats with impaired IDE activity or in IDE-null mice, only insulin and Alzheimer’s beta amyloid peptide have been shown to be physiological IDE substrates [11–13]. IDE is ubiquitously expressed, and therefore is present in tissues that produce relaxin family peptides or express the relaxin family receptors, such as testes, uterus, brain, and kidney [14,15]. The subcellular localization of IDE is predominantly in the cytosol, but it is also found in peroxisomes, mitochondria, and endosomes [1]. IDE has also been identified as a secreted protein in some cells [16,17], as well as associated with the extracellular surface of the plasma membrane [18–20]. Interestingly, relaxin-degrading activity was detected in membrane preparations of rat uterine tissue, and this degradation was blocked by protease inhibitor mix containing iodoacetamide, which is a potent inhibitor of IDE activity [21]. Further study is needed to identify a physiological role of IDE in relaxin, InsL3 and relaxin-3 degradation

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References

1. Duckworth WC, Bennett RG, Hamel FG. Insulin degradation: Progress and potential. Endocrine Reviews. 1998; 19:608–624. [PubMed: 9793760]
2. Becker AB, Roth RA. An unusual active site identified in a family of zinc metalloendopeptidases. Proceedings of the National Academy of Sciences USA. 1992; 89:3835–3839.
3. Ding L, et al. Comparison of the enzymatic and biochemical properties of human insulin-degrading enzyme and *Escherichia coli* Protease III. Journal of Biological Chemistry. 1992; 267:2414–2420. [PubMed: 1733942]

4. Misbin RI, et al. Inhibition of insulin degradation by insulin-like growth factors. Endocrinology. 1983; 113:1525–1527. [PubMed: 6352249]
5. Hamel FG, et al. Identification of the cleavage sites of transforming growth factor a by insulin-degrading enzyme. Biochim Biophys Acta. 1997; 1338:207–214. [PubMed: 9128138]
6. Bennett RG, Duckworth WC, Hamel FG. Degradation of amylin by insulin-degrading enzyme. Journal of Biological Chemistry. 2000; 275:36621–36625. [PubMed: 10973971]
7. Goto S, Kurochkin IV. Alzheimer's β-amyloid peptide interacts with and is degraded by insulin degrading enzyme. FEBS Letters. 1994; 345:33–37. [PubMed: 8194595]
8. MacPhee, CE.; Dobson, CM.; Wade, JD. The formation of amyloid fibrils by relaxin. Third international conferece on relaxin and related peptides; Kluwer Academic Publishers; 2000. p. 399-404.
9. Pilistine SJ, Varandani PT. Degradation of porcine relaxin by glutathione-insulin transhydrogenase and a neutral peptidase. Molecular and Cellular Endocrinology. 1986; 46:43–52. [PubMed: 3519316]
10. Bennett RG, et al. Insulin inhibition of the proteasome is dependent on degradation of insulin by insulin-degrading enzyme. Journal of Endocrinology. 2003; 177:399–405. [PubMed: 12773120]
11. Fakhrai-Rad H, et al. Insulin-degrading enzyme identified as a candidate diabetes susceptibility gene in GK rats. Hum Mol Genet. 2000; 9:2149–2158. [PubMed: 10958757]
12. Farris W, et al. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta -protein, and the beta -amyloid precursor protein intracellular domain in vivo. Proc Natl Acad Sci U S A. 2003; 100:4162–4167. [PubMed: 12634421]
13. Miller BC, et al. Amyloid-{beta} peptide levels in brain are inversely correlated with insulysin activity levels in vivo. Proc Natl Acad Sci U S A. 2003
14. Baumeister H, et al. The rat insulin-degrading enzyme: Molecular cloning and characterization of tissue-specific transcripts. FEBS Letters. 1993; 317:250–254. [PubMed: 8425612]
15. Kuo WL, Montag AG, Rosner MR. Insulin-degrading enzyme is differentially expressed and developmentally regulated in various rat tissues. Endocrinology. 1993; 132:604–611. [PubMed: 7678795]
16. Qiu WQ, et al. Insulin-degrading enzyme regulates extracellular levels of amyloid beta- protein by degradation. Journal of Biological Chemistry. 1998; 273:32730–32738. [PubMed: 9830016]
17. Safavi A, et al. Identification of gamma-endorphin-generating enzyme as insulin- degrading enzyme. Biochemistry. 1996; 35:14318–14325. [PubMed: 8916918]
18. Lynch JA, et al. Insulin degrading enzyme is localized predominantly at the cell surface of polarized and unpolarized human cerebrovascular endothelial cell cultures. Journal of Neuroscience Research. 2006; 83:1262–1270. [PubMed: 16511862]
19. Takeuchi F, et al. Insulin degradation by Madin-Darby canine kidney cells expressing the insulin receptor. Diabetes Research and Clinical Practice. 1997; 37:81–90. [PubMed: 9279478]
20. Vekrellis K, et al. Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. J Neurosci. 2000; 20:1657–1665. [PubMed: 10684867]
21. Mercado-Simmen RC, Bryant-Greenwood GD, Greenwood FC. Characterization of the binding of 125I-relaxin to rat uterus. J Biol Chem. 1980; 255:3617–3623. [PubMed: 6245087]