Structural Characterization of the Insulin-Degrading Enzyme by Microfluidic Modulation Spectroscopy

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Abstract

Insulin-degrading enzyme (IDE) is a ubiquitously expressed Zn^{2+} metalloprotease that digests several key substrates including insulin, glucagon, amyloid-β, and amylin. The structure of IDE under conditions of proteolysis is not known. Here, we used a new bioanalytical technique called Microfluidic Modulation Spectroscopy to directly probe the backbone structure of IDE in the absence and presence of ATP and insulin. In the presence of ATP, the backbone structure of IDE does not change. In contrast, the structure of IDE is altered in the presence of insulin such that the percentage of random coil is increased at the expense of β-sheet. Together, our results show that the interaction of ATP with IDE is localized to sidechains but the interaction of insulin with IDE leads to a perturbation in the backbone structure of the enzyme.

Introduction

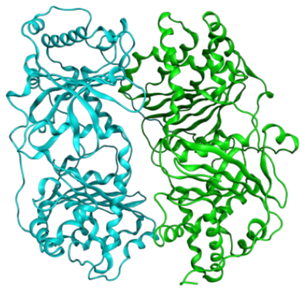


Figure 1. IDE (4PES (1)) is a 110 kDa protein made up of an N-terminal half (cyan) and a C-terminal half (green) that are joined by a flexible linker (purple). Together, these halves form a catalytic chamber with a volume of ~16,000 Å^3, limiting substrates to small polypeptides such as insulin, glucagon, amyloid-β and amylin (2).

- Because IDE degrades biologically important substrates associated with metabolism and amyloid plaque formation, it is an attractive target for the development of novel therapeutic strategies for type 2 diabetes and the amyloid diseases including Alzheimer's disease.
- An important requirement is the identification of regulators that modulate the activity of IDE *in vivo*.
- ATP present in cells at concentrations ranging from 1-10 mM may regulate the activity of IDE towards a particular substrate.
- Recently, we obtained Michaelis-Menten kinetic constants (Table 1) showing that ATP regulates the activity of IDE towards insulin but the addition of $MgCl_2$ abolishes the regulation.

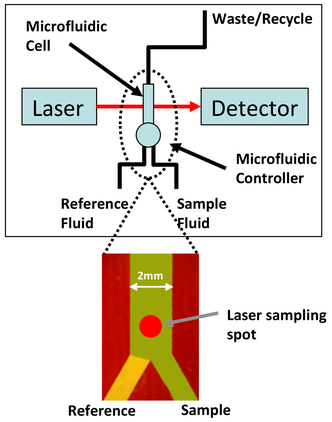
Table 1. Kinetic constants for the IDE-dependent proteolysis of insulin at pH 7.4 and 37 °C.

Small molecule	K_m (M)	k_{cat} (sec^{-1})	k_{cat}/K_m (M^{-1}sec^{-1})
None	$2.0 \pm 0.04 \times 10^{-5}$	$4.8 \pm 0.08 \times 10^{-2}$	$2.4 \pm 0.02 \times 10^{3}$
1 mM ATP	$3.0 \pm 0.2 \times 10^{-5}$	$5.6 \pm 0.2 \times 10^{-2}$	$1.8 \pm 0.07 \times 10^{3}$
1 mM ATP + 1 mM $MgCl_2$	$2.0 \pm 0.05 \times 10^{-5}$	$4.4 \pm 0.07 \times 10^{-2}$	$2.1 \pm 0.02 \times 10^{3}$

- The overall goal of this project is to elucidate the mechanism of the regulation of IDE by ATP. A specific aim is to determine if the regulation by ATP is mediated by structural changes in IDE. To do so, we used a novel bioanalytical technique called Microfluidic Modulation Spectroscopy (MMS) developed by RedShiftBio.

Microfluidic Modulation Spectroscopy

MMS uses a tunable mid-IR laser to probe the amide I band of the sample and advanced software to deconvolute the signal to determine the Higher Order Structures (HOS). In the flow cell, the sample is modulated against the buffer it is dissolved in allowing for accurate baseline subtraction and a drift-free signal (4).



Relative to other bioanalytical techniques, MMS offers the following advantages: wider concentration range (0.1-200 mg/mL), better sensitivity, multiple sample automation, and it is capable of measuring aggregation, quantitation, stability, similarity and structure.

Experimental

- An inactive form of IDE in which the catalytically active glutamate residue (Glu111) was substituted by glutamine was produced.
- The following samples were then prepared in 50 mM Tris pH 7.4: IDE only, IDE + ATP, IDE + ATP + $MgCl_2$, insulin only, and IDE + insulin.
- The concentrations of IDE or insulin was set at 0.57 mg/mL.
- The concentration of ATP or ATP-Mg^{2+} was set at 4 mM.
- IR spectra were recorded using the following parameters: room temperature, scanning every 4 cm^{-1}, using a pressure of 5 psi and modulating at 1 Hz.

Results and Discussion

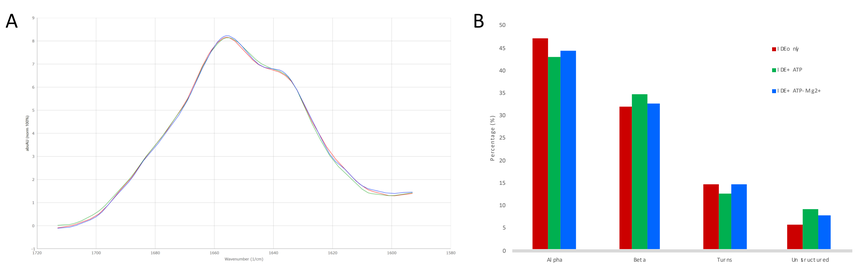


Figure 3. The structure of IDE does not change in the presence of ATP or ATP + $MgCl_2$. Absorbance spectra (A) of IDE only (red), IDE + ATP (green) and IDE + ATP-Mg^{2+} (blue) shows all three spectra overlay very well. Deconvolution (B) verifies that there is very little change in the secondary structure distribution of IDE.

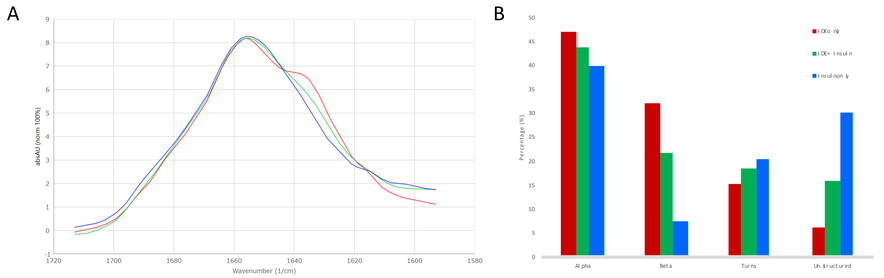


Figure 4. The structure of IDE changes in the presence of insulin. Absorbance spectra (A) of IDE only (red), insulin only (green) and IDE + insulin (blue) shows a significant decrease in the IDE signal at 1638 cm^{-1} when in the presence of insulin. Deconvolution (B) quantifies a decrease in β-sheet and an increase in random coil.

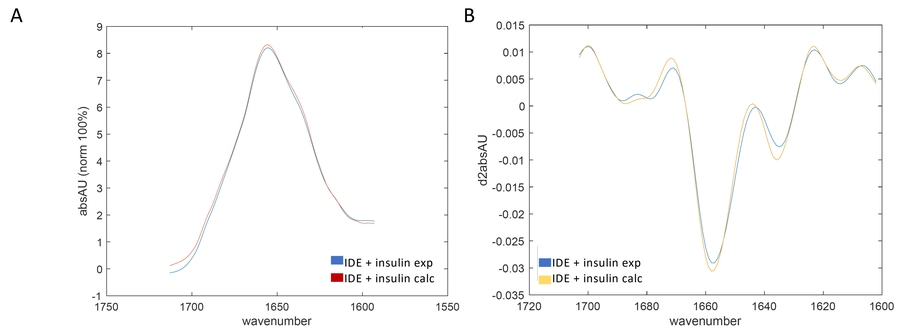


Figure 5. Absorbance spectra (A) of experimentally determined IDE + insulin (exp) and mathematically predicted IDE + insulin (calc) along with the second derivative plot (B). This shows the ability to accurately predict a spectrum of two proteins, and any differences between the calculated spectrum and the experimentally determined spectrum are due to interactions that affect the secondary structure of the proteins.

Conclusions

- Our results show that the regulation of IDE by ATP is not mediated by structural changes in the enzyme.
- The regulation by ATP may be mediated by the binding of the small molecule to positively charged residues in the C-terminal half of IDE. When $MgCl_2$ is present, the binding and thus the regulation is abolished
- In contrast, insulin induces a conformational change in IDE. We propose that this change is due to the accommodation of a much larger molecule in the catalytic chamber of IDE.
- This work clearly shows that MMS can be used to investigate ligand-protein and protein-protein interactions associated with enzymatic reactions.

References

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(3) Patel, L. et al. *Science* 2017, 356 (6339), 753-756.
(4) Ma, E. Microfluidic Modulation Spectroscopy (MMS) Application Note #101.

Acknowledgements

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