Validating the Effectiveness of Proteolytic Enzyme Supplements to Digest Protein

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Introduction

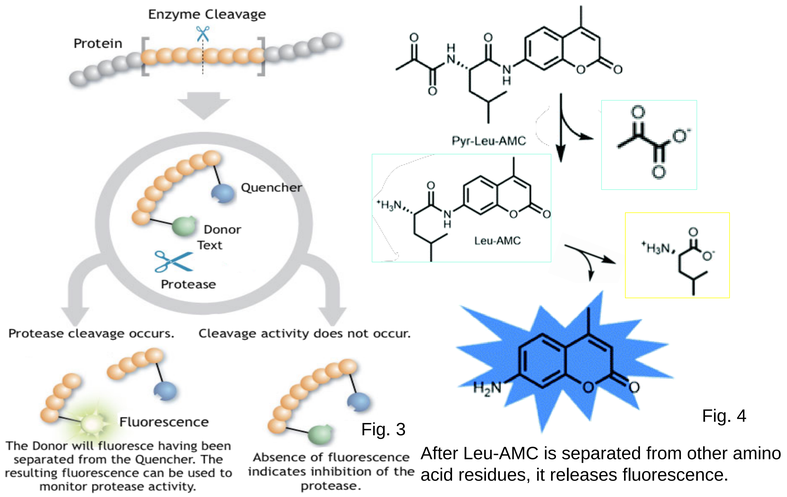


Fig. 3

Fig. 4

After Leu-AMC is separated from other amino acid residues, it releases fluorescence.

Objectives

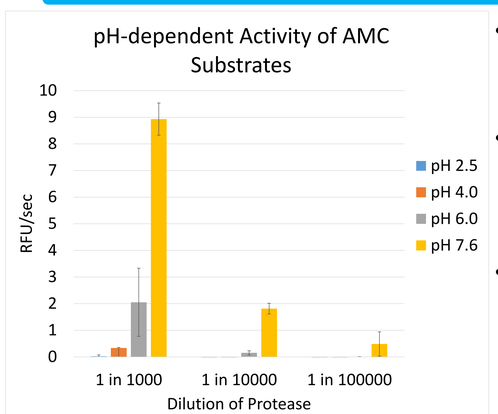
- To determine whether or not proteases from the tablet have detectable activity
- To find the pH range at which the enzyme functions.
- To ascertain if the supplement can digest a broad spectrum of peptides.
- To identify the stability of proteases at different pH's.
- To observe the effectiveness of proteases to denature proteins

Proteolytic Enzymes in Doctor's Best Supplement

Fig. 1, Fig. 2

Name	Source	Enzyme Family	pH Optimum	Substrate Specificity (amino acid)
Bromelain	Pineapple Stem	Cysteine	pH 3.0-6.5	Lysine, alanine, tyrosine, glycine
Papain	Papaya	Cysteine	pH 6.0-7.0	Leucine, glycine (broad range)
a-Amylase	Fungus Aspergillus oryza	Amylolytic	pH 6.7-7.0	X *Only digest carbohydrates
Lipase	Unknown Source	Esterase	pH 4.0-5.0	X *Only digest lipids
Neutral Protease	Seaweed	Metalloprotease	pH 5.9-7.0	Non specific
Fungal Protease 4.0	Fermented Food	Aspartic Acid Protease	pH 4.0-9.0	Cleaves after Phe and Ala residues
Bacterial Protease	Unknown Source	x	Near neutrality	x
Rutin	Citrus Fruits	Glycoside	pH 5.5-6.5	x
Serratiopeptidase	Digestive Tract of Silkworms	metalloprotease	pH 6-8	Cleaves before PHE, ALA residues

Results for Fluorescent Activity in AMC

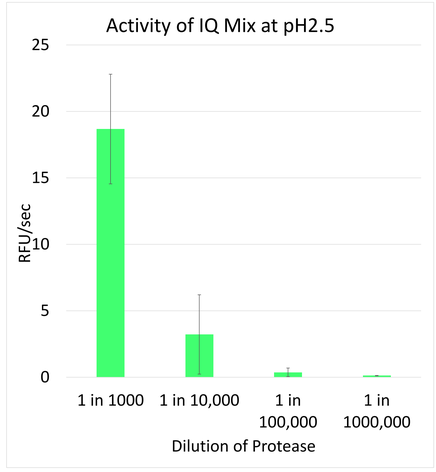


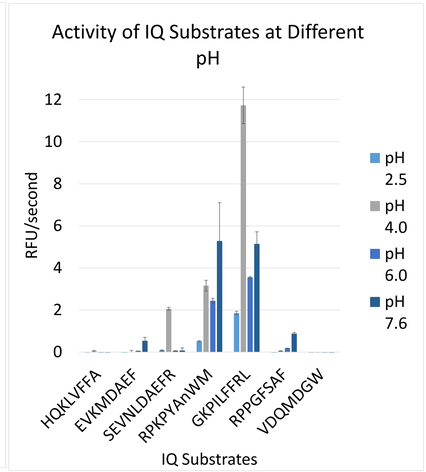
- As the pH lowers, fluorescent activity in AMC substrate decreases due to the acidity inhibiting enzyme catalysis.
- At neutral pH range, rate of enzyme reaction digesting AMC substrate was faster than rest of the pH conditions.
- However, the AMC substrate test is not reliable enough to conclude that neutral pH is the optimum pH for the protease to digest protein well.

Methods

- pH 7.6 assay buffer contains 100 mM of NaCl, 0.01 % of Tween-20, and 20 mM of Tris-HCl
- pH 2.5, 4.0, and 6.0 assay buffers contain 100 mM of NaCl, 0.01 % Tween-20, and 20 mM of citric phosphate.
- All data gathered in RFU/sec was processed in a Synergy | HTX multi-mode

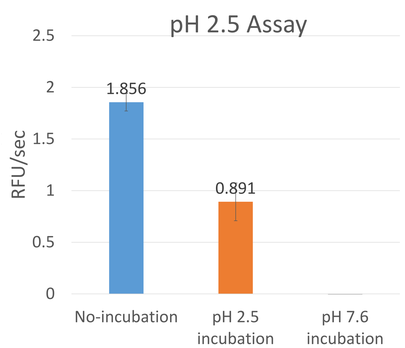
Results for IQ Substrates

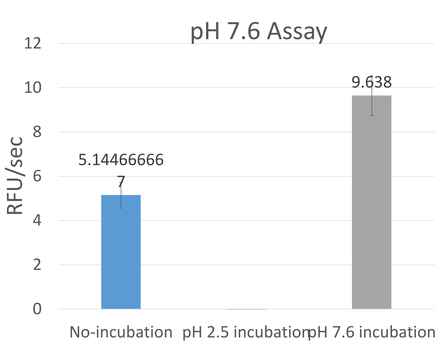




- An assay of serial dilutions of enzyme in IQ mix substrate to distinguish which dilution was suitable to examine protease activity in an MR graph.
- 1 in 10,000 dilution was chosen for its optimum rate of fluorescence at which to view enzyme-IQ activity.
- 1 in 1,000 dilution had a rate of fluorescence at which no crucial data could be detected.
- Tested 7 distinct substrates to determine which substrates would be best suited to detect the protease activity in each pH buffer.
- Substrates EVKMDAEF, RPKPYAnWM, GKPILFFRL, RPPGFSAF displayed activity at all pH's
- These four IQ substrates were chosen for their capability to produce enzymatic reaction under conditions of the stomach.

Stability of Proteases

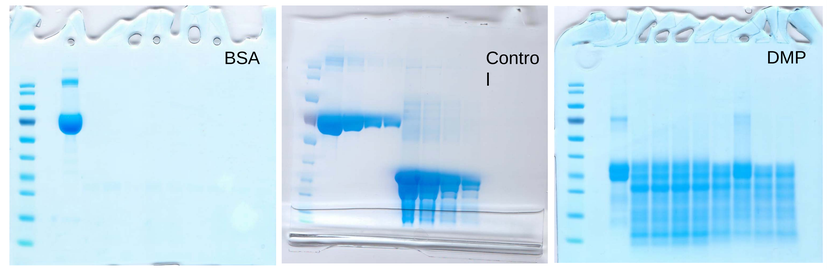




GKPILFFRL IQ Substrate

- Proteases incubated at pH 2.5 then transferred to pH2.5 buffer were still active, while proteases transferred to pH7.6 buffer were not. Proteases incubated at pH7.6 were active when transferred to pH7.6, and inactive when transferred to pH2.5 buffer.
- The graph of no-incubation illustrates higher activity of enzyme, which is caused by diminution of inhibitor.

Effectiveness to Digest DMP, BSA Protein



- Control shows ladder on the left, four lanes of serial dilution of BSA protein in pH2.5 buffer, last four lanes are serial dilutions of milk protein in pH2.5 buffer.
- BSA gel shows ladder on the left, lane of protease, one lane of BSA without protease, eight lanes of BSA protein and enzyme at different incubation times (2, 5, 10, 15, 30, 60, 90, 120 min).
- DMP gel shows ladder on the left, lane of protease, one lane of DMP without protease, eight lanes of DMP protein and enzyme at different incubation times (same as above).
- In BSA gel, 1 in 100 concentration of protease completely denatured the BSA proteins in lanes 8-11.
- In DMP gel, 1 in 100 concentration of protease digested DMP protein.

Discussion and Conclusions

Protease activity was mainly detected in neutral pH conditions for AMC substrates. IQ substrates were used rather than AMC, because proteases active at the low pH of the stomach can produce more fluorescence in IQ substrates. It was found that proteases were unable to survive and function after pH shifts: pH2.5 -> pH7.6, and pH7.6 -> pH2.5. This means that these proteases would not continue to function after traveling from the stomach to neutral-pH small intestines. However, proteases incubated and transferred within the same pH, functioned the same or better when compared to non-incubated enzymes in similar environments. We also found that digestive capabilities of Doctor's Best proteases were unaffected by a 2-hour long incubation within pH2.5 assay buffer, the pH level at which we prepared gel samples. Proteases at 1 in 100 dilutions were able to cut peptide bonds, and produce additional molecular weight bands in the gel in both BSA and milk proteins even after 2 min.

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