Non-Specific Degradation of Peptides is Dependent on End Group Chemistry and Membrane-Type Protease Cleavage of Peptides is Determined by Interior Chemistry

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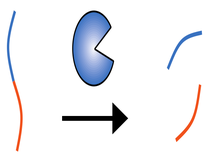
Introduction

Synthetic peptides are used as biomaterials to control a range of biological functions; however, their stability in-situ is almost never validated experimentally. In cell systems, these peptides are suspectable to degradation from cell-secreted peptidases and we have found that peptide degradation can be controlled with the peptides' end group chemistry as well as with specific interior sequences. We have performed a study evaluating a RGD control peptide to evaluate exopeptidase activity. Another study focused on endopeptidases activity on a KLVAD peptide to find interior sequences that are cell membrane specific. A better understanding of how peptide design influences degradation is needed to improve stability of biomaterials.

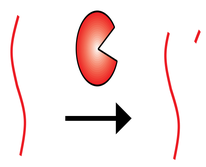
Classes of Proteases

Endopeptidase: *cleaves interior amino acids*



Examples: *MMPs, cathepsins, trypsin, Beta-secretase 1*

Exopeptidase: *cleaves terminal amino acids*



Examples: *aminopeptidase P, dipeptidyl peptidase IV, carboxypeptidase A*

Design Scheme

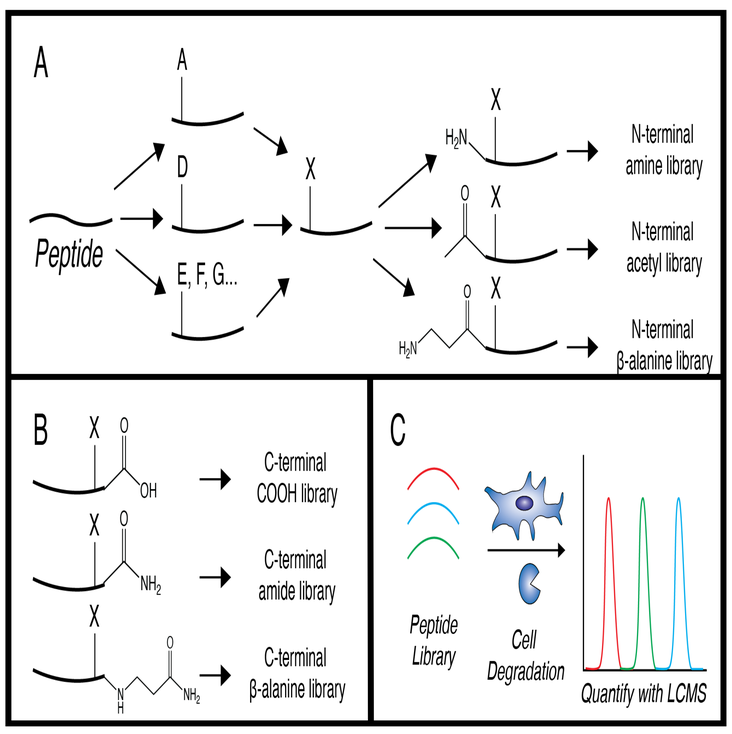
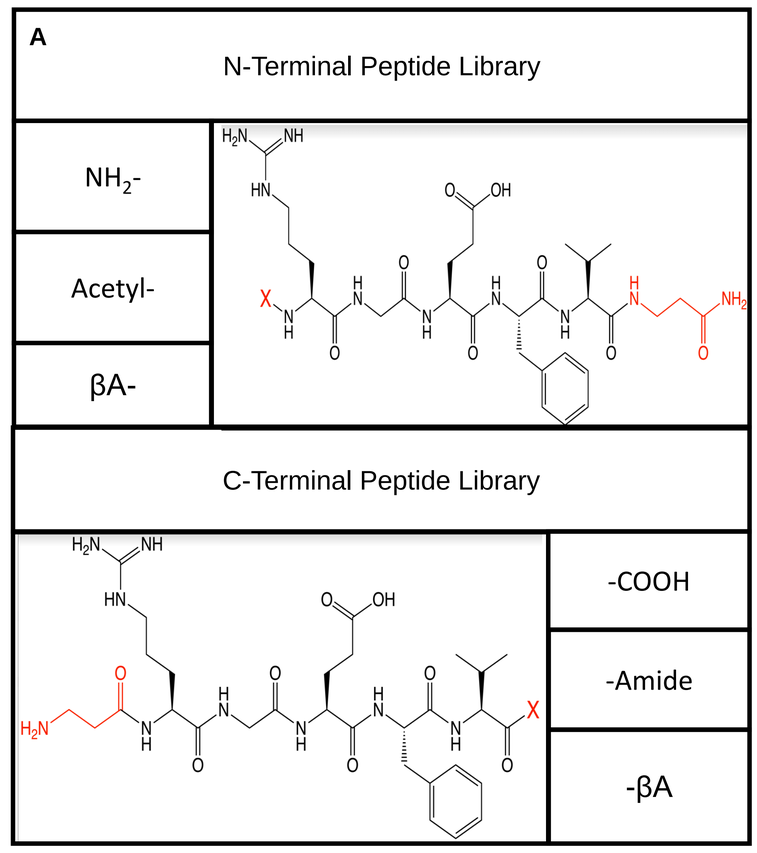


Figure 1. A) Split-pool synthesis paired with solid-phase synthesis was done in which the resin was split into 19 different pools. **B)** These pools had different end group chemistries to study the effects of terminal modification in addition to amino acid sequence. **C)** shows the LCMS peaks representing the relative amounts of peptide left from incubation with cells.

Experimental Setup



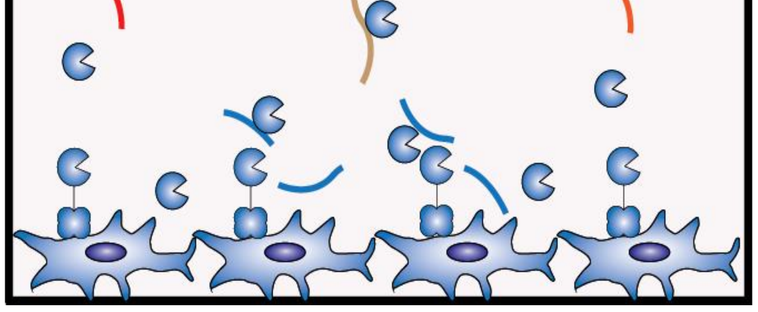


Figure 2 A) Six types of RGEFV peptide libraries were used in this study. X is every amino acid except cysteine. βA is a degradation resistant amino acid. **B)** This peptide library was added to cells after 24 hours of conditioning the media**. C)** Average degradation ratio across all 19 peptides were calculated for M2A macrophages. Averages show significantly greater cleavage of N-terminal NH_2 compared to the other averages ($p<0.001$). βA inhibits degradation regardless of which terminal end it is attached to as the averages for the C and N terminal βA are statistically the same ($p>0.05$).

Membrane Protease Specific Peptides

Figure 3 A) A KLVAD-XX-SAE series of peptides were incubated in either cells or cell-conditioned media. **B and C)** Average macrophage degradation is greater in the cell conditions compared to the media only conditions **D and E)** With M2A macrophage, the sequences shown are particularly more specific to the cell membrane as the cell conditions show greater degradation than the media only conditions.

Conclusion

N-terminal NH_2 peptides are degraded in 24 hours independently of the N-terminal amino acid, while other end terminals had little degradation. Certain sequences shown are membrane specific cleavage sites as cell conditions were degraded more than media in all cases. These results show the non-specific proteolysis of peptides can be largely dependent on end group and interior chemistry. Using non-degradable amino acids like β-alanine may reduce unwanted degradation and avoiding certain sequences may reduce degradation from specifically M2A macrophages.

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