Inhibition of Gliadin-α2 Using Novel Peptide Synthesis

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Purpose

The purpose of this study is to produce a novel peptide sequence that can competitively inhibit tTG-modified gliadin-α2 by having a stronger binding affinity and binding likelihood.

Hypotheses

i) If specific amino acid positions are changed, then the binding affinity of the sequence to a MHC-II molecule will be altered.
ii) If a peptide sequence has a higher binding affinity and likelihood then it will competitively inhibit its natural peptide sequence because it will both bind stronger and be more likely to bind.

Celiac Disease

Celiac Disease (CeD) is a common food-induced inflammatory disease affecting 1% of the global population. It is triggered by ingesting wheat gluten or similar proteins found in cereals like barley and rye. The immune response attacks the small intestine, leading to damage on the villi, which promote nutrient absorption. Treatments include a gluten-free diet, but enzymatic treatments have been suggested for degrading gluten peptides. CeD relies on T-cell recognition (TCR) of gluten-derived peptides bound to HLA-DQ2 or HLA-DQ8 genes, which help the immune system identify harmful agents. Peptides, such as gliadins and glutenins, are non-degradable by most intestinal enzymes and are not degraded by most intestinal enzymes. The gliadin-α2 peptide is of utmost importance in CeD patients as it has the most reported binding and cannot be naturally broken down.

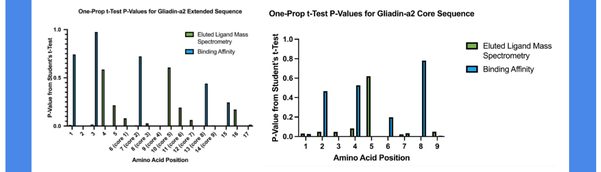
This peptide inhibits the immune system's reaction to the peptides, creating an autoimmune response damaging the patient's body. The goal of this project was determined to create a novel peptide that will competitively inhibit the binding of gliadin-α2 to the MHC-II complex.

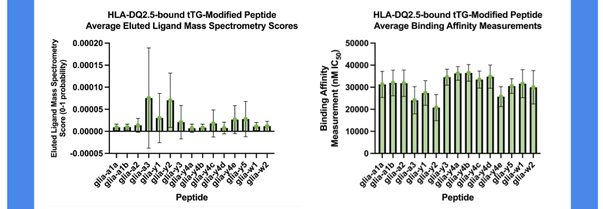
Methods

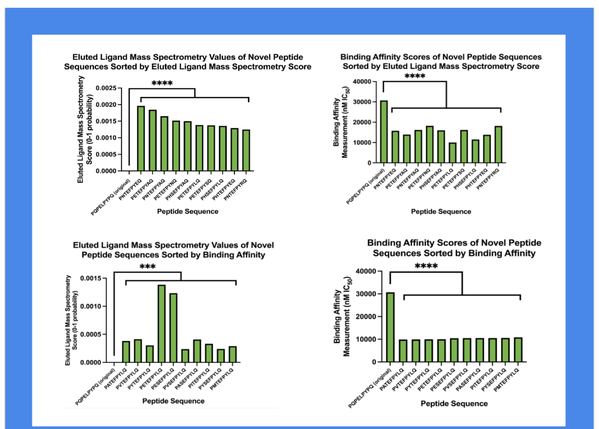
- NetMHCIIpan-4.1 Immune Epitope Database & Tools (IEDB), SYFPEITHI and the RCSB Protein Data Bank (PDB) were used for in silico analysis
- Every permutation combination was generated for the peptide core and analyzed in NetMHCIIpan-4.1 for binding affinity and eluted ligand mass spectrometry to the HLA-DQA10501-B10201 MHC allele
- Peptide core was put into the extended peptide sequence of gliadin-α2. The scores were then re-sorted by highest to lowest score for eluted ligand mass spectrometry and lowest to highest binding affinity, respectively
- One-proportion Student's t-test was used to compare the changes in binding affinity (BA) and eluted ligand mass spectrometry (EL) scores for a modified and unmodified amino acid sequence of the same gluten-derived peptide

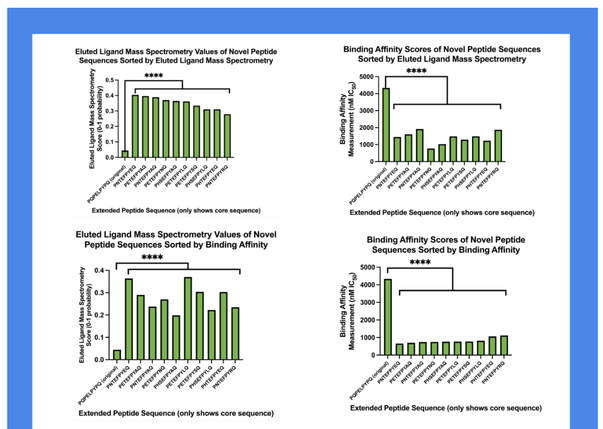
Main Takeaway

This project utilized NetMHCIIpan-4.1 to predict binding affinity and likelihood between peptide sequences and MHC-II molecules. The peptide sequence PETEFPYLQ is in the top 0.001 percent of both binding affinity and likelihood scores.









Conclusions

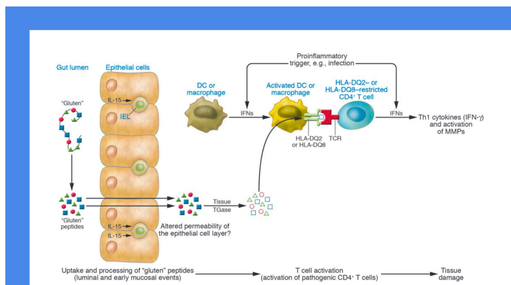
- HLA-DQA10501-DQB10201 MHC-II molecule was the most affected by tTG modification.
- HLA-DQA10301-DQB10302 and HLA-DQA10501-DQB10301 genes were the most affected by tTG modification.
- Gliadin-α3 and -γ2 had the highest eluted ligand scores and the lowest binding affinity measurements, with both sharing a glutamic acid in position 4.
- Positions 1, 4, 6, 7, and 9 in the gliadin-α2 peptide core having hydrogen bonds to the MHC-II molecule.
- The peptide core PETEFPYLQ was the only peptide with a eluted ligand mass spectrometry score and a binding affinity measurement in the top 0.009% of all values, with an 88% change for eluted ligand mass spectrometry and 463% for binding affinity from the original gliadin-α2 peptide sequence.

Significance

CeD is one of the most common diseases caused by eating food, affecting over 70,000,000 people, including over 3,000,000 Americans, where 60-70% of Americans who have the disease are undiagnosed and needlessly suffering (Celiac Disease Foundation, 2016). Little research has been done into CeD treatment because it has long been considered that a gluten-free diet is the only option. However, with newly developed biotechnologies and knowledge, it is now possible to develop a drug that targets the pathology itself. This project presents the possibility for a novel peptide-based approach using the sequence PETEFPYLQ by competitively inhibiting the gliadin-a2 peptide, the most prevalent gluten-derived peptide in CeD patients. This peptide could significantly reduce the effects of gliadin-a2 on the immune system and does not affect any CeD blood tests, proving it useful for CeD therapeutics.

Future Work

As mentioned in the limitations and challenges, an in vitro analysis of the peptide QLQPFPETEFPYLQPQP is the most necessary future research from this project. This would consist of a binding affinity test and eluted ligand mass spectrometry test for T-cell proliferation and mass spectrometry to HLA-DQA10501B10201. Future extensions could link these findings to other peptides related to CeD, or any other autoimmune disease relying on T-cell recognition of antigen-presenting cells.



Pathogenesis of CeD. (Kagnoff, 2007)