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Immunoproteomics characterization of allergenic and non-allergenic tropomyosin orthologs

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Background
The digestion pattern of proteins plays a key role in allergenicity. If the enzymatic digestion is not efficient, bigger peptides/proteins can persist and cause sensitization. Tropomyosin represents the major allergen of crustaceans, which is a highly conserved protein present in muscle cells of vertebrates and invertebrates. The hypothesis of the current work is that shrimp tropomyosin (TM) is not fully digested or exhibits a digestion pattern that generates some peptides that can be immunogenic. To assess this hypothesis, both purified and recombinant chicken and shrimp TM orthologs were in vitro digested and analyzed for their degradation and immunoreactivity pattern.
Methods
One milligram of both shrimp and chicken TM orthologues has been processed through simulated oral, gastric and intestinal digestion using INFOGEST protocol. The cleavage pattern was subsequently analysed by proteomics and immunoproteomics using 1D and 2D tricine gel electrophoresis coupled with mass spectrometry. The immunoreactivity pattern was evaluated using 1D immunoblotting against serum (IgE and IgG) of patients allergic to shrimp TM (allergen Pen a 1).
Results
2D Tricine-SDS-PAGE analysis revealed that chicken TM ortholog was sensitive to proteolytic activity during stimulated gastric digestion with pepsin at low pH, in contrast to shrimp TM ortholog that was not cleaved. These results were consistent with the immunoreactivity assays, which demonstrated a high reactivity of both IgE and IgG against shrimp TM after oral and gastric digestion and no reactivity against chicken TM.
Conclusion
The resistance of shrimp TM ortholog to gastric simulated digestion supports the hypothesis that undigested proteins may enhance the possibility of sensitization process. Considering the importance of protein cleavage before absorption it can be concluded that simulated gastric digestion coupled to the used immunoproteomics approach could represent a starting point for the evaluation of allergenicity of novel foods.
Keywords
Tropomyosin, digestomics, immunoproteomics, allergenic peptides.
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