Bioinformatics-Based Prediction of B- and T-Cell Epitopes in R-Mandelonitrile Lyase, a Recently Described Peach Allergen

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Bioinformatics is a potent tool for the characterization of proteins and prediction of their immunological properties. It also serves as the cornerstone for identifying the IgE-epitome of allergens and its connection with the immune system. In recent years, multiepitope-based vaccines have been developed for COVID-19 and other infectious diseases [1]. Using bioinformatics, we aimed to predict the B epitopes of R-mandelonitrile lyase (RML) [2], a recently described peach allergen, to compare the results with those of an experimental analysis and to predict major histocompatibility complex (MHC) class II–binding epitopes.

Two RML isoforms were identified, namely, A0A251QUN1 and A0A251QUN8 (http://uniprot.org). A0A251QUN8 showed the higher score in mass spectrometry analysis [2] and was selected for this study. The first 21 amino acids (aa) corresponding to a signal peptide were not considered in the protein structure. The tertiary structure was modeled in AlphaFoldDB (https://alphafold.ebi.ac.uk/), and images of the 3D structure were generated with Pymol (Figure S1).

B-cell epitopes of RML were predicted using BepiPred-2.0 [3] and BepiPred-3.0 [4] with scores of 0.5 and 0.04, respectively. Eleven epitopes were predicted with BepiPred-2.0 (Figure S2, Table S1) and 15 with BepiPred-3.0 (Figure S2, Table S2). Only peptides between 5 and 22 aa were considered, because most B-cell epitopes are of this length [5].

To experimentally determine IgE-binding regions of RML, 86 peptides (15-aa peptides labeled with N-terminal biotin) spanning the entire sequence of RML were designed, each overlapping by 9 aa (Table S3). IgE epitopes were identified using ELISA. Briefly, microplates were coated with antihuman-IgE monoclonal antibody, and a pool of 22 sera from patients sensitized to RML was added (1/3 dilution) (Study PI-4513, approved by the Ethics Committee of Hospital

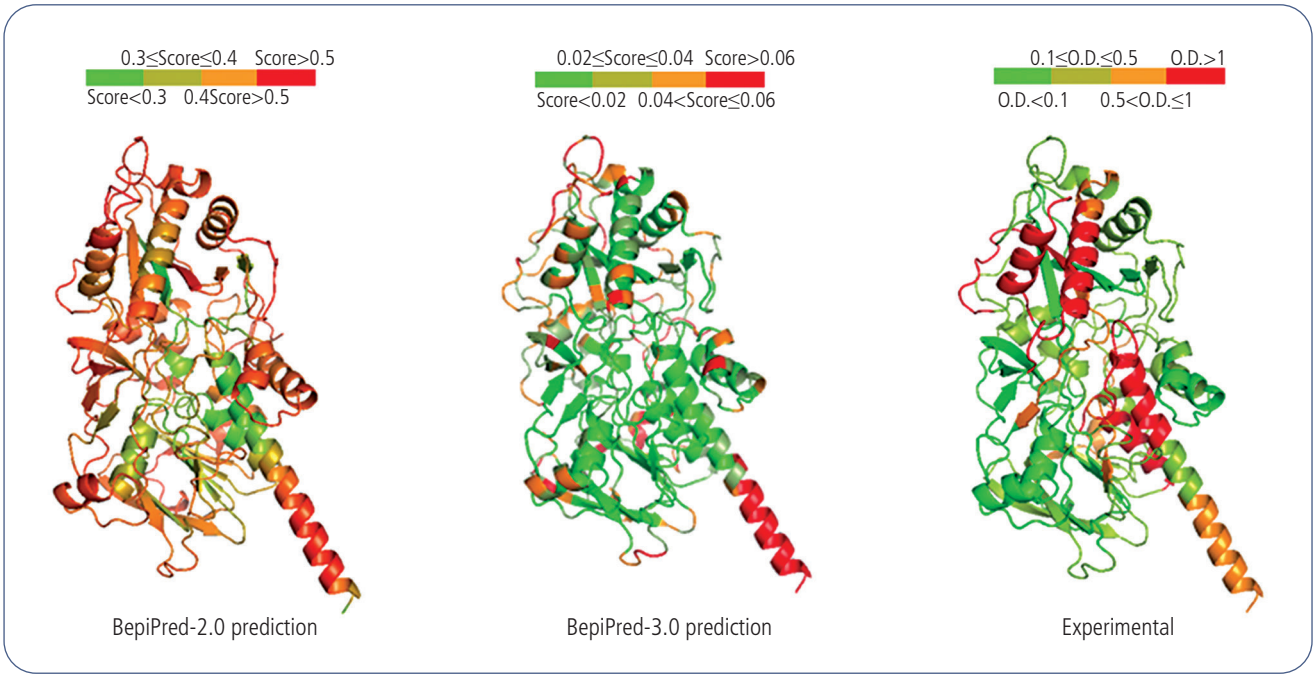

Figure. Epitope location within the 3D structure of the R-mandelonitrile lyase allergen from peach, as predicted by BepiPred-2.0 (left) and by BepiPred-3.0 (center) and determined experimentally (right).

La Paz, Madrid, Spain) [2]. Individual biotin-labeled peptides were added to each well (50 µg/well), and streptavidin-HRP was used to detect peptides bound to IgE. Thirty-seven of the peptides bound to IgE at levels above background (optical density >0.1; 3× negative control values) (Figure S2). Most of the peptides were located in the RML C-terminus.

Comparison of the predicted epitopes with the experimental ones confirmed that 6 peptides of the 11 predicted by BepiPred-2.0 (54.5%) and 11 of the 15 predicted by BepiPred-3.0 (73.3%) bound IgE (Figure S2, S3, Tables S1, S2). Seven epitopes coincided for both prediction tools. Four were predicted only with BepiPred-2.0 and 8 only with BepiPred-3.0 (Figure S3). Predicted epitopes were considered coincident if they shared at least half of the aa with the peptides that were experimentally shown to bind IgE or with the epitopes predicted by the alternative BepiPred tool. Predicted B-cell epitopes and experimental IgE epitopes were then identified within the 3D structure of peach RML (Figure). The number of predicted B-cell epitopes was lower than that of the experimentally confirmed epitopes probably because BepiPred-2.0 and BepiPred-3.0 are fed with experimental data and the systems need more data to increase their precision, especially IgE binding data.

The poor utility of epitope prediction methods for allergens was recently described [6]. BepiPred-2.0 is a tool for sequence-based B-cell epitope prediction that is trained only on epitope data derived from antibody-antigen crystal structures [3]. BepiPred-3.0 is an improved version based on protein language models [4]. We used both tools to test whether BepiPred-3.0 more effectively predicted allergen epitopes. However, in both cases, the data used to train these systems were based mainly on the use of IgG antibodies. Since the study of the allergen-IgE structure has only recently been made possible [7], few structures are included in databases. Despite the limitations of prediction tools, a high percentage of epitopes predicted using BepiPred-3.0 matched with the experimental results.

As a second objective, we predicted epitopes binding to the MHC class II isotypes HLA-DR, HLA-DQ, and HLA-DP [8] using NetMHCIIpan-4.1 based on artificial neural networks. These alleles of MHC class II have been linked to food allergy [9]. T-cell epitopes retain immunogenicity but have low allergenicity, making their identification of great interest in the design of new allergy treatments.

A total of 54 strong binder epitopes (% rank <0.5) were identified within peach RML, distributed among 8 regions of the sequence (Table S4, Figure S4). Seven of the areas with predicted strong binder T-cell epitopes overlapped, at least in part, with predicted B-cell epitopes (Figure S5) (4 predicted with BepiPred-2.0 and 6 with BepiPred-3.0). Five of these epitopes were also in the regions identified as IgE binders in the in vitro assay. All these areas are located on the solvent-exposed side of the protein. Thus, the overlapping B- and T-cell epitopes may be a favorable therapeutic alternative when attempting to induce both types of responses. Experimental studies are necessary to confirm that the predicted T-cell epitopes bind MHC class II in vivo.

Despite the growing relevance of the epitome in various diseases, information about the epitope map for allergens remains limited [6]. The main use of epitope prediction is to determine a protein's immunological capacity. In the case of peach, only lipid transfer protein has been studied in detail [10,11]. The present study is the first to identify the B-cell and T-cell epitopes of peach RML and thus discern its immunogenic capacity. A major limitation of this study was that our experimental method is only capable of identifying linear epitopes. Additionally, since we tested overlapping 15-aa

peptides, the reactivity found within multiple overlapping IgE-binding peptides probably points to the presence of a single epitope. Regardless, higher scores were found in the RML C-terminus by both in silico and in vitro analysis.

Bioinformatics offers the potential to predict the immunological properties of protein regions. Although experimental verification is necessary, these tools allow for rapid searches and, as they become more reliable, will enable the design of new molecules. The challenge is to select the best in silico approach for each step, especially given that different tools are designed for similar purposes. The availability of new tools such as AlphaFold-3 [12] will advance vaccine design, since it enables the prediction of a protein's capacity to bind to a selected molecule.

In summary, we successfully used bioinformatics to predict the linear B- and T-cell epitopes of RML and experimentally confirmed the B-cell epitopes.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Previous Presentation

Some of the results of this study were presented as a flash talk poster at the EAACI Congress, Valencia 2024.

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