RESEARCH ARTICLE

Molecular Nutrition Food Research

Peach Allergen

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Rational Design, Structure–Activity Relationship, and Immunogenicity of Hypoallergenic Pru p 3 Variants

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Scope: Allergies to lipid transfer proteins involve severe adverse reactions; thus, effective and sustainable therapies are desired. Previous attempts disrupting disulfide bonds failed to maintain immunogenicity; thus, the aim is to design novel hypoallergenic Pru p 3 variants and evaluate the applicability for treatment of peach allergy.

Methods and results: Pru p 3 proline variant (PV) designed using in silico mutagenesis, cysteine variant (CV), and wild-type Pru p 3 (WT) are purified from *Escherichia coli*. Variants display homogenous and stable protein conformations with an altered secondary structure in circular dichroism. PV shows enhanced long-term storage capacities compared to CV similar to the highly stable WT. Using sera of 33 peach allergic patients, IgE-binding activity is reduced by 97% (PV) and 71% (CV) compared to WT. Both molecules show strong hypoallergenicity in Pru p 3 ImmunoCAP cross-inhibition and histamine release assays. Immunogenicity of PV is demonstrated with a phosphate-based adjuvant formulation in a mouse model.

Conclusions: An in silico approach is used to generate a PV without targeting disulfide bonds, T cell epitopes, or previously reported IgE epitopes of Pru p 3. PV is strongly hypoallergenic while structurally stable and immunogenic, thus representing a promising candidate for peach allergen immunotherapy.

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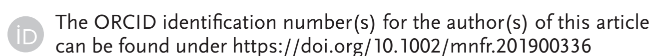
The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.201900336

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DOI: 10.1002/mnfr.201900336

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1. Introduction

According to the WHO, the worldwide number of people suffering from food allergy (FA) is estimated to be 520 million.[1] In Westernized countries, up to 8% of children and 5% of adults are affected by IgE-mediated FA.[2,3] Besides primary sensitization to the offending food, allergic reactions can also be a consequence of IgE cross-reactivity, mainly due to preceding pollen allergies, the so-called pollen food syndrome, which typically presents with milder symptoms. In contrast, patients primary sensitized via the gastrointestinal tract or skin can be confronted with more severe and systemic reactions.[4,5] Since those food allergies are typically persistent in adulthood and can cause life-threatening symptoms, there is a need for an effective and sustainable therapy.

The current approach in FA management—allergen avoidance and rescue medication in case of accidental exposure—strongly impairs the patient's quality of life and might lead to nutritional deficiencies.[6] At present, novel strategies for treatment of IgE-mediated FA through the oral, sublingual, or epicutaneous routes are investigated.[3] However, clinical benefits mostly confine to active treatment regimens, while sustainable tolerance as observed upon subcutaneous allergen immunotherapy (AIT) could not be proven. Based on recent studies, guidelines of the European Academy of Allergy and Clinical Immunology recommend oral immunotherapy (OIT) for cow's milk, hen's egg, and peanut allergic children to induce desensitization.[3] However, FA immunotherapy needs further evaluation regarding standardization of food formulations, and represents a demanding and time-consuming treatment, which should only be carried out in appropriate medical centers due to potential severe side effects. In the past, subcutaneous therapies using food extracts led to severe reactions or limited efficacy.[7,8] An approach to diminish side effects is the rational design of hypoallergens, exploring structural alterations or peptides, thereby reducing the IgE-binding capacity.[9–11] Such strategies were shown to be clinically effective for inhalant allergies and are considered to provide a long-lasting therapeutic effect for food allergies.[12]

Non-specific lipid transfer proteins (LTPs) belong to the prolamin superfamily, which share a common globular alpha-helical fold stabilized by intramolecular disulfide bonds.[13,14] These characteristics confer a strong resistance to heat, harsh pH conditions, and gastric proteolysis, contributing to their ability to sensitize during food ingestion.[15–18] Therefore, they served as models for plant food allergy and were extensively studied during the past decades.[18–20] LTPs are one of the major causes of FA in Mediterranean countries, though some recent studies also suggest LTP sensitization distinct from this geographic location.[18,21–25] Their high structural homology gives rise to the LTP-syndrome, characterized by clinically relevant IgE cross-reactivity between homologs from various fruits and vegetables.[26] Pru p 3 is considered the primary sensitizer for most patients and a positive correlation between Pru p 3 IgE-levels and the number of LTP plant-food causing allergic symptoms was demonstrated.[18,27] A recent study suggested the relevance of the natural Pru p 3-lipid ligand to act as adjuvant for allergic sensitization.[28]

Although usefulness of sublingual immunotherapy (SLIT) with a Pru p 3 quantified peach extract was demonstrated, immunologically characterized, and beneficial for peach and LTP-related peanut allergic patients,[29–31] no patients' data regarding long-term efficacy are available. A recent SLIT study using grass pollen tablets showed a sustained clinical response after discontinuation[32] and induction of long-lasting tolerance for Pru p 3 peptides conjugated to mannose nanostructures was suggested in a murine model.[33] So far, attempts to generate suitable (hypoallergenic) Pru p 3 vaccine candidates for use in AIT proved to be challenging. Mutations of known B- and/or T-cell epitopes of Pru p 3 showed that single mutants still demonstrated significant IgE binding, while exchange of more residues typically affected protein stability.[34–37] Targeting the disulfide bond stabilized structure by Pru p 3 reduction and alkylation resulted in low allergenicity but diminished immunogenicity in a sensitization and therapeutic mouse model.[38,39] Within the framework of the EU-funded Food Allergy Specific Therapy (FAST) project, a panel of Pru p 3 candidate molecules was investigated but did not achieve set criteria of allergenicity, immunogenicity, stability, and solubility.[40] We, therefore, conclude that designing a Pru p 3 hypoallergen is finding a fine balance between eliminating IgE epitopes while preserving sufficient structural integrity.

In this study, we generated two hypoallergenic variants of Pru p 3. Reduced allergenicity was confirmed with a panel of peach allergic patients' sera and immunogenicity was demonstrated in a murine model. In summary, the feasibility of innovative strategies for generation of potential immunotherapeutics was demonstrated and might allow transferring knowledge to other disulfide bond–stabilized allergens.

2. Experimental Section

Detailed protocols used in this study are provided in Supporting Information.

2.1. Patients' Sera

In total, sera from 60 Mediterranean peach allergic patients with clinical symptoms and positive IgE to Pru p 3 were included in the study. Detailed information on demographics, clinical symptoms, and in vitro tests are shown in Table S1, Supporting Information. Usage of the sera was approved by the ethical committee of the clinical centers in Italy (30/CE/2009, ALL-FAST-I EC 2009), Spain (E-09/041), and Greece (6640/15.5.09), and subjects freely accepted to participate after receiving specific information on the study, and signed specific informed consents.

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2.2. Design of Pru p 3 Variants

Pru p 3 proline variant (PV) was designed using ProSa 2003, a tool for validation of protein structures and in silico mutagenesis.[41–43] Substitution of any amino acid besides cysteine in the mature sequence of Pru p 3.0102 were allowed and exchanges with largest z-score increase corresponding to the most destabilizing residues were selected. The other construct, Pru p 3 cysteine variant (CV), was designed to minimally interfere with the disulfide bond–stabilized backbone exchanging two cysteines (C27A, C28A) stabilizing two different disulfide bonds relevant for IgE binding.[44]

2.3. Recombinant Protein Production

Pru p 3 variants were obtained by point mutations of respective amino acids using overlapping PCR products. For expression, PV was cloned into the vector pHis parallel II,[45] while the intein-system was used for the production of CV. Both variants were expressed in *Escherichia coli* Rosetta-gamiB (DE3) pLys S for 20 h at 16 °C and purified to homogeneity. Pru p 3 WT was used as reference and obtained from pET-based expression in *E. coli* Rosetta-gami2 (DE3).

2.4. Physico-Chemical Protein Characterization

Proteins were analyzed by denaturing polyacrylamide gel electrophoresis under reducing and non-reducing conditions. Mass spectrometry analysis was performed, and protein concentration was assessed using amino acid analysis. Circular dichroism was performed for secondary structure analysis; protein homogeneity was assessed by high-performance size-exclusion chromatography (HP-SEC) and dynamic light scattering. For accelerated stability tests, proteins were supplemented with thimerosal as preservative, stored at temperatures ranging from −70 to +40 °C, and analyzed in detail after 2 and 6 months.

2.5. Immunological Characterization of Proteins Using Patients' Sera

IgE reactivity of immobilized Pru p 3 variants and WT was evaluated in ELISA using sera of 33 patients (panel A in Table S1, Supporting Information). In addition, IgE cross-inhibition of PV and CV ($n = 27$) were tested using Pru p 3 ImmunoCAP (f420), and basophil histamine release experiments were performed with serum titrations of 19 patients' samples (panel B in Table S1, Supporting Information).

2.6. Dosage Regimen of Mouse Immunization

Prior to mouse immunization, adjuvant binding kinetics of PV and WT to aluminum hydroxide and phosphate were evaluated in vitro. Endotoxin-free proteins were adsorbed at optimized conditions for 16 h at 4 °C (PV) or 1 h at RT (WT). Female BALB/c mice ($n = 6$ per group) were subcutaneously immunized four times in a 2-week interval using 10 µg protein (= 0.435 mg kg^{-1}) corresponding to 0.035 mg kg^{-1} human equivalent dose. Animal experiments were conducted according to the national guidelines approved by the Austrian Federal Ministry of Science, Research and Economy (BMWF-66.012/0010-II/3b/2013).

2.7. Determination of Murine IgG and IgE Levels and ELISPOT Assay

IgG1 and IgG2a antibody levels were determined in ELISA experiments with serial serum dilutions and determination of end-point limit of detection titers. Murine IgE antibodies were analyzed using rat basophilic leukemia cells, and results are presented as percentage of total β-hexosaminidase release from Triton X-100-treated cells. Murine splenocytes were re-stimulated with either PV or WT and production of IL-4 and IFN-γ was measured by ELISPOT assay.[46]

2.8. Statistical Analysis

Results of the patients' IgE ELISA were statistically analyzed by one-way ANOVA followed by Tukey's multiple comparison post-test using GraphPad Prism. *p*-Values <0.05 were regarded as statistically significant.

3. Results

3.1. Generation and Characterization of Pru p 3 Variants

Using in silico mutagenesis, the four most destabilizing amino acid exchanges were identified to be V6P, L10P, I31P, V34P; thus, the resulting variant was termed Pru p 3 PV. For generation of the CV, cysteine residues 27 and 28 were mutated to alanine (**Figure 1**). Test expressions revealed optimal production rates at lower temperatures (data not shown), and thus, 16 °C was chosen for subsequent expression and purification (**Figure 2**A). The expression band of PV appeared at 12 kDa in gel electrophoresis, and the fusion protein of CV with the intein tag was migrating at a molecular weight around 35 kDa. PV and CV were purified to homogeneity and after auto-cleavage of the CV-intein fusion protein, both molecules were detected at 12 kDa in gel-electrophoresis (Figure 2B). Determination of the protein concentration by amino acid analysis implied a purification yield of ≈15 and 5 mg protein per liter expression culture for PV and CV, respectively. Protein identity was confirmed by mass spectrometry performed with intact proteins as well as peptide mass fingerprints of tryptic digested molecules resulting in sequence coverage of 100% (PV) and 85% (CV).

3.2. Physico-Chemical Protein Characterization

Purified molecules showed neither degradation nor disulfide bond linked oligomerization or aggregation in reducing and non-reducing gel-electrophoresis (Figure 2B). In CD spectroscopy, WT Pru p 3 presented a spectrum typically observed for alpha

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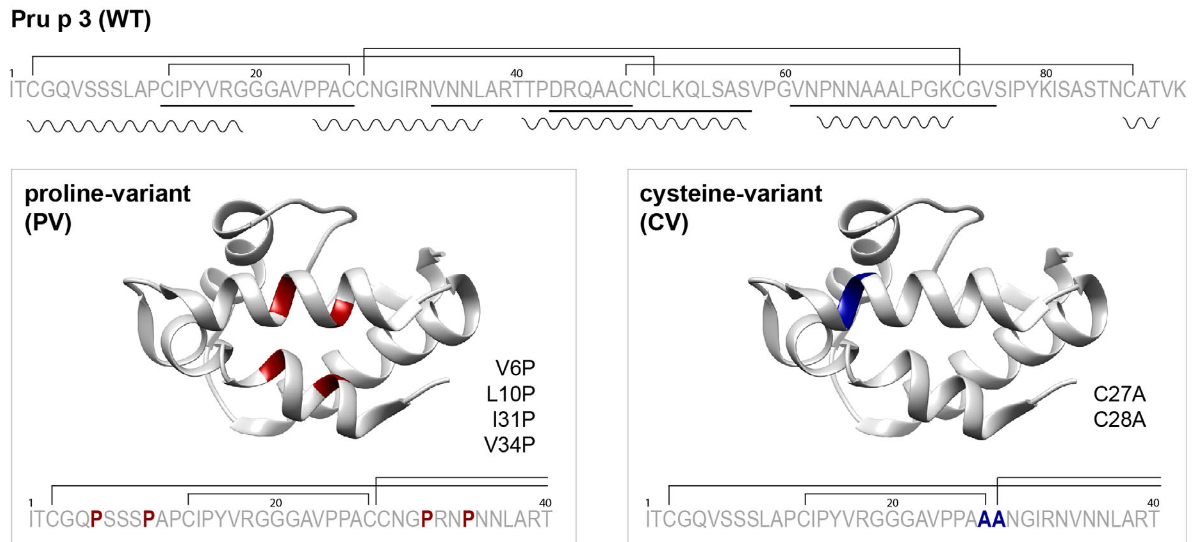

Figure 1. Design of Pru p 3 variants. The disulfide bond pattern, T cell epitopes[50] (solid lines), and alpha helical regions (waved lines) are indicated in the primary sequence of Pru p 3 WT. In the structure of Pru p 3 (PDB:2ALG), amino acids targeted by mutations are indicated in red (PV) or blue (CV). The respective sequence stretch is given below and exchanged amino acids are highlighted in color and bold.

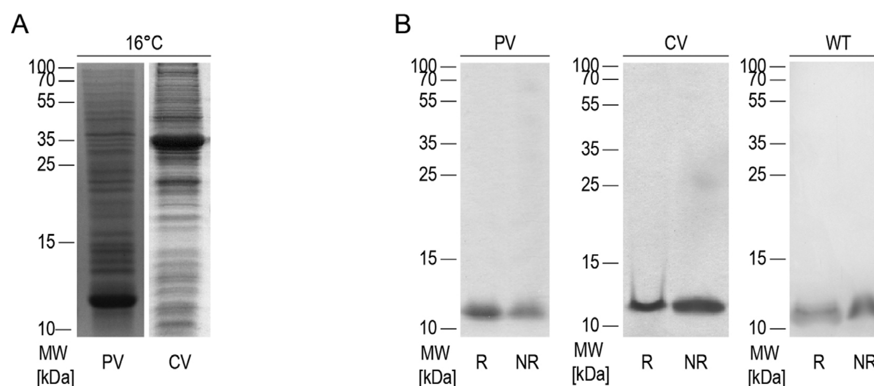

Figure 2. Gel-electrophoresis of Pru p 3 variants and WT molecule. A) Protein expression of PV (migrating at 12 kDa) and CV with a C-terminal intein tag (migrating at 35 kDa) analyzed by SDS-PAGE. B) Gel-electrophoresis of purified Pru p 3 variants and WT under reducing (R) and non-reducing (NR) conditions.

helical proteins like LTPs. In contrast, spectra of the variants resembled irregular structural motifs, and notably, a shift in the composition of secondary structural elements was observed upon heating to 95 °C (**Figure 3**A,B). However, after cooling, both variants were able to regain their conformation as before heat treatment. In HP-SEC, more than 90% of the two variants were observed as monomers showing only a minor tendency toward dimerization or oligomerization, while 98% of WT was present as monomer (Figure 3C). Both Pru p 3 variants showed a slight increase in the hydrodynamic radius compared to the WT molecule, while PV also presented a broader peak suggesting heterogeneous protein conformations (Figure 3C,D).

3.3. Accelerated Stability Test

The shelf life of all proteins was assessed by an accelerated stability test using thimerosal as preservative. Notably, inclusion of thimerosal did not change results of physico-chemical analysis. Samples were analyzed upon storage at different temperatures ranging from −70 to +40 °C after 2 and 6 months (Figure S1, Supporting Information). WT proved to be very stable at all storage conditions. Full integrity of PV was observed at −70, −25, +4 °C, while degradations and aggregations were observed at elevated temperatures (+25 and +40 °C). After 6 months at +40 °C, more than 60% of PV was still detectable as intact molecule in gel-electrophoresis. In contrast, CV was affected by aggregation as well as degradation. While degradation of CV could be prevented by freezing, slight aggregation was observed under all tested conditions. While the performance of CV had some constraints, stability of PV was more similar to WT during shelf life tests (Figure S1, Supporting Information).

3.4. IgE Binding Capacity

The allergenic reactivity of the Pru p 3 variants and WT was investigated using sera from peach allergic patients. Sera from

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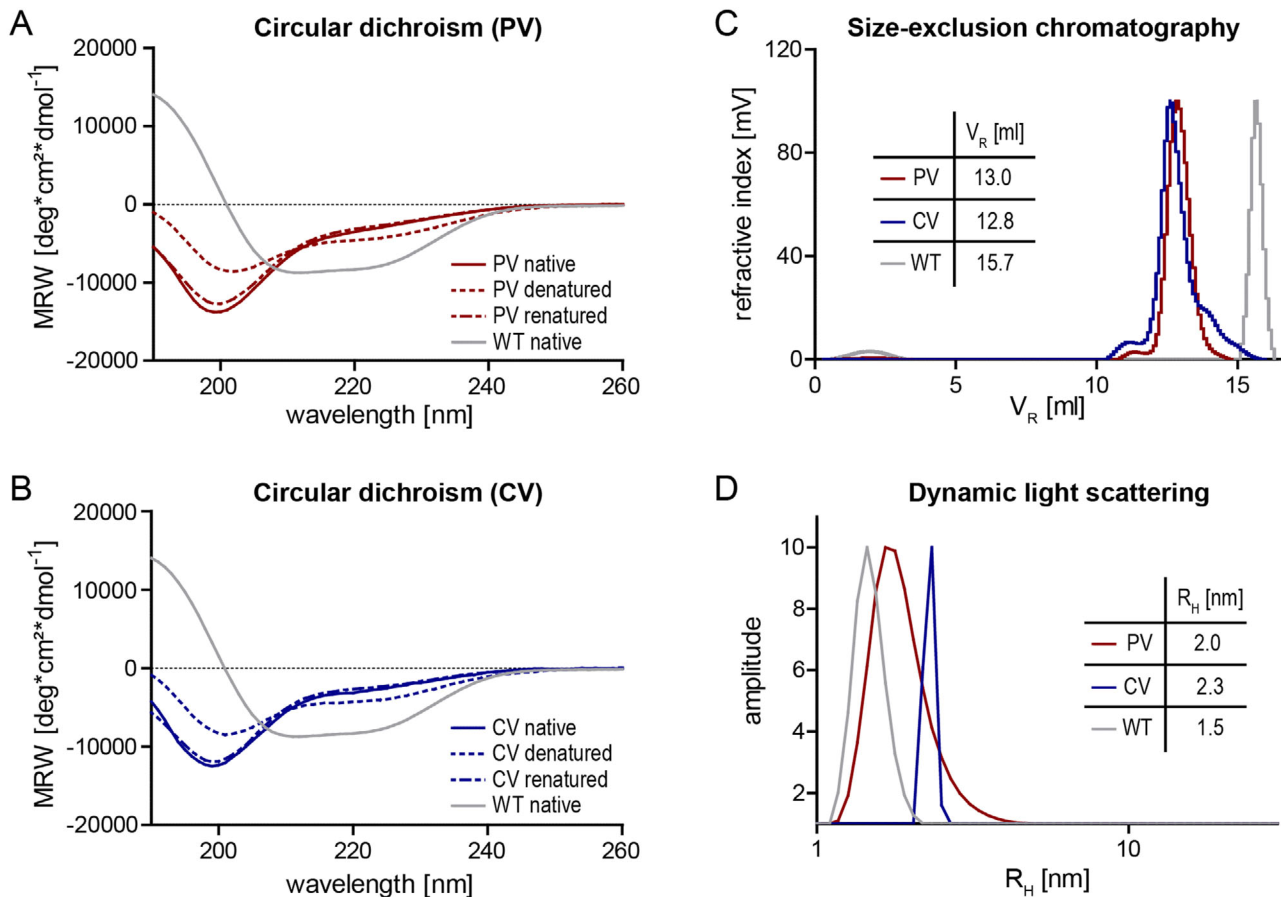

Figure 3. Physico-chemical characterization of Pru p 3 molecules. Purified recombinant PV (red) and CV (blue) were physico-chemically characterized and results were compared to WT (grey). Far-UV spectra of PV (A) and CV (B) were recorded at 20 °C (native, solid line), at 95 °C (denatured, dotted line), and again at 20 °C (renatured, dashed line); WT Pru p 3 measured at 20 °C was included as a reference (grey line). In solution aggregation behavior of protein batches was analyzed by C) size-exclusion chromatography and D) dynamic light scattering.

33 Italian peach allergic patients (panel A) showed a strongly reduced IgE reactivity to both Pru p 3 variants in ELISA (**Figure 4**A). PV presented an average reduction of 97% (min–max 60–100; 95% CI, 93–100) while CV showed a mean reduction of 73% (min–max 0–100; 95% CI, 61–84). Inhibition experiments were performed to further assess hypoallergenicity of the two Pru p 3 variants. Therefore, 27 patients' sera were pre-incubated with PV, CV, and WT, and residual IgE reactivity to Pru p 3 was determined by ImmunoCAP (Figure 4B). WT was able to inhibit IgE reactivity to itself in a dose-dependent manner, with mean inhibition reaching up to 85%. In contrast, IgE cross-inhibition of Pru p 3 variants was very limited, showing in average less than 12% at highest inhibitor concentration. In histamine release assay performed with sera from 19 patients, WT triggered substantial mediator release in a concentration range from 50–50 000 pg mL^{-1} (Figure 4C). Mean release induced by WT was reaching up to 86 ng mL^{-1} of histamine, while those observed for PV and CV were in average considerably lower (0.017 and 0.007 ng mL^{-1}, respectively). Release curves for individual sera showed very low responses for most of the patients, while for some histamine release was detectable at distinct protein concentrations (Figure S2, Supporting Information).

3.5. In vivo Immunogenicity in a Mouse Model

Owing to the fact that PV showed favorable storage capacities as well as strongly reduced IgE binding activity, immunological in vivo studies were focusing on this molecule. WT was included as reference and endotoxin levels measured in both protein preparations were ≤3 EU. In order to optimize immunogenicity, adsorption conditions to aluminum hydroxide (H) and aluminum phosphate (P) were assessed in vitro (Figure S3, Supporting Information). Longer incubation time increased the binding rate of PV, while the adsorption of WT already reached a plateau after shorter incubation period. Based on these results, in vivo experiments' formulation of PV was performed o/n at 4 °C, while WT was incubated for 1 h at RT.

After four immunizations, the humoral immune response of mice was analyzed by ELISA (**Figure 5**A,B; Figure S4, Supporting Information). WT mounted a robust IgG1 production in 6/6 mice immunized with P and 5/6 immunized with H, while higher IgG2a titers were observed with P. The IgG1 immune response of PV was shown to be superior in phosphate-based formulations (6/6) compared to hydroxide-based adjuvants (2/6); 4/6 mice responding to aluminum phosphate–based immunizations

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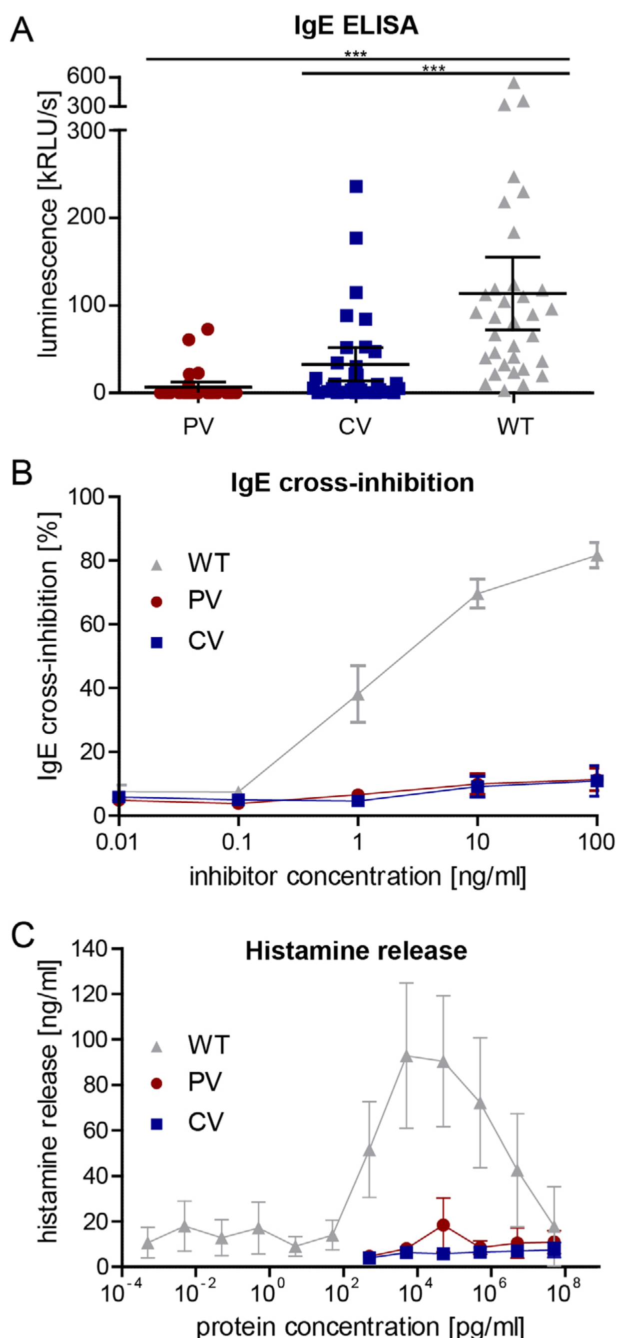

Figure 4. Allergenic activity. A) IgE binding capacity of PV and CV was compared to the WT molecule in ELISA using sera from peach allergic patients ($n = 33$). B) ImmunoCAP inhibition to Pru p 3 (f420) was evaluated by inhibiting reactivity of 27 sera with PV, CV, or WT. C) Histamine release induced by WT and variants was measured using 19 sera.

with PV demonstrated (partially) cross-reactive IgG antibodies to WT. IgG2a titers showed similar profiles but were generally considerably lower. Murine IgE was determined by measuring the β-hexosaminidase release from rat basophilic leukemia cells (Figure 5C). Mice immunized with WT and H-based adjuvant presented a significantly higher (mean 50.8%) mediator release compared to P (mean 28.7%). Immunization with PV generally led to very limited IgE mediator release with the immunogen (Figure 5C), but also when using WT for cross-linkage indicating lack of IgE cross-reactivity (data not shown). Immunogenicity of PV was investigated in ELISPOT assay (Figure 5D). Splenocytes from mice receiving PV formulated with aluminum phosphate showed an IL-4 and IFN-γ upon re-stimulation with either PV or WT. Aluminum hydroxide–formulated PV showed very limited response similar to WT.

4. Discussion

Treatment strategies for FA still predominately rely on allergen avoidance and rescue medication.[6] In the last years, research on therapeutic approaches focused on OIT and SLIT.[3,7,8] However, no long-lasting beneficiary effect after ending the therapy regimen could be proven for OIT, and treatment requires highly motivated patients/families and specialized centers with adequate emergency treatment options. Thus, sustainable therapies with reduced side effects are still desired. AIT using hypoallergenic molecules is one option to allow modulating the immune response with limited risk of severe IgE-mediated side effects.[47,48] The high clinical relevance of LTP allergy prompted us to develop strategies for generation of novel hypoallergenic vaccine candidates of Pru p 3.[18]

In this work, the first approach to generate a Pru p 3 fold variant is based on a robust computational screening previously also used for inhalant allergens.[42,49] Parameters of in silico mutagenesis were set not to affect known T-cell epitopes and cysteine residues were excluded from calculations as they lead to structural collapsing.[38,50] Applying this method, the four most destabilizing amino acids were identified within the alpha-helical regions and based on Z-score results those were exchanged for proline residues. Our second approach involved modification of cysteine residues. As the disulfide bond–stabilized backbone of Pru p 3 was shown to be essential for protein stability and immunogenicity,[38] intervention was reduced to a minimum. It was anticipated that residual disulfide bonds would be able to stabilize the protein since other proteins of the prolamin superfamily contain only three disulfide bonds. In our setup, two neighboring cysteine residues (C27, C28) involved in two independent disulfide bonds were exchanged for alanine. Previously, substantial reduction in IgE binding of the LTP from pellitory pollen was observed upon mutation of bonds C14–C29 and C30–C75, while remaining bonds showed less involvement in epitope formation.[44] In line with that, we specifically targeted analogous cysteine residues in Pru p 3 aiming to keep residual structural features as intact as possible.

For quality control, recombinant molecules were extensively physico-chemically characterized. Both variants presented circular dichroism spectra devoid of the typical alpha-helical LTP fold but rather resembled irregular structural motifs analogous to other Pru p 3 mutants.[36–38,51–53] However, a shift in spectra was observed upon thermal denaturation which was restored upon cooling suggesting a thermostable conformation for both proteins. Presumably, molecules did not undergo extensive disulfide bond rearrangement, which irreversibly occurs when LTPs are heated in neutral buffer conditions.[52,54,55] Both variants

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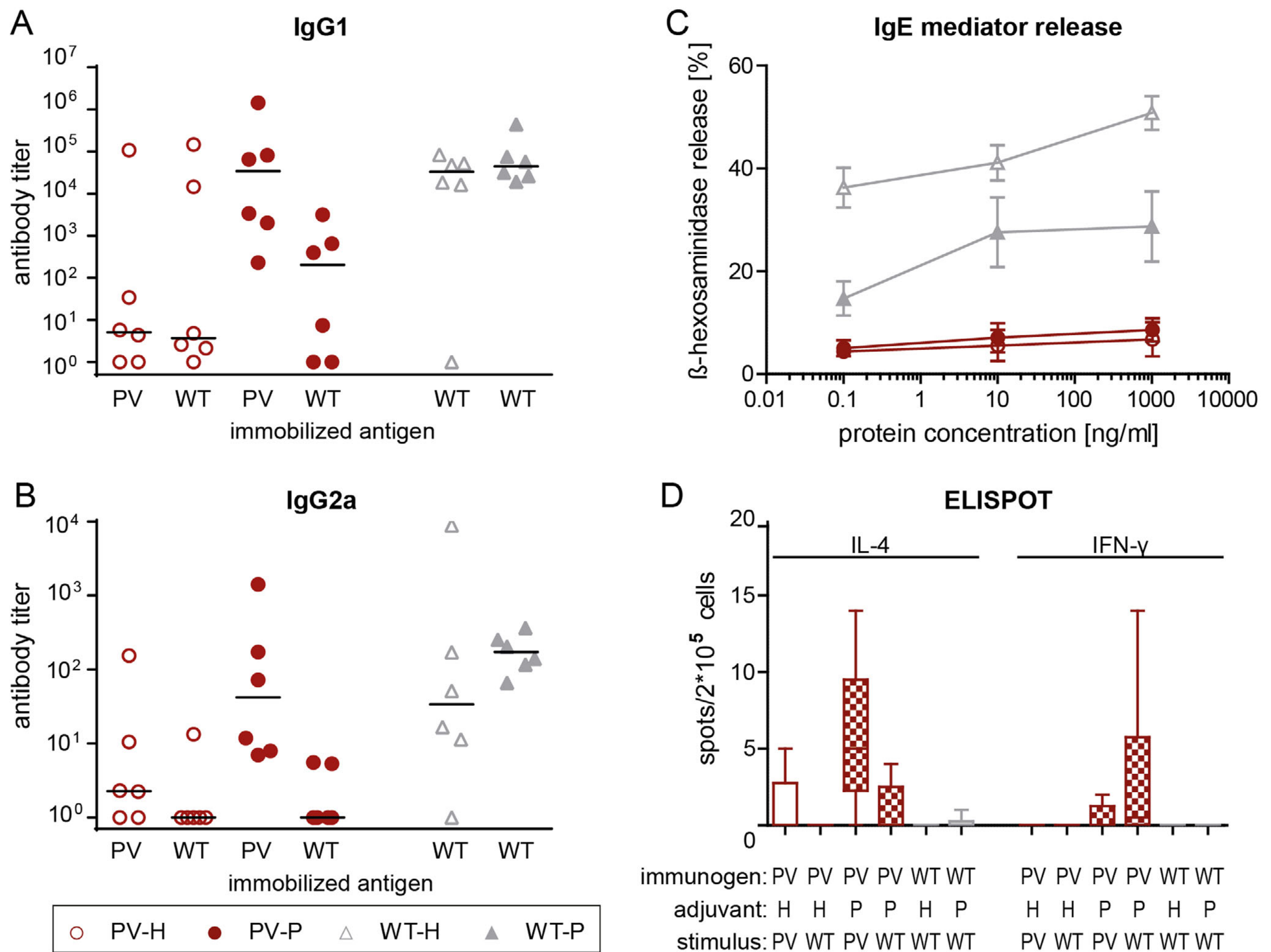

Figure 5. Immunologic characterization of PV and WT in a murine model. PV (red) and WT (grey) were adsorbed to aluminum hydroxide or phosphate at pH 7.5 at 4 °C o/n and RT for 1 h (A,B). Murine IgG1 and IgG2a response upon immunization with PV and WT was analyzed in ELISA. End point LOD titers were calculated and medians are indicated. C) RBL-2H3 cells were loaded with murine IgE antibodies and mediator release upon stimulation with PV or WT is given as mean, including SD of the total release. D) In ELISPOT experiments, cytokine production upon re-stimulation was measured and minimum to maximum box-and-whisker plots are given.

presented a less compact conformation indicated by an altered migration in gel-electrophoresis and increase in the hydrodynamic radius. While the alpha helical structure elements were clearly changed, the overall conformational stability of the molecules seemed to be largely preserved.

A necessity for development of a drug substance is an easily implementable production process. For both Pru p 3 variants, straightforward and robust purification protocols in *E. coli* were established. Shelf life, an essential feature, was addressed by an accelerated stability test. PV displayed remarkable stability, since the protein integrity was not affected upon long-term storage in a cooled environment. In comparison, only freezing assured the stability of CV. Considering those factors, there is a preference for PV due to its enhanced production yield and stability features.[56,57]

A reduced allergenic potency of both Pru p 3 variants was demonstrated in IgE-binding and histamine release assays using sera of peach allergic patients. In ELISA, a strong reduction in IgE binding of PV (97%) and CV (71%) was observed compared to WT. Regarding IgE and cross-inhibition reactivity, our variants were comparable or superior to previously engineered hypoallergens.[36–38] WT showed similar histamine release curves as previously determined with Mediterranean patients' sera.[38] Of note, the allergenic activity of both Pru p 3 variants in this study was calculated to be 10^6-fold lower compared to WT with 19 patient sera tested. Considering the patients' sera from different countries included in our study, the tremendous reduction in IgE-binding and biological activity is strongly suggested.

One crucial factor for successful AIT is the ability to activate an immunological response. This feature was addressed by leaving known T-cell epitopes of Pru p 3 sequentially unchanged.[50] Since PV proved to be superior in production yield, storage stability, and lower allergenicity, in vivo mouse experiments focused on this candidate. Prior to immunization experiments, adsorption kinetics to aluminum-based adjuvants was optimized in vitro. Adsorption to aluminum-based adjuvants is mainly driven by

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electrostatic interaction, and hence the isoelectric point of the adjuvants in relation to the protein is of relevance regarding adsorption efficacy and subsequent immunogenicity.[58,59] Since the isoelectric point of aluminum hydroxide (pI 11) is in a similar range as that of Pru p 3 (9.3), we were specifically interested in aluminum phosphate which presents a pI around 5–7. Binding studies revealed that PV showed different kinetics with higher binding capacities after overnight incubation. Though only four residues were exchanged, the compactness of PV seems to be lower, thus exposing interior amino acids which may explain different binding kinetics.[60]

Similar to other studies, WT mounted a sound immune response independent of the adjuvant formulation used.[38,39] In contrast, the adjuvant formulation strongly impacted immunization with PV. While aluminum phosphate was able to induce an IgG response, only 1/6 mice responded to the hydroxide-based formulation. Thus, changes in the net surface charge of PV potentially allowed favorable binding to aluminum phosphate.[60] Using aluminum phosphate as adjuvant along with a stable structure of our molecule might explain the difference in immunogenicity and antigenicity compared to reduced/alkylated Pru p 3.[38,39] Interestingly, WT immunization with aluminum hydroxide triggered a significantly stronger IgE-mediated release compared to aluminum phosphate while IgG levels were similar. Aluminum-based adjuvants are generally known to drive Th2 polarization, but PV showed only marginal activity in mediator release assays.[60] Although PV was able to induce a humoral response, we observed only limited IgG cross-reactivity with the WT molecule. It is still unsolved if blocking IgG antibodies are crucial during immunotherapy, as T cell epitope peptide therapy also confers clinical efficacy.[61–63] PV was able to trigger a T-cell response in aluminum phosphate–immunized mice, while WT and other adjuvant formulations resulted in generally low reactivity, which might be attributed to the narrow immunization regimen used in our study. Notably, in vitro T-cell reactivity of reduced/alkylated Pru p 3 was maintained in the study by Toda et al. and completely independent of the diminished humoral response.[38] Based on observed facts and previously established protocols for reduced/alkylated Pru p 3 protein and peptide derivatives,[33,39] the performance of PV can now be investigated in a therapeutic mouse model.

The disulfide bond–stabilized structure of LTPs seems relevant for protein sturdiness and even minor interventions, as demonstrated with CV, were affecting protein integrity. Exploiting a robust in silico mutagenesis approach to identify destabilizing amino acids led to the generation of the fold variant PV. Based on the strongly reduced allergenicity and retained immunogenicity, as well as its suitable shelf life, the molecule seems to fulfill first requirements for potential application as therapeutics in LTP-related FA. The in silico concept could be transferred to other food allergens, allowing new treatment options for patients' suffering from adverse reactions to disulfide bond–stabilized allergens.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

G.G., F.F., and R.v.R. were responsible for study concept and design. S.E., A.H., M.S., J.L., B.M.J., S.A.V., I.P., and P.B. performed the experiments. S.E., L.J., L.K.P., P.L., and G.G. analyzed and interpreted the data. N.R., J.A.A., A.P., M.F.-R., N.G.P., and A.M. provided reagents and materials. S.E. and G.G. drafted the manuscript. All authors provided critical revision of the manuscript for important intellectual content and approved the final version for publication. The authors thank Eva Vejvar and Rosetta Ferrara for support in the lab. The study was supported by the FAST–Food Allergy Specific Immunotherapy project (201871) funded by the European Union.

Conflict of Interest

J.A.A. is/was an employee of a biopharmaceutical company in the allergy field. R.v.R. is a consultor for HAL Allergy BV and for Citeq BV and received speaker's fees from HAL Allergy BV and ThermoFisher Scientific. F.F. is a member of Scientific Advisory Boards (HAL Allergy, NL; SIAF, Davos, CH; AllergenOnline, USA). The rest of the authors have no conflict of interest.

Keywords

allergen immunotherapy, hypoallergens, lipid transfer proteins, peach allergies, Pru p 3

Received: April 3, 2019
Revised: May 28, 2019
Published online: June 26, 2019

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