Purification and Characterization of Allergens for Walnut Allergy Analysis
（クルミアレルギー解析のためのアレルゲンの精製とその特性）

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General Introduction

Plant-based proteins have garnered significant attention due to rising global demand for protein supply, environmental sustainability concerns, and the increasing popularity of vegetarian diets. Tree nuts are known for their high protein content. Global tree nut production has steadily increased over the past decade. However, alongside this rise in production and consumption, tree nut allergy has emerged as a significant health issue, with tree nuts being a major causative food. Tree nut allergy remains inadequately researched, highlighting the importance of identifying key allergen components and understanding their potential cross-reactivity. Cases of allergy to walnuts and cashews are frequently reported among tree nuts. Because pecans belong to the same family as walnuts, it should be noted in walnut allergies. In this study, for the analysis of walnut allergy, allergens from walnut, pecan, and cashew are prepared and their properties were examined.

1. Purification of Vicilin N-Terminal Fragments from Walnut, Cashew, and Pecan seeds

Vicilin, a major seed storage protein, is widely implicated in tree nut allergies. The N-terminal fragments of vicilin in seeds often undergo post-translational modifications, resulting in cysteine-rich hydrophilic fragments. These cysteine-rich fragments play a key role in forming disulfide bonds, which stabilize hairpin structures and influence allergenicity. In this chapter, N-terminal vicilin fragments were extracted from walnut, pecan, and cashew seeds using tailored buffer systems to account for varying solubility across these tree nuts. After ammonium sulfate fractionation, vicilin fragments were purified using high-performance liquid chromatography (HPLC). After purification, the amino acid sequences of the fragments were confirmed by Mass Spectrometry.

2. Expression, Purification, and Characterization of Recombinant Vicilin N-Terminal Fragments of Walnut, Pecan, and Cashew

To study the allergenic properties of vicilin N-terminal fragments, recombinant N-terminal fragments of Jug r 2 and Jug r 6 from walnut, Car i 2 from pecan, and Ana o 1 from cashew were expressed in *Pichia pastoris*. DNA encoding these fragments was ligated into pPICZα vectors and introduced into the X33 strain for protein expression. Following methanol induction, the proteins were purified using HisTrap FF columns and further refined with a Superdex 75 16/60 column. SDS-PAGE

and Western blot analysis confirmed the presence of vicilin fragments, which were then tested for IgE reactivity using sera from 24 walnut-allergic patients. ELISA results demonstrated higher positivity rates for the Jug r 2 and Jug r 6 N-terminal fragments and the Car i 2 N-terminal fragment, suggesting strong cross-reactivity between walnut and pecan vicilin fragments. In contrast, the Ana o 1 N-terminal fragment exhibited a much lower positivity rate, indicating weaker cross-reactivity with walnut. These findings emphasize the critical role of structural motifs, particularly conserved disulfide-bonded hairpin structures, in determining cross-reactivity potential.

3. Expression, Purification, and Characterization of PR-10 and Profilin in Walnut

PR-10 and profilin are key allergens linked to pollen-food allergy syndrome, affecting individuals sensitized to pollen when consuming certain plant-based foods. In this chapter, the PR-10 protein (Jug r 5) and profilin (Jug r 7) were expressed using *E. coli* systems with pCold and pGEX vectors, respectively. After IPTG induction, the cells were lysed by sonication and purified using affinity columns and HPLC. The purified proteins were confirmed by SDS-PAGE and Western blotting. For comparison, 2S albumin (Jug r 1) was also expressed and purified, and fractions containing 11S globulin were extracted from walnut seeds. All of these were included in the ELISA tests. While storage proteins (Jug r 1 and 11S globulin) showed high positivity rates in most patients, non-storage proteins such as Jug r 5 and Jug r 7 exhibited significant IgE reactivity in some patients, particularly P7 and P8. In conjunction with results from Chapter 2, P13 also demonstrated higher IgE reactivity to the N-terminal fragments. These findings highlight the importance of both storage and non-storage proteins in walnut allergy, emphasizing variability in individual patient responses.

Conclusion

This study offers important insights into the allergenic properties and cross-reactivity of walnut allergens. Through the purification and characterization of native and recombinant vicilin N-terminal fragments from walnut, pecan, and cashew, it was shown that structural motifs, particularly disulfide-bonded hairpin structures, play a crucial role in cross-reactivity. ELISA results indicated high IgE reactivity for walnut and pecan N-terminal fragments, with lower reactivity for cashew, underscoring the relationship between structure and allergenicity. The research also explored PR-10, profilin, 2S albumin, and 11S globulin, highlighting variability in patient responses, particularly to non-storage proteins in patients P7, P8, and P13. These findings emphasize the importance of personalized approaches in allergy diagnosis and treatment, providing a strong foundation for improving diagnostic methods and developing targeted therapies for walnut and other tree nut allergies.