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Haptenic Side Chains as Posttranslational IgE-Binding Determinants among Pollen Protein Allergens

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Key Words

Haptens · Allergens · Pollen

Introduction

Scarce attention has so far been given to the idea that pollen proteins may owe their allergenicity to low-molecular-weight compounds of defined chemical structure associated as haptenic side chains to carrier proteins [1, 2]. Such IgE- or IgG-binding antigenic determinants might derive from pro-hapten precursors (e.g. by hydrolysis during extraction), giving rise to reactive chemical intermediates combining with proteins under mild conditions. The hypothesis of the possible nature of pollen allergens as protein-hapten conjugates gained momentum when it was found that the <10-kD dialysable constituents of aqueous pollen extracts contain all the essential IgE-binding epitopes [3]. We therefore decided to probe the interaction of the weakly allergenic <10-kD components from pollen extracts with suitable >10-kD non-allergenic protein carriers.

Results and Discussion

During preliminary experiments, the dialysable <10-kD components separated from aqueous extracts of *Betula alba* pollen were made to react in sodium bicarbonate at pH 9.5 with the non-IgE binding >10-kD proteins of *Corylus avellana* or *Urtica dioica*. The mixtures were stirred vigorously with free access of air for 3 h at room temperature and were subsequently redialysed for 18 h through 10-kD cut-off membranes and recovered by lyophilization. The resulting >10-kD hapten-protein conjugates exhibited considerable

capacity to react with human anti-*Betula* IgE antibodies relative to the parent proteins. Inhibition experiments against *Betula* allergens showed that the virtually inactive *Corylus* >10-kD proteins had been converted into antigens with the IgE-binding potential and specificity of a birch pollen allergen: the semisynthetic product was over 200× more active than the *Corylus* parent proteins and only 47 times less potent than the native birch pollen allergens.

Similarly, artificial adducts of low-molecular <5-kD *Betula* subcomponents (separated by molecular sieving on Sephadex G25 columns) and milk whey proteins as carriers acquired the specific IgE-binding properties of the natural *Betula* protein allergens (fig. 1). We also noted that the low molecular *Betula* components kept in concentrated solution for 6 years at room temperature had lost much of their protein binding power (fig. 1).

As demonstrated in figure 2, potent semisynthetic IgE-binding allergens could likewise be produced using a <3-kD chromatographically separated subfraction of *Parietaria officinalis* pollen coupled chemically to cow's milk whey proteins (mainly β-lactoglobulin and α-lactalbumin).

'Hybrid' molecules were also prepared by cross-coupling the <5-kD *Parietaria* compounds to the proteins of *C. avellana* and, in like fashion, the <5-kD *Betula* components to the proteins of *U. dioica*. Ongoing experiments show that it is possible to produce mixed hybrids featuring both *Parietaria* and *Betula* specificity by reacting the pertinent pro-haptenic materials with *Urtica* or cow's milk proteins as carriers.

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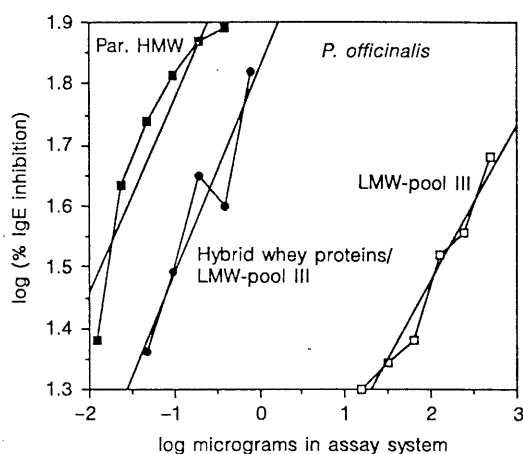


Fig. 1. Dose-response curves for inhibition of specific IgE-binding observed with prototypes of a semisynthetic allergen prepared by reacting the <3-kD low-molecular-weight chromatographic subfraction LM-III from *B. alba* pollen with the nonpollen >10-kD casein-free whey proteins of cow's milk. For comparison, the potency of the traditional >10-kD allergenic proteins isolated directly from the pollen of *B. alba* is included. The aged LM-III preparation had been stored for 6 years at room temperature.

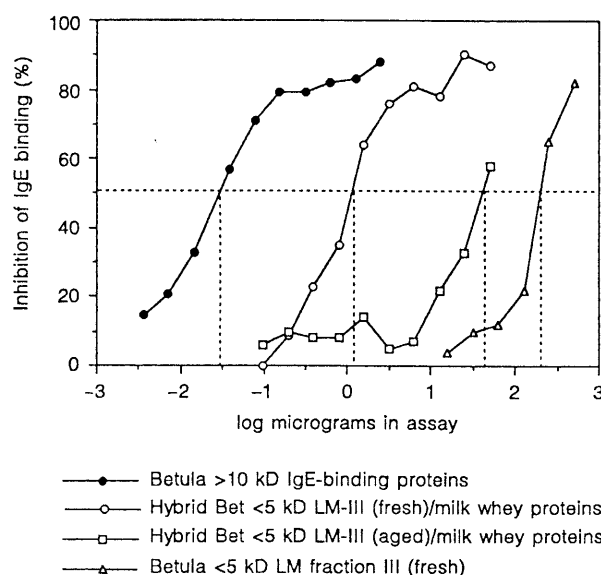


Fig. 2. Dose-response curves for inhibition of specific IgE-binding observed with an experimental allergenic 'hybrid' prepared by reacting the <3-kD low-molecular-weight chromatographic subfraction 'LMW-pool III' from *P. officinalis* pollen with the >10-kD whey proteins of cow's milk. The potency of the traditional >10-kD allergenic proteins (Par. HMW) isolated from the pollen of *P. officinalis* is included for comparison.

UV spectroscopic analysis demonstrates that the semi-synthetic products had relatively strong absorption at about 350 nm, indicating the presence of chemically conjugated or strongly adsorbed (3',4'-biphenolic) flavonols. Our results therefore seem to confirm the erstwhile observations of Benjamins et al. [4] and Long and Teller [5], who reported that small-molecular-weight pollen components adsorbed to a variety of proteins could acquire an over 100-fold increase in skin-reactive potency.

Basically, adducts of proteins and (quinoid) flavonols, carbohydrate derivatives or other haptenic structures, represent degradation products featuring de novo epitopes, as has been proposed before by Storm van Leeuwen [6] and Berrens [7]. Apart from the theoretical consequences, our results may therefore open the way to the manufacture of an entirely new generation of chemically defined (semi) synthetic allergens.

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