

IgE binding to a human profilin-containing fraction caused by contamination with yeast-derived material

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Profilin is a protein found in all eukaryotic cells. Profilin in pollen from grasses, trees, and weeds was found to be largely responsible for cross-allergenicity between pollen and vegetable foods.^{1,2} Valenta et al.³ described cross-reactivity for human IgE antibodies between profilin from birch pollen and profilin from human platelets, using immunoblots as an assay system. We identified 11 sera with IgE to a human profilin-containing fraction in a preliminary investigation.⁴

In this study we re-investigated the occurrence of IgE against human profilin in view of the possibility that human profilin, acting as an autoallergen, might be implicated in IgE-dependent histamine-releasing activity.⁵ Human profilin was isolated from human platelets by its affinity to poly-L-proline.³ IgE to profilin, as described by others^{2,3} and by us,¹ reacts with profilin from pollen and vegetable foods. It was therefore unexpected to find IgE binding to the fraction containing human profilin in sera that were negative for IgE to pollen allergens.⁴ Because all of these sera were positive in the RAST for *Aspergillus fumigatus*, other molds, and yeast, we assumed that this might indicate cross-reactivity between profilin from molds and human profilin; alternatively, we considered the possibility that tissue damage by the mold was

Abbreviation used

ATP: Adenosine triphosphate

responsible for the release of autoallergenic human profilin.

However, none of these sera were positive on an immunoblot of an extract of human platelets. A rabbit serum sample obtained by immunization with the human profilin-containing fraction showed IgG binding with both a 14 kd band (presumably human platelet profilin) and a 41 kd band (presumably human platelet actin; data not shown).

The profilin preparation used in the RAST was prepared with an adenosine triphosphate (ATP) containing buffer and was subsequently dialyzed. The results of control experiments suggested the presence of a nondialyzable contaminant in one of the buffer components. Indeed, a high molecular weight contaminant in the ATP proved to be responsible, as was shown by RAST with Sepharose-coupled ATP (using CNBr-activated Sepharose from Pharmacia, Uppsala, Sweden) and by RAST inhibition. The commercially obtained ATP (Boehringer catalog no. 127531; Boehringer, Mannheim, Germany) was found to be isolated from yeast. The IgE binding to ATP was inhibited by yeast extracts (Fig. 1). We have no indication that the reaction with yeast is caused by yeast profilin. The protein content of the dialyzed ATP used for the profilin isolation was 0.45 mg of protein per gram of ATP. For 50% inhibition of the ATP RAST, 6 μ l of 1 mmol/L ATP was required (Fig. 1). On a protein basis, this corresponds to 1.6 ng. We therefore consider it unlikely that the inhibitory activity is caused by a protein. Purified yeast mannan had little inhibitory effect (Fig. 1).

Spectroscopically, no difference was found between the ATP used for the purification of profilin

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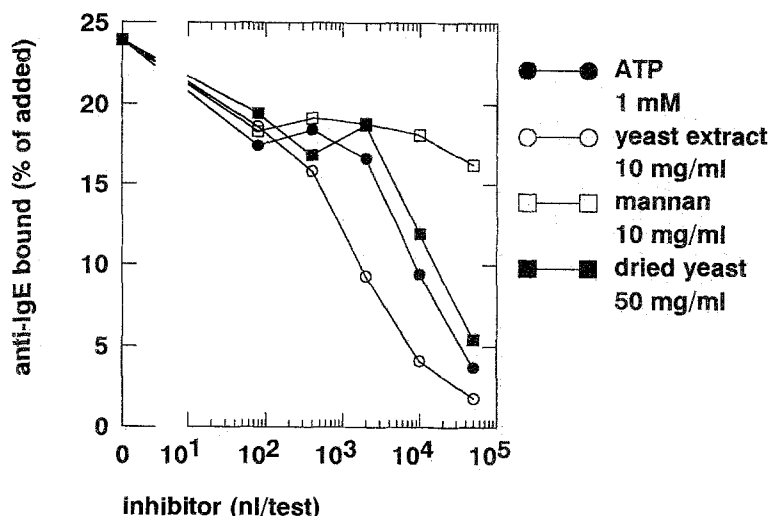


FIG. 1. RAST inhibition assay of the IgE binding of one serum to ATP-Sepharose by ATP, yeast extract, and mannan.

and a more purified ATP (Boehringer catalog no. 519987), either at 260 or at 280 nm. After purification of human profilin with the more purified ATP, no IgE binding to human profilin was demonstrated in: (1) the 11 sera positive in the RAST for *A. fumigatus*, (2) 23 sera positive in a RAST to grass profilin, and (3) in 16 sera positive in a bioassay for the IgE-dependent histamine-releasing activity⁵ in supernatants derived from mononuclear cells. As a positive control we used the rabbit antiserum to human profilin that demonstrated IgE binding with human profilin but not with grass pollen profilin.

In conclusion, the results obtained by direct RAST and RAST inhibition indicate that the IgE binding to human profilin, which we previously described, was in fact caused by yeast-derived material in the ATP used during the isolation of profilin. We did not find IgE binding to human profilin in 23 sera with IgE to grass pollen profilin.

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