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Localization of group-1 allergen Zea m 1 in the coat and wall of maize pollen

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Summary

The pollen surface consists of an outermost coat and an underlying wall. It makes the initial contact with the stigma surface during sexual reproduction. To date, only two proteins have been identified from the maize pollen coat. Zea m 1 (β-expansin 1) is the major group-1 allergen in maize pollen, but its presence and localization in the pollen coat and wall has not yet been explored. In the present study, immunoblot analysis using an antibody directed against group-1 allergen revealed that a small amount of Zea m 1 exists in the pollen coat fraction prepared using a diethyl ether wash. Immunogold labeling also showed that the extracellular localization of Zea m1 was mainly restricted to the tectum and the foot layer of the exine (the outer pollen wall), and gold particles immunolabelling Zea m 1 were unevenly dispersed throughout the pollen coat and wall. Moreover, a substantial amount of Zea m 1 was localized in the cytoplasm of the pollen interior. The presence of Zea m 1 in the pollen coat and wall suggests that Zea m 1 may play a potential role in pollen germination on the stigma.

Introduction

The group-1 allergens are pollen-specific glycoproteins that are present at high levels in pollen of many grasses. Their immunological aspects have been fairly intensively studied (Singh et al. 1991; Broadwater et al., 1993; Knox et al., 1993; Laffer et al., 1996; Petersen et al., 1996), but the biological functions of group-1 allergens remain, to a large extent, obscure. Previously, immunogold labeling with electron microscopy showed that the localization of the group-1 allergens in pollen differed in different grass species. For example, the major group-1 allergen (Lol p 1) in rye-grass pollen was predominantly present in the electron-opaque regions of the vegetative cell (Taylor et al., 1994). In timothy grass, the two major group-1 allergens Phl p 1 and Phl p 5 were localized to different subcellular pollen compartments after

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anhydrous fixation and immunogold staining (Grote et al., 1994). In particular, Phl p 5 was found in the cytoplasm and on the exine, and phl p 1 in the intine (Behrendt et al., 1999). In maize (*Zea mays* L.) pollen, the major group-1 allergen Zea m 1 (ca. 31 kDa) was recently recognized as β-expansin 1, based on its wall-loosening activity, which is specific to grass cell walls (Cosgrove et al., 1997; Li et al., 2003). Moreover, four major isoforms of Zea m 1 can be discriminated using chromatography (Li et al., 2003) or by two-dimensional (2D) gel/blot analysis after deglycosylation (Wang et al., 2004). It is generally accepted that Zea m 1 is a cell-wall protein because it is easily released from maize pollen in a liquid medium; however, its localization on maize pollen surfaces has still not been explored.

The pollen surface consists of an outermost coat and an underlying wall. The pollen surface makes the initial contact with the stigma surface during sexual reproduction. Recent reports revealed that the pollen surface contains proteins and other components needed for efficient pollination and pollen germination (Bih et al., 1999; Mayfield et al., 2001; Wu et al., 2002; Suen et al., 2003). In maize, several proteins released from the pollen coat and walls have been characterized and their potential roles in pollen tube growth on the stigma and through the style have been proposed (Suen et al., 2003). Nevertheless, based on molecular mass and GenBank accessions none of these proteins was recognized as Zea m 1.

In the present study, for the first time, the ultrastructure of the pollen surface of maize pollen was examined, and the presence and localization of Zea m 1 in maize pollen surface was determined using protein blot analysis and immunogold labeling for electron microscopy.

Materials and methods

Plant material

Maize plants were grown in a glass greenhouse with supplemental lighting. At anthesis, fresh pollen was collected by shaking the tassels gently over a sheet of paper. Pollen samples were sifted to remove debris and then used for biochemical and microscopic studies.

Pollen coat preparation

Pollen grains were mixed with diethyl ether (1 g of pollen/10 ml of ether) for 1 min in a capped tube by repeated inversions (Bih et al., 1999). The ether layer was collected after centrifugation for 10 min at 8000*g*, and then transferred into a small mortar and allowed to air-dry under a chemical hood. The protein constituents in the dry coat materials were extracted using a phenol-based protocol (Wang et al., 2003). Proteins from mature pollen and the pollen remains after diethyl ether washing were extracted as above. Protein was quantified according to the method described by Bradford (1976), using bovine serum albumin as a protein standard.

Electrophoresis and immunoblotting

SDS-PAGE was performed in 12.5% polyacrylamide gels using Bio-Rad Mini-Protean II equipment. Resolved proteins in gels were visualized using Coomassie Blue R250 staining, or electrophoretically transferred onto polyvinylidene difluoride membranes (Hybond-P, Amersham, Uppsala, Sweden) using a standard protocol described by Towbin et al. (1979). Protein blots were blocked overnight with 5% milk powder in TBST buffer (10 mM Tris–HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween 20), and then incubated for 1 h with the monoclonal antibody HB 7, which was raised against group-1 pollen allergen by Grobe et al. (2002), diluted 1:200. After washing, the blots were incubated with peroxidase-labeled anti-mouse IgG (Amersham), at a 1:5000 dilution, for 1 h. The detection system was the ECL Plus Western Blotting Reagents kit (Amersham), employed according to manufacturer's instructions. The chemiluminescent signal was exposed onto Kodak X-OMAT film (Sigma) and then scanned and processed using a Fluor-S Multi-Imager system (Bio-Rad) (Wang et al., 2004).

Transmission electron microscopy

For ultrastructural studies of the wall, fresh pollen was fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at room temperature, dehydrated with ethanol and embedded in Spurr's resin using routine protocols. Ultrathin sections were cut using an ultramicrotome and collected onto grids. Dried sections were stained with 1% uranyl acetate according to the protocol of Reynolds (1963) and observed using a Philips Morgagni transmission electron microscope at 80 kV.

Immunogold labeling

Prior to fixation, fresh pollen grains were rehydrated for 1 h at 100% relative humidity and