Posttranslational modification of Bioaerosol protein by common gas phase pollutants; NO_2 and O_3

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Objective: To investigate the posttranslational modification of highly allergenic pollen species that are common in Europe.

Introduction

✧ Pollen grains of interest are Birch and Ragweed with in the diameter of 19–26 µm. These particles range in diameter from 5-150µm with most airborne pollen ranging from 20-45µm (Pope 2010).

✧ Pollen protein often shows visible auto fluorescence excited by UV or violet light. Amino acids (Particularly tryptophan, tyrosine and phenylalanine), nucleic acids, and some coenzymes are the molecules responsible for the fluorescence in most cell (Hill et al., 2013).

✧ Allergies to pollen have rapidly increased globally especially within Westernised urban areas. It has been hypothesized that exposure of the pollen to common gas phase pollutants increases the allergenicity of the pollen and thus increases hay fever incidence (D'Amato et al. 2013).

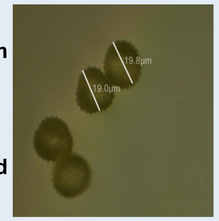

Figure 1: Microscope image of Ragweed pollen (*Ambrosia artemisiifolia*).

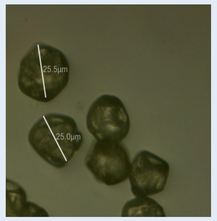

Figure 2: Microscope image of Birch pollen (*Betula pendula*).

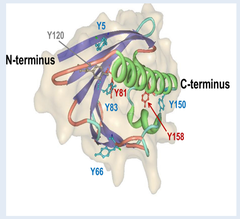

Figure 3: Position of tyrosine residues in the 3-D structure of crystallized unmodified Bet v 1.0101 (Reinmuth-Selzle et al. 2014)

Analysis

✧ Within the laboratory, we expose pollen grains to atmospherically applicable exposures of gas phase NO_2, O_3 and other common gas phase oxidants under a range of environmentally relevant conditions (temperature and relative humidity).

✧ The modification on the biochemistry of the pollen grains were probed using a proteomic approach (liquid chromatography coupled ultra-high resolution spectrometer).

✧ The untreated and treated pollen sample where viewed under Fluorescence microscopy to study any visual changes.

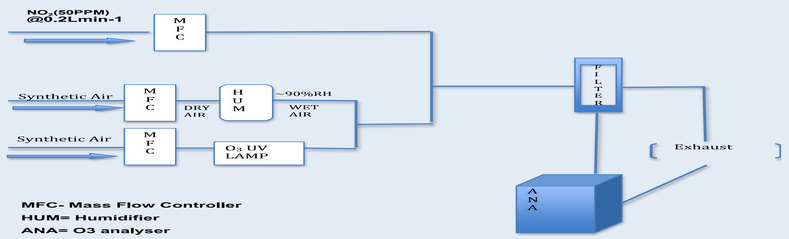

Figure 3- Schematic Setup of the experimental chamber for the exposure of the pollen sample on a 47mm (0.15mm)poly carbonate filter paper to NO_2 and O_3 under differing environmental conditions.

Discussion

✧ The PTMs of Birch and Ragweed pollen upon exposure to gas pollutant NO_2 and an O_3/NO_2 mixture was investigated.

✧ Results showed NOx-mediated modification of both allergen protein of the pollen upon exposure to the atmospheric gases .

✧ The posttranslational modifications (PTMs), Nitration and Nitrosylation, occurred on a specific sites, tyrosine (designated as Y within a hydrophobic environment) and cysteine (C) of the protein respectively.

✧ Reduced intensity of visible auto fluorescence of the Birch pollen upon exposure to the gases was observed.

✧ These modifications may affect human immune response to the pollen protein, which may suggest the possible reason for increased allergies in hay fever sufferers.

Findings

✧ We observe NOx mediated modification on the pollen protein.

✧ Our results supports other research findings, however, we used real pollen samples rather than allergen protein expressed in E. coli (Reinmuth-Selzle et al. 2014).

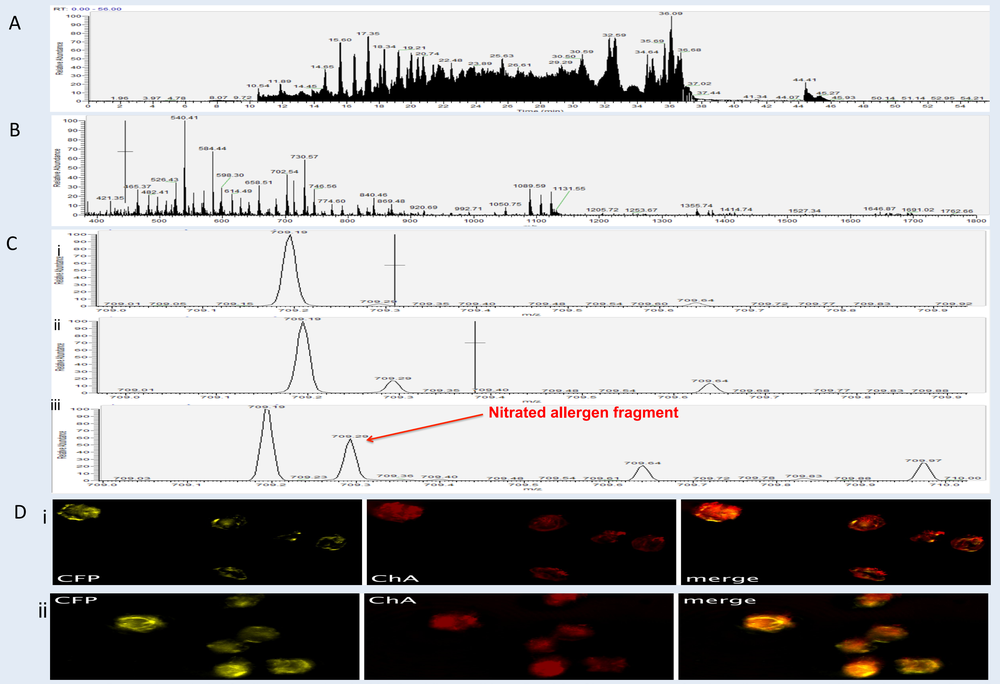

Figure 4. Proteomic analysis of pollen exposed under high humidity conditions. Panel A – Chromatogram of entire pollen proteome (all retention times). Panel B – Mass spectrum of entire pollen proteome (at different m/z values). Panel C – Fragment of mass spectrum of three samples exposed to different gases (High Rh- panel Ci, O3- panel Cii and NO2- panel Ciii respectively). The m/z value of interest got nitrosylated (upon exposure to NO2- panel Ciii) on cysteine residue of Bet V1 allergen protein (retention time = 25.98 mins charge=3, mol weight= 2127.89 and m/z= 709.29). Panel D I & ii- images of untreated and treated Birch pollen respectively with the untreated having a sharper images.

References

D'Amato, Gennaro, et al. "Climate change, air pollution and extreme events leading to increasing prevalence of allergic respiratory diseases." *Multidisciplinary respiratory medicine* 8.12 (2013): 1-9.

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Reinmuth-Selzle, Kathrin, et al. "Nitration of the Birch Pollen Allergen Bet v 1.0101: Efficiency and Site-Selectivity of Liquid and Gaseous Nitrating Agents." *Journal of proteome research* 13.3 (2014): 1570-1577.

What next?

✧ Relative quantification of the degree for PTMs using Tandem Mass Tag (TMT) system.

✧ Imaging of real-time exposure of the pollen samples and Birch sampling campaign.

✧ The laboratory-derived results will be supported with a time series analysis of asthma incidence rates for the London,which will take into account the pollen count and pollutant concentrations.