

A Serine Proteinase from *Parietaria Judaica* Pollen: Partial Purification, Characterization and Clinical Implications

CARLOS FARO¹, MARGARIDA COSTA-PINTO¹, CÉLIA ANTÓNIO¹, LUÍSA CORTES¹,
PAULA VERÍSSIMO¹, ANA TODO-BOM², EUCLIDES PIRES¹

INTRODUCTION

The pollen of the weed *Parietaria* is the main cause of allergic reactions in the Mediterranean countries, representing about 40% of the cases in pollen-allergic patients. Several proteins that elicit IgE production in sensitive individuals have been identified by immunoblotting and the major allergens are characterized biochemically and immunologically (Cocchiara et al. 1989; Duro et al, 1996). Recently it was shown that in addition to the well characterized allergens, the pollen of *Parietaria judaica* contains serine proteinase activity (Costa-Pinto, 1997).

The presence of proteolytic enzymes in other allergenic extracts has also been reported (for review see Kraft and Schon, 1993). Cysteine and serine proteinases were found in dust mites - *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* - (Yasueda et al, 1993, King et al, 1996) and more recently a chymotrypsin-like serine proteinase was isolated and characterised from ragweed (*Ambrosia artemisiifolia*), whose pollen is the major cause of late summer hay fever in the United States and Canada (Bagarozzi et al, 1996).

Here, we describe the identification, partial purification and characterization of a chymotrypsin-like serine proteinase from pollen of *Parietaria judaica*. The possible clinical implications of the presence of a proteolytic enzyme in these pollens are also discussed.

DETECTION OF PROTEOLYTIC ACTIVITY IN *PARIETARIA* POLLENS

As a first approach to address the question of whether or not *Parietaria* pollens display proteolytic activity, a

standard assay using k-casein as substrate was performed as described by Costa-Pinto (1997). Pollen extracts of *P. judaica*, *P. officinalis* and *P. lusitanica* were tested, and the results obtained clearly showed that they all contain proteolytic activity. The highest activity was detected in *Parietaria officinalis* pollen extracts, although the pollen of *P. judaica* also displayed a significant level of proteolytic activity.

Proteinases have been classified as to the amino acid residues or cofactors involved in the proteolytic activity and the classification is usually based on the sensitivity to various specific inhibitors (Barrett, 1998). The main classes include serine proteinases, cysteine proteinases, aspartic proteinases and metalloproteinases. To determine to which class of proteinases could the proteolytic activity of *Parietaria* pollens be ascribed, proteolytic activity assays were carried out in the presence of selective inhibitors. Proteolytic activity of the *Parietaria* pollen extracts was totally inhibited by PMSF, a well known inhibitor of serine proteinases, indicating therefore that serine proteinase activity is present in these pollens.

PARTIAL PURIFICATION OF A SERINE PROTEINASE FROM *P. JUDAICA* POLLEN

Even though the pollen of *P. officinalis* was the one with highest activity, further studies in this work were carried out with *P. judaica* pollen. The main reason for this choice is the abundance and more widely distribution of this latter species as compared to that of *P. officinalis*.

The enzyme was extracted from *P. judaica* pollen with 0.1 M ammonium bicarbonate pH 8.0 and partially purified by gel filtration on Superdex 200 and ion-exchange chromatography on DEAE-Sephacel. Although the active fraction obtained upon gel filtration still contained a yellow color, this step was found to be essential to remove large amounts of pigments and phenols. Further purification was then achieved in the DEAE-Sephacel column where a major peak of activity

(1) Departamento de Bioquímica, Faculdade de Ciências e Tecnologia, e Departamento de Biologia Molecular e Biotecnologia do CNC, Universidade de Coimbra;

(2) Unidade de Imunoalergologia, Hospitais da Universidade de Coimbra, Portugal.

was recovered. SDS-PAGE analysis of this active fraction revealed that the enzyme was not yet pure. A major band corresponding to a 60 kDa protein was observed in the gel, but at least five faint bands could be visualised as well.

To identify the band corresponding to the proteinase, a zymogram for detection of proteolytic activity was performed. This technique allows the detection of a specific or a class of enzymes in a protein mixture after electrophoretic separation on a suitable matrix (Paech and Christianson, 1994). Gelatin was used as substrate and activity was detected in the zone corresponding to the 60 kDa protein, suggesting therefore that is likely to be the active form of the proteinase.

ENZYMATIC PROPERTIES OF THE PROTEINASE

The partial purified proteinase was then characterized from the enzymatic point of view. In a first stage, the inhibition profile of this enzyme was studied with several proteinase inhibitors. Results showed that incubation of k-casein with chymostatin led to a total loss of activity, supporting therefore the previous observation that the proteinase belong to the class of serine proteinases. This result also indicates that the proteinase is probably of the chymotrypsin type, since chymostatin is a specific inhibitor of this subclass of serine proteinases. To investigate further this hypothesis, specificity studies using insulin B chain as substrate were performed. Cleavage was found to occur at bonds between hydrophobic residues which is in agreement with the reported specificity of a chymotrypsin-like proteinase.

These results indicate therefore that *Parietaria judaica* pollen contain a chymotrypsin-like serine proteinase like the one previously found in ragweed pollen (Bagarozzi et al. 1996). This latter proteinase has no activity towards protein substrates, with the exception of (-1 proteinase inhibitor, but rapidly hydrolyses, neuropeptides, vasoactive intestinal peptide (VIP) and substance P, which are required for normalized lung functions. It remains, however to determine whether the *Parietaria* proteinase may also display a similar activity.

CLINICAL IMPLICATIONS

Proteolytic enzymes present at the *Parietaria* pollens may play a significant role in the pathobiochemical reactions that occur during pollinosis. Probably the herein

described proteinase is not responsible for initiating hypersensitivity reactions, since no reactivity was observed with sera of allergic patients. Eventually it could be involved in causing disruption of the neuronal control exert by some neuropeptides that leads to chronic airway inflammation, or in the molecular mechanisms leading to the penetration of pollen allergens across the airway and lung epithelium. In any case, it will be interesting to see whether the proteinase may be a target for a drug-based therapeutic approach to the *Parietaria* pollinosis.

REFERENCES

- Bagarozzi, D.A., Pikes, R., Potempa, J. and Travis J. (1996) Purification and characterization of a novel endopeptidase in ragweed (*Ambrosia artemisiifolia*) pollen. *J. Biol. Chem* 271: 26227-26232.
- Barret, A. (1998) Handbook of Proteolytic Enzymes, Academic Press, London.
- Cocchiara, R., Locorotondo, G., Parlato, A., Guarnotta, G., Ronchi, S., Albeggiani, G., Amoroso S., Falagiani, P. and Geraci, D. (1989) Purification of Par jI, a major allergen from *Parietaria judaica* pollen. *Int. Arch. Allergy Appl. Immunol.* 90: 84-90.
- Costa-Pinto, M. (1997) Allergenic and proteolytic activity of *Parietaria* sp pollens. Msc thesis, University of Coimbra, Portugal.
- Duro, G. Colombo, P., Costa, M.A., Izzo, V., Porcasi, R., Difiore, R., Locorotondo, G., Cocchiara, R., Geraci, D. (1997) isolation and characterization of two cDNA clones, coding for isoforms of the *Parietaria judaica* major Par j 1.0101. *Int. Arch. Allergy Immunol.* 112: 348-355.
- Duro, G. Colombo, P., Costa, M.A., Izzo, V., Porcasi, R., Difiore, R., Locorotondo, G., Mirisola, M.G., Cocchiara, R., Geraci, D. (1996) cDNA cloning, sequence analysis and allergological characterization of Par j 2.0101, a new major allergen of *Parietaria judaica* pollen. *FEBS Lett.* 399: 295-298.
- King, C., Simpson, R.J., Moritz R.L., Reed, G.E., Thompson, P.J. and Stewart, G.A. (1996) The isolation and characterization of a novel collagenolytic serine protease allergen (Der p 9) from dust mite *Dermatophagoides pteronyssinus*. *J. Allergy Clin. Immunol.* 98: 739-747.
- Kraft, D. and Sehon, A. (1993) Molecular Biology and Immunology of Allergens CRC Press Inc. Boca Raton, Florida pp 135-307.
- Paech, C and Christianson, T. (1994) Zymography of proteases In: Cell Biology: a laboratory handbook Vol 3 (J. Celis ed.) Academic press, Inc san Diego, California pp 264-271.
- Yasueda, H., Mita, H., Akiyama, K., Shida, T., Ando, T., Sugiyama, S. and Yamakada, H. (1993) Allergens from *Dermatophagoides* mites with chymotryptic activity. *Clin. Exp. Allergy.* 23: 384-390.