

Expression cloning of a dust mite cysteine proteinase, *Der p1*, a major allergen associated with asthma and hypersensitivity reactions

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The house dust mite (HDM), *Dermatophagoides pteronyssinus*, is a major source of allergens, implicated in diseases such as asthma and atopic dermatitis. About 75% of children with atopic diseases are sensitised to HDM allergens by the age of ten years [1].

The group I allergens often account for 50% or more of the anti-mite specific antibodies [2]. We have recently modelled the 3D-structure of a major allergen in this group, *Der p1* [3]. The modelled structure contains active centre features characteristic of the cysteine proteinase family of enzymes (see [4] for a review). As a first step in the elucidation of the structure of *Der p1* by X-ray crystallography and the analysis of its catalytic characteristics and active centre chemistry, we have expressed mature *Der p1* in *Escherichia coli*.

Total RNA was prepared from 0.1 g of a culture of *Dermatophagoides pteronyssinus*, after snap-freezing the mites in liquid nitrogen and RNazol extraction. After cDNA synthesis using reverse transcriptase and a specific oligonucleotide to *Der p1*, the cDNA was amplified by the polymerase chain reaction (PCR) incorporating restriction enzyme sites in the primers. The PCR product was digested with *HpaI* and *KpnI* and ligated to the pin-point expression vector (Promega) which had been cut with *NruI* and *KpnI* to generate compatible ends. The product was cloned at the 3' terminus of the β -galactosidase gene, a component of the expression vector. The latter also contains a cleavage site for Factor Xa endoproteinase at the junction of the β -galactosidase gene and the inserted cDNA. In addition, the β -galactosidase contains a lysine residue which is biotinylated and serves as a purification tag for fused protein.

Prior to expression, the sequence of four clones was determined and all the inserts were in the correct reading frame. A number of mutations were identified which do not correspond to those in published variants [5]. These included Ala3Gly, His72Ser, Val124Ala in one clone, His72Ser in another and Val124Ala in the other two. Two silent mutations were observed; Ala149 and Ser178. The clone used for expression was identical to the published sequence except for Val124Ala.

Expression was monitored by using streptavidin coupled to alkaline phosphatase, which bound to the biotin residue. A fusion protein of the expected size (13 kDa β galactosidase and 25 kDa *Der p1* mature protein) was generated (Fig. 1). The product showed some immunoreactivity with a panel of monoclonal and polyclonal antibodies (results not shown) but was catalytically inactive. No evidence for catalytic activity was obtained either with or without 40 mM cysteine or 100 μ l of 2-mercaptoethanol at pH 7.4 (phosphate buffer) using the following substrates at a concentration of 150 μ M: D L-BAPNA, NAc-Phe-Gly-pNa, Z-Arg-pNA, α -N-Ac-Arg-pNA, α -N-Ac-Lys-pNA, 0.6% azocasein (over a 2h incubation period).

These results in all probability suggest that the enzyme is not

folded properly. Another possibility is that the catalytic site may be blocked in some way although probably not as a mixed disulphide. In an attempt to obtain active enzyme, we have adapted a protocol for solubilisation, denaturation and renaturation which has been used successfully with papain [6] but this was unsuccessful with *Der p1*.

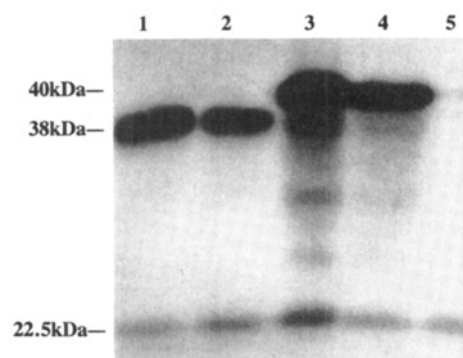


Fig. 1 Expressed fusion protein. Lanes 1 & 2 *Der p1*, lanes 3 & 4 are a positive control for CAT protein and lane 5 is a negative control. 22.5kDa is an endogenous *E. coli* protein.

There are several possible reasons for this; it has recently been recognised that it is important to incorporate the pro-sequence for proper folding. The cysteine proteinase papaya proteinase Ω was expressed as active enzyme when the pro-sequence was included and the protocol detailed above was followed [7]. A further potential problem is the presence of a portion of the β -galactosidase gene at the amino terminus. In addition, as is often the case, the expressed protein may be insoluble. Further studies are in progress to express *Der p1* incorporating the pro-sequence in both *E. coli* and *Aspergillus*. Production of substantial quantities of enzyme by these methods would facilitate structural and mechanistic studies which may provide new strategies in the treatment of individuals sensitised to *Der p1*. It needs to be established whether active enzyme plays a role in the disease process and if modification of active site residues could be used to desensitise hypersensitive individuals.

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1. Carswell, F. & Thompson, S. (1986). *Lancet* 1, 13-15.
2. Chapman, M.D., Rowntree, S.R., Di Prisco Di Feunmajor, M.C., Mitchell, E.B. & Platts-Mills, T.A.E. (1983). *J. Allergy Clin. Immunol.* 72, 27-33.
3. Topham, C., Srinivasan, N., Thorpe, C.J., Overington, J.P. & Kalsheker, N.A. *Prot. Eng.* (in press)
4. Brocklehurst, K., Willenbrock, F. & Salih, E. (1987) in *Hydrolytic Enzymes* (Neuberger, A. & Brocklehurst, K. eds.) pp. 39-158, Elsevier, Amsterdam.
5. Thomas, W.R., Chua, K-Y. & Smith, W-A. (1992). *Exp. Applied Acar.* 16, 153-164.
6. Taylor, M.A.J., Pratt, K.A., Revell, D-F., Baker, K.C., Sumner, I.G. & Goodenough, P.W. (1992). *Prot. Eng.* 5, 455-459.
7. Revell, D.F., Cummings, N.J., Baker, K.C., Collins, M.E., Taylor, M.A.J., Sumner, I.G., Pickersgill, R.W., Connerton, I.F. & Goodenough, P.W. (1993). *Gene*, 127, 221-225