

Effect of Reduction and Heat on the Detection of House Dust Mite (*Dermatophagoides pteronyssinus*) Allergen *Der p I* by Protein Blotting¹

Stephen A. Ford^a, Euan R. Tovey^a, Brian A. Baldo^{a, b}

^a Kolling Institute of Medical Research, Royal North Shore Hospital, St. Leonards;

^b Department of Medicine, University of Sydney, Australia

Abstract. Employment of reducing conditions during sample preparation alters the mobility of the house dust mite (*Dermatophagoides pteronyssinus*) allergen *Der p I* as determined by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) from an apparent MW of approximately 25 kD (unreduced) to an apparent MW of approximately 30 kD. Probing of nitrocellulose transfers with sera from subjects allergic to *D. pteronyssinus* showed that reduction of *Der p I* was accompanied by a substantial loss of IgE-antibody-binding capacity by this allergen. An important consequence of the effect of reduction on *Der p I* is that the electrophoretic mobility of this protein becomes very similar to a closely spaced pair of protein-staining bands, probably *Der p III*, of MWs 30–31 kD. These bands bind IgE antibodies strongly and with high frequency and exhibit the same electrophoretic mobilities under both reducing and non-reducing conditions. Thus, for the clear resolution of allergen *Der p I* from other IgE-binding components in the same MW region, including *Der p III*, house dust mite samples for analysis by SDS-PAGE and blotting should not be reduced.

Der p I, an important house dust mite allergen, was initially described as a glycoprotein of MW 24 kD following analysis by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [1]. Subsequent studies of this allergen demonstrated apparent MWs of 25 kD [2], 27 kD [3] and 30 kD [2, 4] by SDS-PAGE and 16–17 kD [5] and 24 kD [5] by column chromatography. In one of these investigations [2], it was reported that the MW of *Der p I* was not altered by reduction. In two studies of *Dermatophagoides pteronyssinus* extract by protein blotting [6, 7] the MW of *Der p I* was either given as, or can be estimated from the photograph shown, to be about 30–31 kD. In both of these studies, reducing conditions were employed in preparing the samples for SDS-PAGE examination and *Der p I* was identified by reference to a purified preparation of the allergen [6] or by immuno-

probing with a *Der p I*-specific monoclonal antibody [7]. Recently, the isolation of a cDNA clone coding for *Der p I* has led to a derived MW of 25,371 for this allergen [8].

In a previous study of house dust mite extract by protein blotting we found that *Der p I* had a MW of approximately 25 kD when gels were run under non-reducing conditions [9]. When, however, reducing conditions were used for sample preparation, the 25 kD component on the SDS-PAGE was no longer visible [10]. By contrast, the positions of other IgE-binding components in the gels remained essentially unchanged.

In view of these findings, and the disparity in the reported MWs of *Der p I*, we set out to examine the effects of reducing conditions and heat on the appearance, mobility, resolution and detectability of this allergen on gels and protein blots. Purified *Der p I* was provided by Prof. T.A.E. Platts-Mills, Charlottesville, Va., USA.

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Mite whole-body extracts prepared, separated on SDS-PAGE and electroblotted as previously described [9, 11], were resolved into at least 50 components (fig. 1b) when non-reducing conditions were employed – that is, when 2-mercaptoethanol (2-ME) was omitted from the sample preparation buffer. When 2-ME was included, 46 components were resolved (fig. 1c). The apparent MWs of a purified sample of *Der p* I examined under non-reducing and reducing conditions were approximately 25 and 30 kD, respectively (fig. 1d, e).

Electroblotting of the SDS-PAGE-separated mite proteins followed by probing with a pooled serum from subjects allergic to the house dust mite resulted in the resolution of 17 and 14 IgE-binding components when the gel electrophoresis was carried out under non-reducing (fig. 1f) and reducing (fig. 1g) conditions, respectively. Two IgE-binding components in the former sample disappeared or changed position following reduction. One of these, *Der p* I, was found to decrease in mobility and migrate to a position in the gel corresponding to a MW of approximately 30 kD (fig. 1h, i). IgE-antibody binding to *Der p* I decreased following the employment of reducing conditions.

The effect of heating (100°C for 5 min) on *Der p* I during the preparation for electrophoresis was tested. No differences were observed in the distance of migration in the gel, or the binding of IgE antibodies to blots, of *Der p* I in both unreduced heated and unheated mite extracts using 5 sera which had previously detected *Der p* I and 4 sera which had not detected *Der p* I in heated extracts. Examples of this are shown in figure 1j–m.

In view of the findings in this study, the effects of reduction on both the resolution and the IgE-binding capacity of components of complex allergenic extracts should be investigated in any protein blotting study designed to identify allergens. In the present case, the change in electrophoretic mobility of allergen *Der p* I induced by reduction created a potential problem in clearly distinguishing IgE-binding mite components in the region around 30 kD. Since many mite-allergic subjects have IgE antibodies that react with *D. pteronyssinus* proteins in this MW region [9, 12], in particular the so-called *Der p* III allergen, it is clear that analyses of house dust mite allergens should be carried out using non-reduced samples. Differences in sample preparation of house dust mite extracts for SDS-PAGE analysis, and in particu-

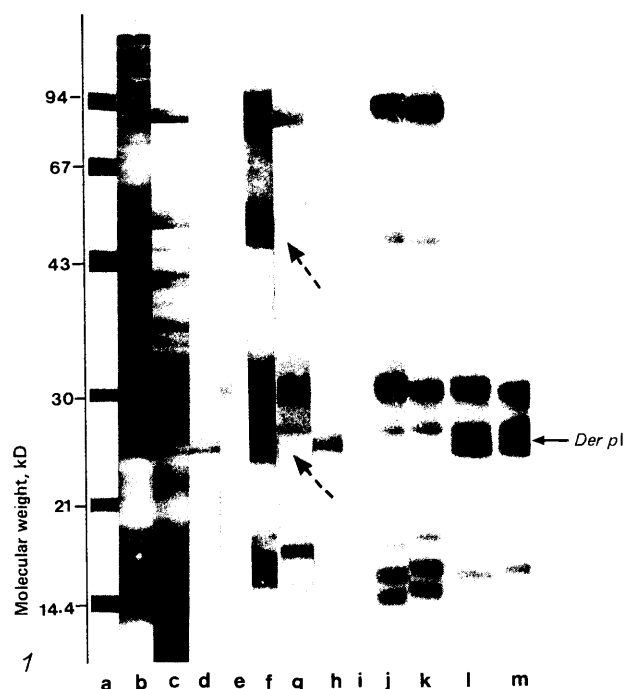


Fig. 1. The effect of reducing conditions and heat on the mobilities and IgE-binding properties of *D. pteronyssinus* allergen *Der p* I and other protein components separated by SDS-PAGE. **a–e** Coomassie-brilliant-blue-stained strips from SDS-PAGE. **f–m** Autoradiographs of nitrocellulose transfers after treatment with human serum followed by ^{125}I -anti-human IgE. **a** Low-molecular-weight markers (Bio-Rad). **b, c** Separated mite components from extracts prepared for electrophoresis in the absence or presence of 2-ME (5%), respectively. **d, e** Purified *Der p* I prepared in the absence or presence of 2-ME, respectively. Mite components on strips **f, g** were treated as for **b, c** and *Der p* I on strips **h, i** was treated as for **d, e**. Dashed arrows on strip **g** indicate the positions of two mite components that disappear after reduction. Strips **f–i** were probed with a pool of sera from mite-allergic subjects. The unreduced mite extract in strips **j, l** was heat-treated (100°C for 5 min) while the extract used for strips **k, m** was maintained at room temperature. Strips **j, k** were probed with a serum from a house-dust-mite-allergic subject who did not have IgE antibodies to *Der p* I while the serum used to probe strips **l, m** was from a subject who had IgE antibodies to *Der p* I. The band position of *Der p* I (~25 kD) on strips **j–m** is indicated by an unbroken arrow.

lar the greater IgE-binding capacity of non-reduced protein components, may explain why our blotting studies [9, 12] have resolved more IgE-binding components (up to 32) than others, where as few as 7 [6] and 9 [7] have been identified. A change in the apparent MW of an IgE-binding component from 26 to 29 kD on reduction was also observed in protein blotting studies on the related mite species *Dermatophagoides farinae* [13].

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Correspondence to: Dr. B.A. Baldo
Kolling Institute of Medical Research
Royal North Shore Hospital
St. Leonards, NSW 2065 (Australia)