

Correspondence

The metal binding site of the major house dust mite allergen Der p 1

To the Editor:

In their recent article, de Halleux et al.¹ reported the crystal structure of mature Der p 1. We have previously published an article describing the structure of the same allergen containing the propeptide.² Our article is correctly acknowledged and referenced in the de Halleux article. However, unfortunately, the authors claim to have discovered a metal binding site that they state has not been described in our article: "Recently, the 3-dimensional structure of the pro-form of Der p 1 has been presented by Meno et al. Careful examination of their data shows that a metal binding site is also present in pro-Der p 1, though it is not mentioned in their article." This metal site is clearly described in our article:

Strong $F_o - F_c$ electron density was observed in a depression on the surface of rproDer p 1-CN. A Y^{3+} ion complex-bound to the side chain carboxylic acid groups of D136, E139, and E171, the backbone carbonyl oxygen of L137, and three water molecules was placed at this position, and it returned nice electron density with a final B-factor of 16.7 Å². Two of the complex-bound water molecules hydrogen bond to a symmetry-related rproDer p 1-CN molecule through a hydrogen bonding network involving several other water molecules and a well ordered sulfate ion. This could explain the major improvement in crystal quality observed when YCl_3 was included in the crystallization.²

The amino acid residues that coordinate the yttrium ion are the same as those that coordinate the magnesium ion in the de Halleux article (the 80-residue discrepancy in the residue numbering is due to the presence of the propeptide). We find this misrepresentation most unfortunate because it is a substantial finding and because we believe that the statement in the de Halleux article suggests that they have examined our data and located a metal ion that we overlooked. This is certainly not the case because it is described both in our article, as well as in the corresponding Protein Data Bank entry (1XKG). Having said this, the biologic significance of the metal-binding site remains obscure. Enzyme kinetic measurements on Der p 1 are

usually performed in the presence of EDTA to prevent cation-facilitated inactivation of the active site cysteine residue, and therefore the metal binding site is unlikely to be occupied under these conditions. Omitting the EDTA and adding magnesium or calcium ions increases the enzymatic activity of the homologous protease papain somewhat,³ but this enzyme does not harbor the metal-binding site in question. It is tempting to speculate that the metal ion plays a role in stabilizing the Der p 1 structure, as suggested in the de Halleux article. However, magnesium or calcium ions have been shown to decrease the thermal stability of papain,³ and therefore the effect of metal ions remains to be investigated further for Der p 1. The observation that both yttrium and magnesium ions can bind at this site despite their different ionic radii suggests that the metal binding site is flexible.

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Disclosure of conflict of interest: K. Meno, P. B. Thorsted, H. Ipsen, J. N. Larsen, M. D. Spangfort, and K. Lund are all employed by ALK-Abelló. The rest of the authors have declared that they have no conflict of interest.

Editor's note: de Halleux et al.¹ have acknowledged their omission in not citing the work of Meno et al.² and extend their sincere apology to the authors.

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