Ligand Binding: an Intrinsic Property of Cockroach Allergen Bla g 2?

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Rationale: Bla g 2 is an inactive aspartic protease similar to pregnancy-associated glycoproteins. We investigated the effects of amino acid substitutions in the catalytic site and the "flap" region of Blag2 on enzymatic activity and binding to pepstatin, a specific aspartic protease inhibitor.

Methods: Recombinant Blag2 mutant (Mut1) was expressed in Pichia pastoris after site-directed mutagenesis of the wild type (Wt) allergen. De-glycosylated mutant (N93Q) was produced for crystallography. The Wt and Mut1 were assayed for enzymatic activity (milk-clotting-MCA- or hemoglobin Assay-HA-) or for binding to pepstatin.

Results: The Wt and Mut1 allergens did not show aspartic protease activity at 10microg/ml and 80microg/ml for MCA and HA, respectively, while pepsin was active at <0.3microg/ml and <10microg/ml. Approximately 70% of pepsin bound to pepstatin-agarose, and was fully recovered by elution. rBlag2 Wt and Mut1 needed more stringent conditions for elution, indicating a stronger binding to pepstatin than pepsin. 2-5 fold more rBlag2 Mut1 than Wt was eluted, suggesting that the inhibitor binds stronger when the resemblance to the active site of aspartic proteases is higher. Crystals were obtained with the N93Q mutant, and diffraction data were collected at 2.2A resolution. Structure determination is in progress.

Conclusions: Since engineering of rBlag2 did not convert the allergen into an active aspartic protease, we conclude that pepstatin binding to Blag2 indicates that this allergen may function as a ligand binding molecule, rather than as a protease. Determination of the crystal structure of Blag2 will help in clarifying this matter and in the search for natural ligands of Blag2.