Poster Presentation

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Structural and mechanistic studies of human chitinase

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Chitinases are enzymes that hydrolyze chitin, a glucosamine polymer synthesized by lower organisms for structural purposes [1]. While humans do not synthetize chitin, they express two active chitinases, Chitotriosidase (hCHIT1) and Acidic Mammalian Chitinase (hAMCase). Both enzymes attracted attention due to their upregulation in immune system disorders [2,3]. They consist of a catalytic domain of 39 kDa and a chitin binding domain, joined by a hinge. The active site shows a cluster of three conserved acidic residues, E140, D138 and D136, linked by H-bonds, where D138 and E140 are involved in the hydrolysis reaction [1,3]. To increase our knowledge on the catalytic mechanism of human chitinases, we conducted a detailed structural analysis on hCHIT1. For this, we have improved the X-ray resolution of the apo hCHIT1 catalytic domain to 1Å. We investigated the protonation state on the catalytic site and detected a double conformation of D138, one in contact with D136 and a second one in contact with E140. Our analysis revealed for the first time different protonation states for each conformation of D138 (fig1). Interestingly, our X-ray data suggest that the catalytic E140, supposed to donate a proton in the catalytic reaction, is deprotonated in the apo form. To gain insight on the proton transition pathway during the hydrolysis, we have solved the X-ray structure of hCHIT1 complexed with the substrate at 1.05 Å. In comparison with the apo form, this structure shows a rearrangement of the protonation states of the catalytic triad triggered by the binding of the substrate. Our results led us to suggest a new hydrolysis model involving changes in the hydrogen bond network of the catalytic triad accompanied by a flip of D138 towards D136. This contributes to protonate E140, which then donates the proton to the substrate. To confirm the role of the active site's hydrogen network, we are currently studying CHIT1 by neutron crystallography and quantum mechanics.

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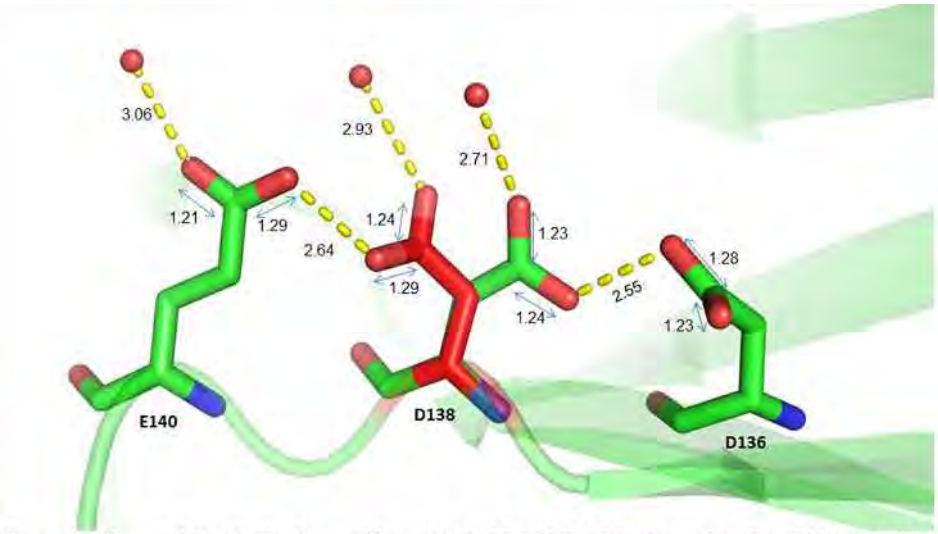

Figure 1 : The catalytic cluster formed by E140, D138 and D136 in the active site of hCHIT1. D138 shows a double conformation (one oriented to D136 in green and the one oriented to E140 in red). The distance between each amino acid and the water molecules are indicated with yellow dotted lines. The distance of the bonds between the carbon and the oxygen are indicated with blue arrows. These distances define the protonation state of the cluster. Structure Refined by shelx, R= 14,19.

Keywords: chitin hydrolysis, X-ray crystallography, protonation state