Are Cysteine-lipases Involved in the Immune System?

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Lipases, esterases and proteases constitute superfamilies of hydrolases not only play an important role in the immune system, but also as catalysts in biotechnology and organic chemistry. Mechanistically, they all involve a similar catalytic triad. The mechanism of lipase-catalysis is defined by the catalytic triad Ser-His-Asp in which activated serine adds nucleophilically to the carbonyl function of an ester or lactone substrate in the rate-determining step with formation of a short-lived oxyanion which then fragments into an alcohol and a covalent acyl-enzyme intermediate, the latter rapidly undergoing reaction with water and liberating the respective carboxylic acid (Figure 1) [1-5].

In contrast, both serine-proteases with Ser-His-Asp as the catalytic triad and cysteine-proteases characterized by Cys-His-Asp have been identified as enzymes with high activity. It has also been shown that in the case of serine-proteases, mutation to the respective cysteine-proteases leads to a partial or complete breakdown of activity, and the same applies to the opposite scenario in which a cysteine-protease is mutated into a serine-protease, which has led to lively discussions concerning the origin of these effects [6-12]. Recently, we demonstrated that the conversion of a serine-lipase into an artificial cysteine-lipase also induces significant loss of activity [13].

Today, it is well known that the human digestive tract has a prominent influence on the immune system. As already alluded to in the above short introductory information, lipases play an important role in a number of health problems. Only a few typical studies are cited here, the focus in these cases being on such enzymes as monoacylglycerol lipases, triglycerol lipases, and phospholipases [14-26]. In these and other works available in the extensive literature, sequence information, lipases play an important role in a number of health problems. Only a few typical studies are cited here, the focus in these cases being on such enzymes as monoacylglycerol lipases, triglycerol lipases, and phospholipases [14-26]. In these and other works available in the extensive literature, sequence information was generally presented, showing that serine-lipases are indeed involved; in some studies, this was just assumed by the authors and no mention of possible cysteine-lipases as alternatives was made.

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**Figure 1:** Generally accepted mechanism of lipases [1-5]. The residue numbers correspond to the catalytic triad Asp-His-Ser of the lipase from *Candida antarctica* B (CALB).
In our study describing the transformation of a serine-to-a cysteine-lipase, Candida antarctica lipase B (CALB) was used as the model hydrolase [13]. The catalytic triad of this standard and in biotechnology often applied lipase is Asp187-His-224-Ser105 (Figure 1) [1-5,27-28]. In our study, mutant Asp187-His-224-Cys105 as a cysteine-lipase was shown to have a very low activity for a number of structurally different substrates. In order to regain and perhaps even to surpass the activity of wildtype (WT) CALB in a model transformation involving the hydrolytic kinetic resolution of a racemic ester, we utilized the techniques of directed evolution [29-32]. Specifically, saturation mutagenesis at rationally chosen residues surrounding the binding pocket according to the Combinatorial Active-site Saturation Test (CAST) [33] was performed, followed by Iterative Saturation Mutagenesis (ISM) [33-34] at other hotspot residues around the binding pocket [13]. The best evolved mutant, W104V/S105C/A281Y/A282Y/V149G, showed a 40-fold enhancement of activity in the model reaction, and was even more active than WT CALB in the hydrolysis of further substrates.

The combination of X-ray structures, kinetics, molecular dynamics (MD) simulations and QM/MM computations revealed dynamic effects upon going from cysteine-CALB to the best mutant [13]. It was shown that the three additional mutations cause, inter alia, the re-adjustment of the now active catalytic triad Asp187-His-224-Cys105 in a way that produces the zwitterion Cys105−/His224+, thereby enforcing a novel 2-step mechanism rather than the traditional concerted addition process [13]. Changing the mechanism of an enzyme by introducing mutations is a rare event in protein engineering. The overall results demonstrate that cysteine-lipases can in fact be active, but they do not prove that such lipases occur in nature.

The distinction between lipases and esterases continues to be a subject of considerable interest. Traditionally it was believed that lipases have a lid which opens upon interaction with a hydrophobic substrate by interfacial activation, in contrast to esterases [1-5,35-36]. However, uncertainties remain, and a sharp distinction may not matter [37-38]. In view of our recent study [13], we believe that an intensive search for natural cysteine-lipases (or esterases) could be a fruitful venture in different areas of lipase research, immunology and virology.

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