EUPERGIT C® ACTIVATION FOR THE IMMOBILIZATION OF CPO THROUGH SPECIFIC REACTIVE GROUPS

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Key words: chloroperoxidase, immobilization, Eupergit C.

Introduction. Chloroperoxidase (CPO) from *C. fumago* is one of the most attractive peroxidases due to its catalytic versatility. Many efforts have been directed to immobilize CPO, in most cases resulting in dramatic loss of enzymatic activity (1). In this work, we explored the covalent immobilization of CPO in Eupergit C®, a commercial acrylic-based support with a high density of oxirane groups, through insertion of appropriate functional groups in the enzyme and/or into the support. The above groups are reactive under enzyme´s stability conditions and their location allows immobilization without affecting access to the active site or the enzyme´s structure.

Methods. Reactive side chains far from the active site, exposed to the solvent and non-blocked by hetero-groups such as glycosyl moieties, were K145, K211, Y144, Y287 and Y288. Lys residues were modified with thiol groups (CPO-thiol), while Tyr residues were modified with a nitro group (CPO-nitro). Eupergit C was activated with maleimide, diazonium ions and glutaraldehyde and are specific for CPO-thiol, Tyr and Lys residues in native CPO, respectively. CPO-nitro was reacted directly with Eupergit C.

Results. Only 5% of the Tyr residues in CPO were modified with nitro groups. Thus, CPO-nitro was not immobilized into Eupergit C, probably due to this low degree of modification. Regarding modification of Lys residues, thiol groups were successfully introduced into CPO. CPO-thiol immobilized in E-maleimide retained 100% of catalytic activity, albeit a low load was observed (0.4 mg/g), resulting in a specific activity of 201 U/g. On the other hand, native CPO immobilized in E-diazonium resulted in a preparation with high specific activity (15691 U/g). This preparation showed enzyme leaching, with a half-desorption time of 2 h. During immobilization at pH=6, CPO is negatively charged and the support is positively charged, thus favoring ionic interactions. The observed diazonium degradation in aqueous solution could explain the interaction instability. The preparation was stabilized in a *t*-butanol: buffer 80:20 (v/v) mixture with a $t_{1/2}$ of 29 h. Native CPO immobilization in E-glutaraldehyde resulted in a preparation retaining 14% of original activity, a load of 43 mg/g, a specific activity of 6874 U/g, and a half-desorption time of 20 h.

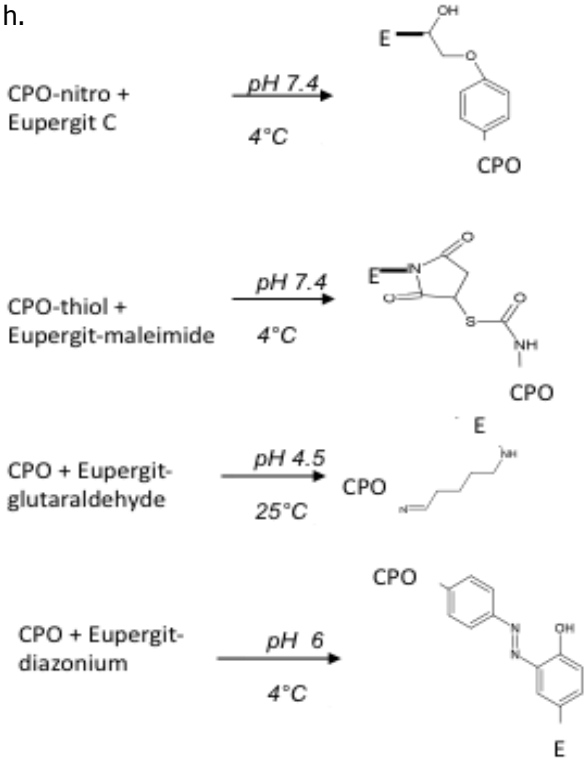

Figure 1. CPO immobilization strategies. E: activated-Eupergit C.

Conclusions.
CPO was immobilized through covalent and non-covalent interactions in different activated supports, in some cases retaining very high activity, highlighting the importance of selecting the chemical nature and localization of the target residues.

Acknowledgements. CONACYT and PAPITT IN201612 for financial support and Rosa Román for technical support.

References.
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