Poster GC20

Regioselectivity of Peptides Acylation by Candida antarctica lipase B (CALB) in Supercritical Carbon Dioxide: Comparison with Observations in Organic Solvent (CALB) or Aqueous Solvent (Acylase)

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Acylated peptides or amino acids are widely used, mainly as biosurfactants, in pharmaceutical and ecologically friendly cleaning products. Their industrial synthesis is currently based on the Schotten-Baumann chemical reaction bearing several drawbacks (temperature, very basic pH, use of some toxic solvents, salt as by-products, yield). To date, no attempts for peptides acylation in supercritical carbon dioxide have been reported. In this study, enzymatic acylation of the dipeptide Lys-Phe, which can potentially be acylated on amino groups of Lysine in position α and/or ϵ (figure 1a), were carried out during 24 hours under batch conditions in a 10 mL magnetically stirred reactor. Two pressures (150 and 200 bars) and four temperatures (45°C, 55°C, 65°C and 90°C) were investigated. 100 mg of *Candida Antarctica* lipase B (CAL B) immobilized on macroporous acrylic beads (a_w=0.12) were mixed together with Lys-Phe HCl (0.02 M), oleic acid (0.04 M) and triethylamine (0.2 M). Thin layer chromatography (TLC) using ninhydrin for revealing free primary NH_2 groups revealed mono-oleyl Lys-Phe at 65°C-150 bars only (figure 2) while serious peptide degradation was evidenced by lysine and phenylalanine spots at temperature above 65°C. Further LC-MS^2 investigations, while confirming TLC observations, also revealed the formation of dioleyl-Lys-Phe (impossible to see in TLC), meaning that CAL B, when used in supercritical CO_2, can acylate Lysine both in α and ϵ position (figure 3) . TLC and LC-MS^2 also revealed that, in supercritical CO_2, CAL B could N-acylate the tripeptide Ile-Lys-Pro (Figure 1b) in position α of Ile and/or ϵ of Lys, generating position isomers (figure 4). As can be seen in table 1 ,the originality of such results lies on the fact (i) that N-acylation of Lysine in position α or α+ ϵ was never observed when using CAL B in organic solvents and (ii) that enzymatic diacylation in α+ ϵ positions was also previously never observed in water when using acylase as a biocatalyst (acylation in α position only). These differences may be explained by the specific characteristic of supercritical carbon dioxide, which increase intermolecular collisions and the accessibility of the CAL B active sites to the peptides and oleic acid.