EICOSAPENTAENOIC ACID CONVERSION BY CYTOCHROME P450 BM-3 AND ITS MUTANTS TO BIO-ACTIVE EPOXIDE DERIVATIVES

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Oxidized polyunsaturated fatty acids such as resolvin and protectin are promising functional lipids because they have strong anti-inflammatory effect[1]. Recently, dietary eicosapentaenoic acid (EPA) was found to exert anti-allergic effect through the conversion to 17,18-epoxyeicosatetraenoic acid in the gut[2]. These findings promoted the studies on enzymatic EPA epoxydation to bio-active derivatives. We screened P450 BM-3 and its mutants with rationally modified substrate binding site for conversion of EPA with existence of NADPH regeneration system and ROS decomposing system, catalase. Through the screening, some mutants were found to produce several products (UK1, 2, 3, and 4). Then, these products were purified and identified with LC-MS, NMR, and GC-MS. Finally, these products were identified: UK1 was 14,15:17,18-diepoxy-eicosatrienoic acid (14,15:17,18-DEpETr), UK2 was 17,18-epoxy-eicosatetraenoic acid (17,18-EpETe), UK3 was 14,15-epoxy-eicosatetraenoic acid (14,15-EpETe), UK4 was 11,12-epoxy-eicosatetraenoic acid (11,12-EpETe). The reaction conditions were optimized with P450 BM-3 mutants, and under the optimized conditions, mutant Al4_Ile converted 0.5 mg/ml EPA to 0.20 mg/ml 11,12-EpETe (conversion rate: 38.0% mol/mol). Mutant F87A converted 0.5 mg/ml EPA to 0.19 mg/ml 14,15-EpETe (conversion rate: 36.1% mol/mol). Wild type P450 BM-3 converted 0.5 mg/ml EPA to 0.38 mg/ml 17,18-EpETe (conversion rate: 72.2% mol/mol). Mutant L7V converted 0.5 mg/ml EPA to 0.075 mg/ml 14,15:17,18-DEpETr (conversion rate: 13.5% mol/mol).

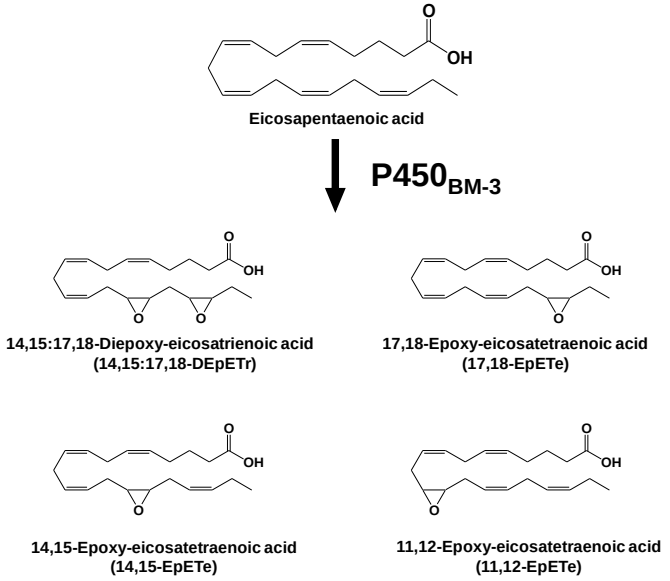

Figure 1 – EPA conversion by P450 BM-3 and its mutants

1) Schwab JM *et al. Nature* (2007)
2) Kunisawa J *et al. Scientific reports* (2015)