

Oligomerization and kinetic characterization of purified human FMO3 and FMO5 expressed as maltose binding protein fusions

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Abstract

The flavin-containing monooxygenase (FMO) family of enzymes oxygenates nucleophilic xenobiotics and endogenous substances. FMO3 and FMO5 are the prominent isoforms in adult human liver. To improve solubility and facilitate characterization of FMO3 and FMO5, *N*-terminus MBP fusions of both enzymes were prepared to >90% purity in the presence of detergent. Based on size-exclusion chromatography, MBP-FMO3 was found to exist as a monomer in the presence of 0.5% Triton X-100 and as a hexamer in the presence of 0.5% CHAPS. The different oligomeric states of MBP-FMO3 correlated to different steady-state kinetic parameters.

Addition of 0.5% Triton X-100 to MBP-FMO3 incubation mixtures resulted in approximately 3-fold improved k_{cat}/K_m values for both methimazole and L-methionine. Similarly, addition of 0.5% Triton-X-100 to commercially available FMO3 Supersomes resulted in a 2.5-fold improvement in k_{cat}/K_m for methimazole. The apparent K_m values were the same for FMO Supersomes and corresponding MBP-fused FMO enzymes but MBP-FMO enzymes afforded a lower k_{cat} compared with Supersome FMO3 (i.e., k_{cat} of 11.5 min⁻¹ vs. 22.0 min⁻¹ for methimazole) and Supersome FMO5 (i.e., k_{cat} of 0.64 min⁻¹ vs. 1.01 min⁻¹ for 10-(*N,N*-dimethylaminoethyl)-2-(trifluoromethyl) phenothiazine). A comparison of kinetic parameters and sensitivity to detergents suggest the overall protein folding, coenzyme association, and oxygenation properties of Supersome FMOs are retained in the MBP-FMO fusion enzymes. With the ease of constructing and producing variant forms of MBP-FMO in high purity, these enzymes should be of great utility for studying both drug efficacy and drug-metabolism induced idiosyncratic disease.