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Identification of Cytochrome P450 Enzymes in Phenobarbital-treated Mouse Liver Microsomes using 2-DE and MALDI-TOF/TOF

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Cytochrome P450s (CYPs) constitute a large superfamily of membrane-bound proteins that participate in metabolism of environmental contaminants, drugs, and endogenous compounds. Advances in mass spectrometry (MS)-based proteomics have provided a potential for the identification and expression profiling of CYP proteins. However, the application of MS-based proteomics is still limited due to the high amino acid sequence homology and insolubility of CYP proteins. Only a few studies have successfully identified CYPs by combination of 1-dimensional electrophoresis (DE) and liquid chromatography tandem mass spectrometry (LC-MS/MS). In previous studies, CYP proteins have been poorly separated in 2-DE. The objective of this study is thus to establish a method using 2-DE and a high-performance matrix-assisted laser desorption/ionization- (MALDI) time of flight/time of flight (TOF/TOF) tandem mass spectrometry for the comprehensive analysis of CYP isozymes expressed in animal tissues. Microsomal fractions were isolated from the liver of MRL/lpr female mice treated with phenobarbital (PB) (80μg/g body weight x 3 times). Total CYP content in the liver microsomes of PB-treated mice was significantly induced 5.4-fold, compared with those of vehicle-treated mice. Moreover, O-dealkylation activities of alkoxyresorufins including methoxyresorufin (MROD), ethoxyresorufin (EROD), pentoxyresorufin (PROD), and benzyloxyresorufin (BROD) were determined by fluorescence assays. MROD, EROD, PROD, and BROD activities were significantly induced at 3.8, 6.4, 17, and 97-fold, respectively. 2-DE showed high resolution of microsomal proteins in the range of the predicted gel migration of CYPs (40-60 kDa, pH 7-10). Peptide mass fingerprinting and MS/MS ion searchusing MALDI TOF/TOF allowed successful identification of some CYP2 isozymes.

Biography

Maria Claret Lauan is currently a first year PhD student at Ehime University, Matsuyama, Japan under the supervision of Prof. Hisato Iwata at Laboratory of Environmental Toxicology, Center for Marine Environmental Studies, Ehime University. Her research interests include wildlife toxicology, proteomics, and bioinformatics. One of the goals of Prof. Iwata's laboratory is to develop novel bioassay methods to explore bioactive/toxic contaminants and its effects in a variety of wild and model animals.