

## SL-2

# Membrane Insertion of Cytochrome P450 1A2 Promoted by Anionic Phospholipids

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The role of phospholipids in the membrane binding and subsequent insertion of the microsomal protein rabbit cytochrome P450 (P450) 1A2 into phospholipid bilayers was investigated. The insertion of P450 1A2 into phospholipid bilayers was determined by the amount of quenching of Trp fluorescence of P450 1A2 by pyrene and brominated and doxyl-labeled phospholipids. When the phosphatidylcholine (PC) matrix was replaced with acidic phospholipids (phosphatidic acid (PA), phosphatidylserine, and phosphatidylinositol) and phosphatidylethanolamine (PE), the extent of insertion into lipid bilayer was strictly dependent on the type of acidic phospholipids. All anionic phospholipids caused the penetration of P450 1A2 into lipid bilayers. PA was the most efficient in facilitating the deep penetration of P450 1A2 into phospholipid bilayers. On the other hand, the binding of P450 1A2 to liposomes was increased by acidic phospholipids to the same degree regardless of the type of acidic phospholipids. PE was found to act as an inert matrix phospholipid, similar to PC, as it exerted very little effects on the insertion of P450 1A2 into lipid bilayers and the binding of P450 1A2 to membranes. It was also found that the phospholipid-dependent membrane insertion of P450 1A2 was associated with altered enzyme activity, and the conformational change including raised the  $\alpha$ -helix content and intrinsic Trp fluorescence of P450 1A2. These results indicate that negative charges on the acidic phospholipids are important for the initial binding of P450 1A2 to membranes but the penetration of P450 1A2 into lipid bilayers is regulated by the type of acidic phospholipids present in membranes. The phospholipid-dependent insertion of P450 1A2 is accompanied by a structural change of P450 1A2 induced by phospholipids.