Complexation of Membrane-Bound Enzyme Systems

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The effect of changes in the N-terminal membrane-binding domain of cytochrome P450 forms and NADPH-cytochrome P450 reductase types on the cytochrome P450-dependent monooxygenase activities, has been examined. The nifedipine oxidase activity of two human P450 forms (CYP3A4, CYP3A4NF14) which differ only in their primary structure by ten amino acid residues in the N-terminal membrane-binding domain, yields nearly the same catalytic cycle time τ =2.65 ± 0.15 s, due to their identical cytosolic catalytic protein structure. In contrast, the complex formation process ([P450]+[reductase] ↔ [complex]) described by the dissociation constant K_D at high substrate concentration ($[S] >> K_S$) and low product concentration ($[P] << K_P$) is determined to be $K_D/[P450]_o$ = 0.3 and 2.0, respectively. These values reflect large differences in the affinity of both P450 forms for the same type of reductase which is only due to their modified membrane-binding domains. In the present work, it has been shown for the first time, that the membrane-binding domain of cytochrome P450 enzymes determines the complexation process of the binary P450:reductase system. Furthermore, the nifedipine oxidase activity of the human CYP3A4 form reconstituted with two different types of reductase from human and rabbit also has the same catalytic cycle time τ = 2.65 ± 0.15 s. This result is based on the similarity of the primary structure of the cytosolic catalytic domain of both reductase types. However, the complex was formed with different dissociation constants of $K_D/[P450]_o$ = 0.3 and 4.7, respectively. This different affinity of both reductase types to the same P450 form is interpreted as a consequence of the substantial alteration of the amino acids in the N-terminal primary structure of their membrane-binding domains. 7-Ethoxycoumarin O-deethylase activity of two rat P450 forms (CYP2B1 and CYP1A1) were reconstituted with the same rat reductase. The catalytic cycle time for each P450 form is τ = 1.8 and 0.6 s, respectively. Correspondingly, the complex formation process controlled by the dissociation constant K_D has changed from $K_D/[P450]_o$ = 2.3 to 1.7, respectively. This is because both forms differ in their cytosolic as well as in their membrane-binding domains.