The common I172N mutation causes conformation chang of P450c21 revealed by systematic mutation, kinetic and structural studies.

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Abstract

We have investigated the structure and function of P450c21 with regard to a conserved site around Ile-172 by site-directed mutagenesis making single amino acid substitutions of residues 169-173. Substitutions of Ile-171 and −172 resulted in production of mutant proteins with dramatic reductions in enzymatic activities, indicating the importance of these two residues in maintaining the structure and function of P450c21. The I171N protein was present at a slightly lower level, due to a decreased rate of protein synthesis. The I172N apoprotein was synthesized at the normal rate, but its heme-bound P450 form was present at a much lower level. This I172N protein was tightly integrated into the membrane of endoplasmic reticulum, similar to the wild type P450c21, as shown by immunofluorescence detection, alkaline extraction, and cellular fractionation. Kinetic studies indicated that I172N had a lower V value. In addition, the I172N protein was more sensitive to proteinase K digestion, indicating a possible alteration of conformation. This conformational change may result in the lower yield of the I172N hemoprotein and the reduced catalytic activity.