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Purification of a human cytochrome P-450 isozyme catalyzing lanosterol 14α -demethylation*

Yoshiko Sonoda, Masayuki Endo, Kaori Ishida, Yoshihiro Sato, Naomi Fukusen** and Morio Fukuhara**

園田よし子、遠藤雅之、石田 香、佐藤良博、福泉直美**、福原守雄**

An isozyme of cytochrome P–450 catalyzing lanosterol 14α –demethylation was purified from human liver using column chromatography, including immunoaffinity chromatography. The purified protein exhibited a single protein band (53 kDa) on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. When reconstituted with NADPH–cytochrome P–450 reductase, the purified protein showed an activity of 14α –demethylation of 24,25–dihydrolanosterol (3.20 nmol/min/mg protein). The apparent Km value for 24,25–dihydrolanosterol was found to be 27 μ M. This enzyme converted in the reconstituted system, the oxygenated intermediates of 24,25–dihydrolanosterol 14α –demethylation, 32–hydroxy–24,25–dihydrolanosterol and 32–oxo–24,25–dihydrolanosterol, to the 32–nor compound, 4,4–dimethylcholesta–8,14–dien–3 β –ol.

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^{**} 国立公衆衛生院