A Direct, Highly Sensitive Fluorometric Assay for a Microsomal Cytochrome P450-Mediated O-Demethylation Using a Novel Coumarin Analog as Substrate

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Z. Naturforsch. **55 c,** 915–922 (2000); received June 21/July 27, 2000

Direct Fluorometric Assay, Cytochrome P450, Demethylation of 3-Chloro-7-methoxy-4-methylcoumarin

A highly sensitive fluorometric assay for the determination of monooxygenase activity in liver microsomes is described. The assay is based on the use of 3-chloro-7-methoxy-4-methylcoumarin which is demethylated to 3-chloro-7-hydroxy-4-methylcoumarin. The rate of formation of 3-chloro-7-hydroxy-4-methylcoumarin was recorded as an increase of fluorescence (λ_A = 380 nm, λ_F = 480 nm) with time. When 3-chloro-7-methoxy-4-methylcoumarin was incubated in the presence of $MgCl_2$ and NADPH with rat liver microsomes, a continuous increase of the fluorescence could be measured. The reaction proceeded linearly for about 10 min and at least up to a concentration of 0.1 mg/ml of microsomal protein. Besides 3-chloro-7-hydroxy-4-methylcoumarin a hydroxylated derivative of the substrate was formed as a second metabolite during the incubation. Using an excitation wavelength of 380 nm and a fluorescence/emission wavelength of 480 nm, the fluorescence of this substance (λ_A = 338 nm, λ_F = 422 nm) amounted only to about 1% of the fluorescence of the main product. The use of 3-chloro-7-methoxy-4-methylcoumarin as substrate enables the fluorometric determination of the O-dealkylation activity of a cytochrome P450-dependent monooxygenase system in rat liver which is inducible by phenobarbital but not by 3-methylcholanthrene.