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Hill Parameters and Heterogeneity of alpha-Naphthoflavone Binding to Human Cytochrome P450 3A4 by Fluorescence Spectroscopic Analysis BENJAMIN CARLSON, GLENN MARSCH, Grove City College, MARTHA MARTIN, F. PETER GUENGERICH, Vanderbilt University School of Medicine — Human cytochrome P450 3A4 (CYP 3A4) is an alpha-helical membrane-bound protein that metabolizes approximately 50% of all drugs. The interaction between CYP450 3A4 and alpha-naphthoflavone (ANF) was characterized using fluorescence methods. ANF quenched fluorescence from tryptophan residues in CYP 3A4, and CYP 3A4 quenched bound ANF. The ANF emission energy was unchanged upon binding to CYP 3A4, implying that enzyme-bound 3A4 is completely quenched. Fluorescence difference spectra were fit to the Hill equation by varying the parameters K_d and n. For quenching of tryptophan fluorescence by ANF, no significant sigmoidal behavior was observed with n=1, and the spectral dissociation constant revealed a strong ANF-CYP 3A4 interaction with K_d=27nM. Modest cooperativity and very tight binding was observed in the quenching of ANF by CYP 3A4, with n=1.4 and K_d= 4.9nM. Fluorescence polarization anisotropy <r> decreased at low ANF/CYP 3A4 molar ratios; then $< r >$ increased at higher ratios. Compared to substrate-free CYP 3A4, adding substrate at low molar ratios increases the CYP 3A4 rotation, suggesting the molecular volume decreases.

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