

Probing the Cytochrome P-450 2B1 Active Site with Diamantoid Compounds

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Abstract. Hydrocarbonyl diamantane has been shown to be a specific substrate with a high affinity for the binding site of PB-inducible cytochrome P-450 2B1 (Hodek et al. 1988). Using a difference spectroscopy approach, a battery of diamantane analogues and diamantane oxygen containing derivatives were examined for their interaction with P-450 2B1 active site. Of the compounds (diamantane and its analogues, adamantane and triamantane) tested, diamantane had the lowest value of a spectral dissociation constant $K_s = 0.5 \mu\text{mol/l}$, indicating that diamantane was accommodated well to the cytochrome P-450 2B1, hence values of 0.46 nm and 0.66 nm for the width and length of the diamantane molecule, respectively, were used to describe of the dimensions the cytochrome P-450 binding site. Adamantane ($K_s = 1.3 \mu\text{mol/l}$) is relatively small and thus it binds loosely whereas triamantane ($K_s = 4.3 \mu\text{mol/l}$) is bulky enough to fit the binding site. This conclusion has been confirmed by spectral competition experiments as well as metabolic studies.

Of all oxygen containing derivatives diamantane 1,6-dicarboxylic acid dimethyl ester only exhibited a pronounced ligand interaction with cytochrome P-450. Using molecular dimensions of this derivative the distance of 0.56 nm from the heme iron to the center of the substrate binding site was estimated.

Key words: Cytochrome P-450 — Diamantane — Active site — Difference spectroscopy

Introduction

Cytochrome P-450 (P-450) are hemoproteins which catalyze monooxygenation of foreign compounds and endogenous substrates (Ortiz de Montellano 1986; Guengerich 1991; Porter and Coon 1991). P-450 plays an important role in detoxification and elimination of hydrophobic foreign compounds. However, in some