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Steroid bioconjugation to a CYP3A4 allosteric site and its effect on substrate binding and coupling efficiency

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Abstract[Abbreviations]

Human cytochrome P450 3A4 (CYP3A4) is an important drug metabolizing enzyme involved in a number of drug-drug and food-drug interactions. As such, much effort has been devoted into investigating its mechanism of interaction with ligands. CYP3A4 has one of the highest levels of substrate promiscuity for an enzyme, and can even bind multiple ligands simultaneously. The location and orientation of these ligands depend on the chemical structure and stoichiometry, and are generally poorly understood. In the case of the steroid testosterone, up to three copies of the molecule can associate with the enzyme at once, likely two in the active site and one at a postulated allosteric site. Recently, we demonstrated that steroid bioconjugation at the allosteric site results in an increase in activity of CYP3A4 toward testosterone and 7-benzyloxy-4-trifluoromethylcoumarin oxidation. Here, using the established bioconjugation methodology, we show how steroid bioconjugation at the allosteric site affects the heme spin state, the binding affinity (K_S) of CYP3A4 for testosterone, as well as the enzyme coupling efficiency.

Keywords

Cytochrome P450 3A4; allostery; progesterone; testosterone; coupling; heme spin-state

Introduction

Human cytochrome P450 3A4 (CYP3A4) is responsible for the metabolism of over 50% of existing drugs [1]. It belongs to a large and ubiquitous family of enzymes that is also involved in the biosynthesis of many diverse molecules (*e.g.* vitamins, steroids, secondary metabolites, bile acids) [1]. The clinical importance of CYP3A4 in drug metabolism and its implication in drug-drug interactions [2,3] are major incentives to fully elucidate the ligand-binding mechanism of this enzyme.

Abbreviations: BFC, 7-benzyloxy-4-trifluoromethylcoumarin; CPR, cytochrome P450 reductase; CYP3A4, human cytochrome P450 3A4; DTT, 1,4-dithiothreitol; HFC, 7-hydroxy-4-trifluoromethylcoumarin; NADPH, nicotinamide adenine dinucleotide phosphate reduced; STM, a steroid maleimide derivative; TCEP, tris(2-carboxyethyl)phosphine; TST, testosterone; TST-OH, hydroxytestosterone.