Modeling mitochondrial cytochrome P450 enzymes: applying theory to reality.

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Cytochromes P450 are key enzymes responsible for the oxidative metabolism of a variety of structurally different endogenous and exogenous compounds including steroid hormones, drugs and xenobiotics. The P450s constitute a remarkably diverse superfamily of heme-containing monooxygenases, some dedicated to the metabolism of a single substrate into a single product, while others convert a number of dissimilar compounds into multiple products. Valuable insight into the catalytic mechanism of P450s has been gleaned from homology modeling with soluble bacterial P450 structures since mammalian steroidogenic P450s have not been crystallised. In the first step of steroid hormone biosynthesis P450 side chain cleavage (CYP11A1) converts cholesterol to pregnenolone. In the terminal step P450 11β-hydroxylase (CYP11B1) converts deoxycorticosterone and deoxycortisol to corticosterone and cortisol respectively. Both the mitochondrial CYP11A1 and CYP11B1 have been cloned from baboon adrenal tissue, enzymatically characterised and homology modeled using structural templates of bacterial P450s with the Insight II suite of programs, Accelrys (San Diego,CA). Expression of CYP11A1 and of constructs obtained with site directed mutagenesis identified specific amino acid residues within the active pocket affecting substrate affinity. Two genes encoding baboon CYP11B1 exhibit different substrate affinities towards deoxycorticosterone and deoxycortisol. Homology modeling identified amino acid changes near the surface that may effect structural elements of the protein, possibly altering substrate affinity and substrate turnover. Results will be presented to show that molecular modeling is a useful aid to understand and visualize changes in the structure that can alter the conformation of the protein thus influencing enzyme/substrate interaction.