Baboon Cytochrome P450 Side-chain cleavage: a structure/function investigation.

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Cytochrome P450 side-chain cleavage (CYP11A1) catalyses the first and 'rate-limiting' step in steroidogenesis, the conversion of cholesterol to pregnenolone in three consecutive mono-oxygenations. Pregnenolone is the precursor to all steroid hormones, including the mineralocorticoids, glucocorticoids and androgens. In an effort to gain further insight into the structure/function relationship of this key enzyme, CYP11A1 was characterised in the Cape baboon (Papio ursinus), a species closely related to humans.

Baboon cDNA was isolated from adrenal tissue and direct sequence analysis revealed mature baboon and human CYP11A1 share 98 % deduced amino acid homology. The cDNA was subsequently amplified and cloned. Selected constructs were sequenced and expressed in nonsteroidogenic mammalian COS1 cells. Apparent Km values were determined with 25-hydroxycholesterol as substrate and used to investigate the effect of point mutations on enzyme activity. Homology modelling was carried out using bacterial cytochromes P450 CYP102 and CYP2C5 as templates. Structure/function relationships were determined by comparing the kinetic data and the three-dimensional structure of the enzyme.