J. Pharm. Pharmacol. 1998, 50 (Supplement): 252

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Derivation of an enhanced representation of the active site of the P-450 enzyme aromatase (AR) from the consideration of the reaction mechanism

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The conversion of androgens (C_{19}) to estrogens (C_{18}) occurs in a number of cell and tissue types, such as ovarian granulosa, adipose tissue and placenta. The extragonadal synthesis of estrogens has great pathophysiological importance, for example, those produced by adipose tissue have been shown to play a rôle in the pathogenesis of certain forms of breast cancer and endometrial adenocarcinoma. The biosynthesis of estrogens is mediated by the enzyme aromatase (AR), a member of the heme-containing cytochrome P-450 family of enzymes which requires a mol of O_2 and NADPH to function (Thompson & Siiteri 1974). This particular enzyme has been the target of extensive research for some time, resulting in the design and synthesis of a variety of inhibitors for the possible treatment of breast cancer.

Due to its localisation on the inner membrane of the endoplasmic reticulum, attempts to create solubilised forms have proved difficult. We have previously reported a novel substrate-heme complex approach (Ahmed & Davis 1995) in an effort to gain further information regarding the active site of AR. This approach proved useful in rationalising the activity of a number of inhibitors, and their enantiomers, however, the mechanism of AR proved difficult to elucidate. In a search for models which may help to explain the probable mechanism, we initiated a search for an enhanced substrate-heme complex, the initial results of which are reported here. The novel model considers the groups involved during the aromatisation reaction, in particular the possible role of the dioxygen and NADPH moiety in the positioning of the steroid substrate. That is, it has been proposed that as enzymes are flexible bodies, they are able to cope with the movements of substrates - thereby allowing the use of both the peroxy and ferroxy radicals in the mechanism of AR. Using the enhanced model we have considered such hypotheses in an attempt to consider whether it is possible for both the ferroxy and peroxy radicals to be utilised during aromatisation.

The molecular structures of androstenedione (AD), the heme and part of the NADPH molecule, were all constructed and minimised (using the fastest minimisation routine available - cycles of 300 iterations were attempted until the gradient dropped below 10^{-3}) within the CACHE molecular modelling software. The structures were refined using ZINDO procedures. For the inhibitor binding study, Alchemy III molecular modelling software was used.

The results of the present study suggest that AD is orientated about the active site such that the C(2) moiety of the steroid backbone is positioned close to the heme, as such steric hindrance may be postulated with inhibitors possessing bulky groups - this has been previously observed in inhibition studies where groups larger than ethyl were found to be disfavoured. Binding inhibitors to the new complex, we observe that the potent inhibitors produce small steroid C(17) or C(3) carbonyl to inhibitor polar group distances, for example, with CGS-16949A, a steroid C(17) to inhibitor C≡N group distance of 1.5Å is observed. From a mechanistic point of view, we propose that the involvement of both the ferroxy and the peroxy radicals in the mechanism of AR is unlikely as it requires extensive movement of the components.

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