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Complete derivation of the active site of the cytochrome P-450 enzyme 17α-hydroxylase/17,20-lyase (P450 17α) using the novel substrate–haem complex approach

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The P-450 enzyme 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$) mediates the conversion of pregnenolone to 17α-hydroxypregnenolone which then undergoes C-C lyase to dehydroepiandrosterone. The specific characteristics of the active site of this enzyme are not known as it is membrane bound. In an effort to gain further information about this enzyme, we have previously constructed individual substrate-haem complexes for each component (Ahmed 1995), i.e. for the 17,20-lyase and the 17α-hydroxylase component of this enzyme and have shown them to be useful tools in drug design. Also, in previous studies, it has been shown that this enzyme undertakes reactions which result in by-products (Akhtar et al 1994). This characteristic has yet to be explained. Here we report the results of a study where we have combined our individual complexes to give an overall complex, and using this we have attempted to consider the proposed mechanisms and to explain the observed by-products.

Both substrate-haem complexes were constructed using the previously reported novel approach (Ahmed & Davis 1995) and the molecular modelling software Alchemy III (using atoms/fragments/groups available within the Alchemy structure libraries). In the construction of the substrate-haem complexes, we hypothesised that the attacking oxygen species must be positioned within approximate attacking distance (and angle) to the appropriate C atom, thus in the case of the lyase, the ferroxy based haem was placed close to the C(20)=O such that attack on the C(20) carbonyl group can take place. We therefore attached the terminal oxygen of the Fe^{IV}-O• species to the C(20) carbonyl carbon atom of 17α-hydroxypregnenolone and carried out an initial minimisation of the 'complex' until the gradient fell to below 10^{-5} (resulting, in general, in 300 or more iterations per structure), the C(20) sp^2 carbon was therefore converted to a sp^3. This then resulted in the ferroxy haem-based 17,20-Lyase substrate-haem complexes (a similar technique was utilised to give the peroxy based substrate-haem complex). Conformational analysis was performed on flexible parts of the substrate-haem complex i.e. about the Fe-O• and O-C(20) in the case of the ferroxy substrate-haem complex or the O-O and O-C(20) bonds in the peroxy based substrate-haem complex (using the systematic search method with energy window of 5Kcal/mol and bond rotations of 30-60°) using the conformational analysis software Powersearch. Points on the haem were then used for the superimpositioning resulting in the overall representation of the $P450_{17\alpha}$ enzyme active site.

From the consideration of the results, we observe that the overall structure is an approximate slanted L shape, i.e. one structure is found to be larger than the other. This result is supported by a recent homology based study (Burke et al 1997) where a similar two lobed structure was observed. With regards to the numerous by-products observed from the action of this enzyme on pregnenolone, we suggest that this behaviour can be rationalised on the basis of loose binding, i.e. several positions of the steroid backbone being presented to the Fe^{IV}-O• radical. Thus, in the formation of androsta-5,16-dien-3β-ol, we postulate that the 16α-hydrogen is positioned close to the Fe^{IV}-O• resulting in C(16)-α-H abstraction and the eventual C=C formation and acetate. The results of this and previous studies (Ahmed & Davis 1995) appear to support the hypothesis that an overall mechanism exists for the P-450 family of enzymes.

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