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THE MECHANISM OF AROMATASE - A MOLECULAR MODELLING PERSPECTIVE

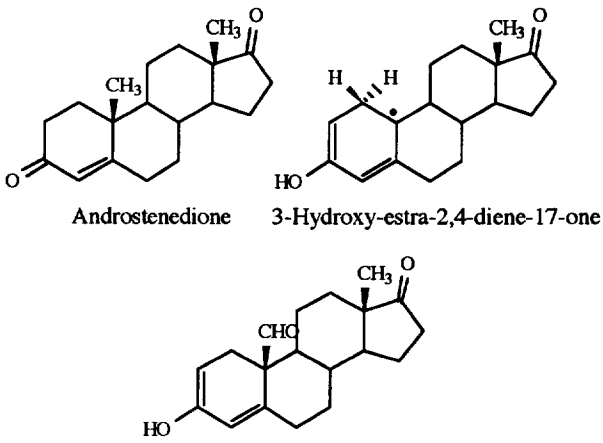
Sabbir Ahmed* and Paul J Davis

Department of Pharmacy, University of Brighton, Cockcroft Building, Lewes Road, Brighton, BN2 4GJ, UK.

Abstract : The three possible mechanisms for the final step of aromatisation, as proposed by Wright and Akhtar[1], are studied using a molecular modelling approach utilising the ferroxy 'substrate-heme complex' previously reported[2] and a novel peroxy complex. The study concludes that only a ferroxy radical is involved in all three steps of aromatase and not a mixture of ferroxy and peroxy as previously suggested. An alternative mechanism for the final step is also proposed.

The enzyme Aromatase (AR) mediates the conversion of androstenedione to estrone. The overall mechanism of AR is known to involve three sequential oxidation steps, each one requiring a mol of O_2 and NADPH[3]. The first two steps involve the hydroxylation of the C(19)-methyl group to give the C(19) gem diol which then undergoes rearrangement to the C(19) aldehyde and is removed as formic acid[4]. Extensive work has been carried out on the mechanism and it has been proposed that radical mechanisms are probably involved due to the energetics of polar reactions[1]. Although radical mechanisms may be postulated, the nature of the attacking group has not been established, however, three possibilities have been suggested - a ferroxy radical (Fe-O•); a peroxy radical (Fe-O-O•) or; a mixture of both[1].

Most of the studies of the mechanism of AR have revolved around chemical points of view, and the rôle of the active site of AR and the rest of the enzyme has not generally been considered, mainly due to lack of knowledge concerning the position of the heme and therefore the iron. We have recently reported a novel molecular modelling study[2] where we produced a representation of the AR active site with respect to the steroid substrate backbone, resulting in the 'substrate-heme complex'. We now seek to apply this complex and the general approach to study the mechanism of AR and we thus constructed (using the molecular modelling package Alchemy III[5]) a representation of both the ferroxy and peroxy equivalents of the substrate-heme complexes as well as the presumed intermediates[1] in the aromatisation reaction scheme (Figure 1). It should be noted that the theoretical studies reported have taken note only of the interactions between the substrate and the porphyrin whilst 'ignoring' the remainder of the 60kDa protien moiety.



Androstenedione 3-Hydroxy-estra-2,4-diene-17-one

19-Al-3-hydroxy-androst-2,4-diene-17-one

Figure 1. Androstenedione and two proposed intermediates of the final step of aromatisation.

From the consideration of the peroxy substrate-heme complex, we observed that, as would be expected, the 'extra' oxygen caused a downward movement of the steroid backbone as compared to the ferroxy substrate-heme complex. Fitting the two complexes (using the heme moiety) we discovered that the steroid backbone of the ferroxy complex was 1.6Å higher than the steroid backbone of the peroxy complex. From this result it would seem unlikely that both ferroxy and peroxy radicals are involved in aromatisation, as this would require the intermediates of the reaction to reposition themselves for the final bond cleavage step. If such repositioning did occur, we would need to presume the existence of numerous hydrogen bonding sites about the active site such that the intermediates could move from one site to another in order to maintain the position of the C(19) near the heme. Also, the movement required from ferroxy to peroxy would need to be in the vertical plane, as such a 'ladder arrangement' of hydrogen bonding groups would be required - an observation which has not been reported in any detailed study of AR and which seems unlikely. That the presence of numerous hydrogen bonding sites is unlikely can be further supported by the observation that specific stereochemistry and geometry is required by non-steroidal irreversible inhibitors of AR and that if these inhibitors do not conform to these requirements, inhibitory activity is reduced or lost[2]. Furthermore, if numerous hydrogen bonding sites did exist, then most compounds (observed to possess low inhibitory activity) could be expected to be good inhibitors as they could utilise these favourable interactions and so further increase their binding to the active site. Indeed, using the assumption that only two hydrogen bonding sites are available to AD [corresponding to the steroid C(3) and C(17) carbonyl groups], we have explained the observed inhibitory activity of the enantiomers of several inhibitors such as 3-ethyl-3-(4'-pyridyl) piperidine-2,6-dione (PYG), aminoglutethimide (AG) and 10-thiiranylestr-4-ene-3,17-dione. Thus, we conclude that aromatisation involving a mixture of a ferroxy and peroxy radical is unlikely.

To further investigate this conclusion, we utilised the ferroxy and peroxy substrate-heme complexes to consider the mechanisms proposed by Wright and Akhtar[1]. The first and second steps of aromatisation have been widely accepted as involving ferroxy radicals. Thus, only the final step remains unclear. In considering the mechanisms put forward by Wright and Akhtar for the final step, we hypothesised that the hydrogen abstraction (leading to the formation of the final carbon-carbon double bond) is an important step. Thus, the basis of our study involves the determination of the feasibility of this step from a geometric point of view. We therefore built the appropriate intermediates and considered the distance of the C(1)-βH bond with respect to the attacking oxygen in each mechanism. Also, we hypothesise that as a result of the conversion of the C(3) carbonyl to the hydroxy, hydrogen bonding interaction with the active site via the C(3) polar group is lost (as a result of hydrogen atom 'donation' by the active site) and the remaining C(17) carbonyl group of androstenedione becomes important in binding the intermediates to the active site. Thus, in fitting the intermediates to the substrate-heme complexes, points on the D-ring of AD [and in particular the C(17)=O group] were utilised.

Considering the mechanism involving the peroxy radical and fitting the intermediate 3-hydroxy-estra-2,4-diene-17-one onto the peroxy substrate-heme complex, it was discovered that the βC(1)-H bond was some 2.5Å away from the appropriate oxygen atom for βH abstraction (Figure 2). As mentioned earlier, hydrogen abstraction from the C(1) position of the intermediate appears to be an important step, the distance obtained giving an indication as to the feasibility of this step. In this case, the C(1) hydrogen is presumed to be too far away for abstraction to occur and therefore the mechanism involving the peroxy radical in the C(10)-C(19) bond cleavage seems unlikely. This suggests that all three overall steps in the Aromatisation reaction therefore involve a ferroxy species.

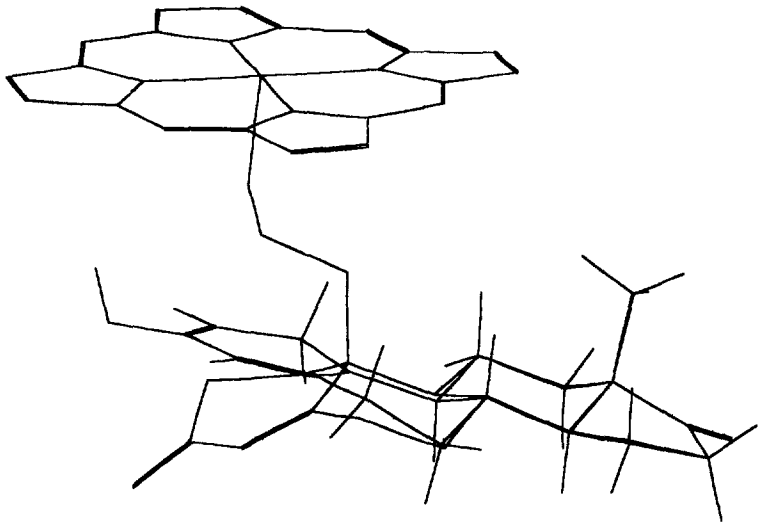


Figure 2. 3-Hydroxy-estra-2,4-diene-17-one fitted onto peroxy substrate-heme complex.

Considering the remaining two mechanisms put forward by Wright and Akhtar, involving a ferroxy radical, we conclude that the mechanism involving H abstraction from an intermediate containing the C(19) aldehydic group is also unlikely. When the intermediate 19-al-3-hydroxy-androst-2,4-diene-17-one (Figure 1) is fitted onto the ferroxy-based substrate-heme complex (Figure 3), we find that the C(1)-βH is 2.4Å from the ferroxy oxygen atom (similar to the result obtained with the peroxy mechanism), a distance which is considered too large for H abstraction.

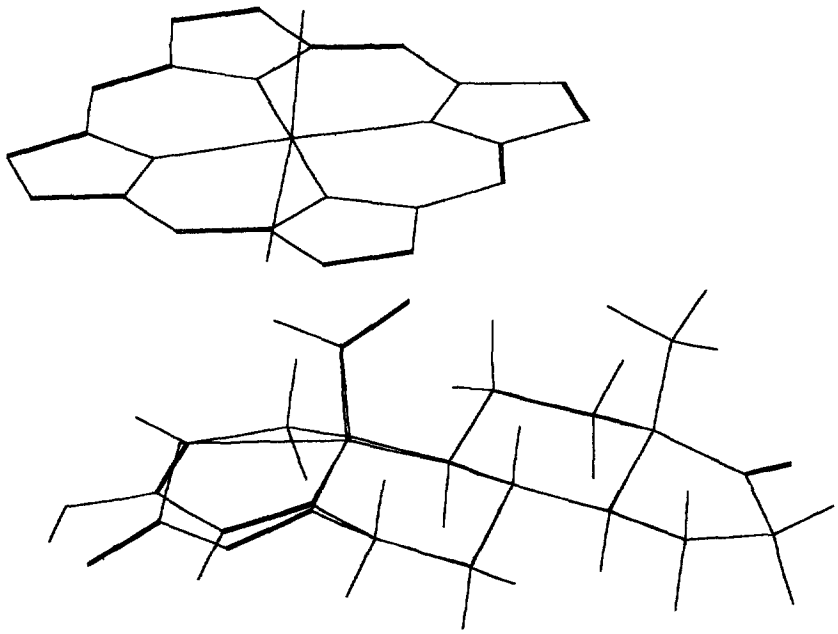


Figure 3. 19-Al-3-hydroxy-androst-2,4-diene-17-one fitted onto ferroxy substrate-heme complex.

Considering the third and final mechanism, we observe that this also involves hydrogen atom abstraction from C(1), although the intermediate involved is a C(10) radical (also proposed in the peroxy mechanism). After the homolytic fission of the C(10)-C(19) bond, there is a pronounced upward movement of the C(1) atom from below the plane of the steroid as the C(10) radical becomes planar (Figure 4) - similar to the peroxy mechanism. Fitting the 3-hydroxy-estra-2,4-diene-17-one intermediate onto the ferroxy substrate-heme complex results in a ferroxy oxygen to C(1)-βH distance of 1.2Å, i.e. H abstraction from the intermediate is possible. Wright and Akhtar, however, considered this mechanism to be doubtful as it involved addition of the ferroxy radical to the C(19) aldehyde as opposed to hydrogen abstraction, however, the peroxy mechanism which they considered probable, involved a

similar step. We therefore conclude that of the three proposed mechanisms suggested by Wright and Akhtar, the mechanism involving the attack by a ferroxy species on the C(19) aldhydic group appears to be the most likely from a consideration of distances between attacking species.

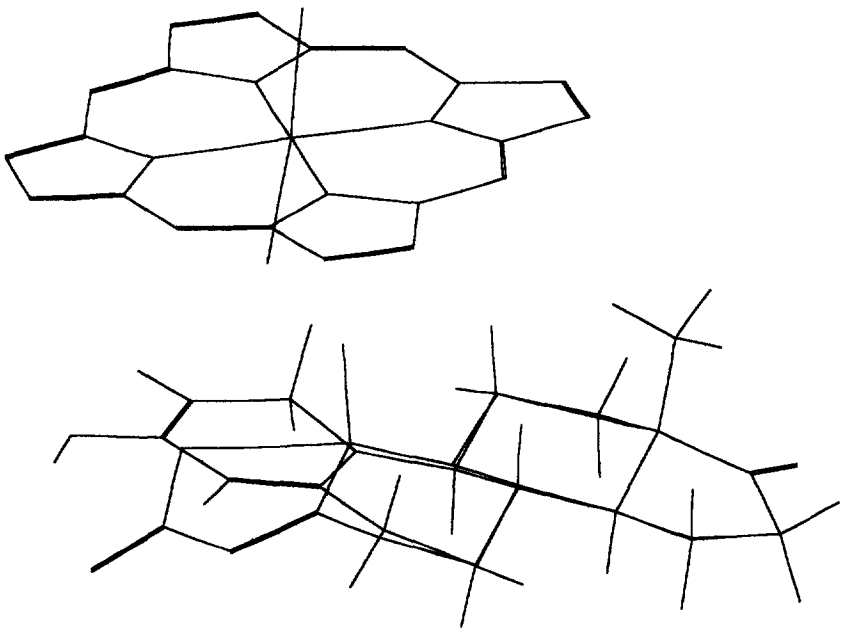


Figure 4. 3-Hydroxy-estra-2,4-diene-17-one fitted onto ferroxy substrate-heme complex.

A detailed consideration of mechanism 3 shows that it relies upon the formation of •CHO. It has been postulated that this radical is too small to be contained within the active site for conversion to formate at a later stage of the reaction[1]. We therefore suggest an alternative 'route' where the breakup of the 'iron-formate complex' together with hydrogen abstraction from C(1) of 3-hydroxy-estra-2,4-diene-17-one, occurs via a concerted mechanism, without formation of the •CHO radical (as shown in Figure 5).

In conclusion, the present theoretical study has allowed us to model the three possible mechanisms for the final step of aromatisation as suggested by Wright and Akhtar. From the study, we conclude that the mechanism of AR involves a ferroxy radical during each step of aromatisation and that the combined action of two ferroxy radicals and a peroxy radical appears to be unlikely due to the reorientation required by the intermediates, as well as the distances involved for the peroxy species to abstract hydrogen from the steroid C(1) position.

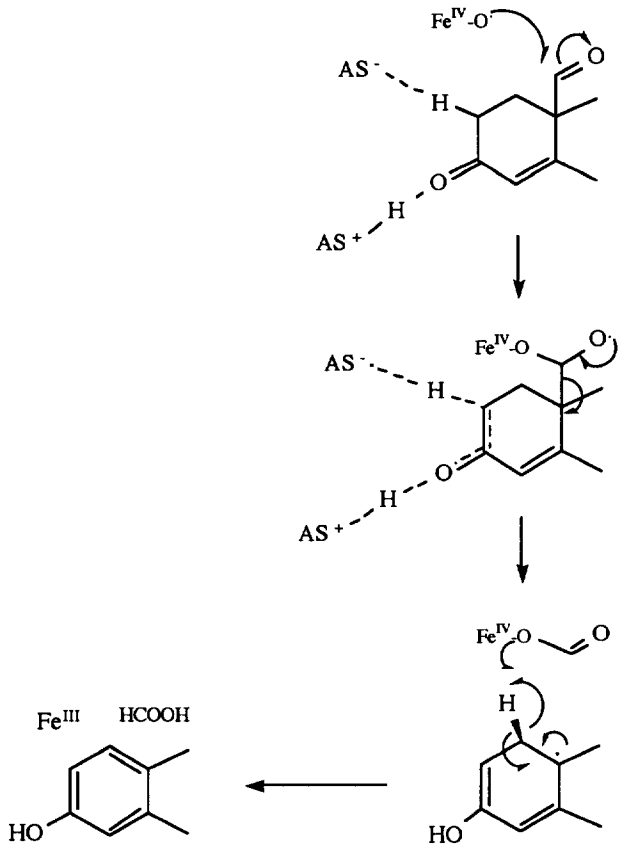


Figure 5. Alternative mechanism for the third step of Aromatisation.
AS = group at active site.

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