Mimicking Cytochrome P-450 2B4 and Aromatase: Aromatization of a Substrate Analogue by a Peroxo Fe(III) Porphyrin Complex

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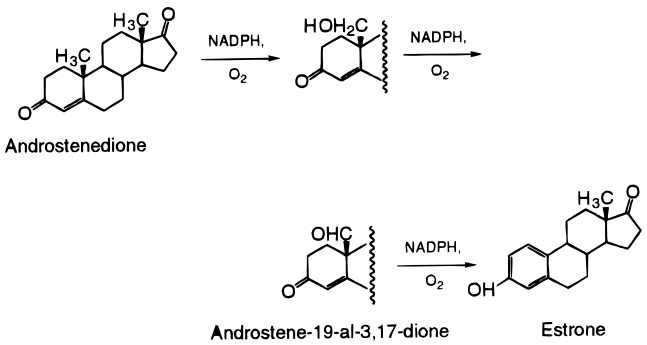
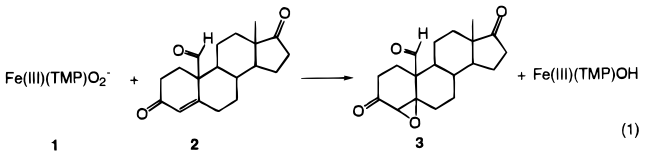
Most of the reactions catalyzed by cytochrome P-450 enzymes are believed to involve iron oxene or high-valent iron oxo heme intermediates that are formed after protonation and O−O bond cleavage of an iron-bound peroxide ligand.[1−6] In some cases, however, it appears that these same enzymes proceed via a different mechanism, namely direct nucleophilic attack on the substrate by a ferric heme-bound peroxo ligand.[7] One example of such an enzyme is aromatase, the enzyme responsible for the conversion of androgens to estrogens in humans (Scheme 1). The mechanism by which aromatase achieves this remarkable transformation has been the subject of much debate.[8−12] Although the first two steps are typical P-450 type hydroxylations thought to occur via an iron oxene type intermediate, the third step involves loss of the C-19 aldehyde as formate and aromatization of the A ring of the steroid. There is now growing evidence that the species responsible for the oxidative aromatization of the third step is a ferric heme-bound peroxo ligand.[13−20]

Ferric porphyrin peroxo complexes are potential synthetic analogues of the reactive intermediate implicated in the third oxidative step of aromatase.[21] We have recently shown that ferric peroxo complexes of electron-rich porphyrin ligands, including the biological protoporphyrin IX dimethyl ester, are unusually reactive nucleophiles capable of epoxidizing electron-deficient olefins.[22−24] This species is analogous to the intermediate proposed for nucleophilic attack on the aldehydic substrate in the aromatase reaction.[7] We report here that the ferric porphyrin peroxo complex reacts with androstene-19-al-3,17-dione, an aromatase substrate, to give epoxidation rather than aldehyde deformylation and aromatization. However, reaction of that same peroxo complex with an aldehyde designed to mimic the enolized A and B rings of the steroid substrate gives the desired result, suggesting that enolization of the substrate may play an important role in the enzymatic mechanism.

Scheme 1



Reactivity of Androstene-19-al-3,17-dione with [Fe(TMP)O_2]$^-$. Addition of androstene-19-al-3,17-dione to a solution of the peroxo ferric porphyrin complex, [Fe(TMP)O_2]$^-$ (**1**), in acetonitrile resulted in the gradual disappearance over a period of 1 h of the UV−vis spectrum characteristic of the peroxo complex.[25] The ^{1}H NMR spectrum of the resultant solution indicated the presence of epoxide **3**, as well as unreacted **2**, and the presence of several minor side-product peaks (eq 1).[26] The yield of steroid epoxide obtained (14 ± 4%)[27] was similar to the value (23 ± 2%)[22] reported for epoxidation of 2-cyclohexen-1-one by **1**.



(1)

Reactivity of Aldehyde 4 with [Fe(TMP)O_2]$^-$. The observation of epoxidation rather than aromatization when the natural substrate of aromatase was reacted with [Fe(TMP)O_2]$^-$ prompted us to consider the possibility that the substrate might be enolized in the enzymatic reaction. It has in fact been suggested that enolization of the 3-keto group occurs via 2-H abstraction by

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(25) The final porphyrin product was established to be Fe(TMP)OH by UV−vis [λ_{max} = 418 nm (Soret)] and paramagnetic ^{1}H NMR, which showed the characteristic peaks for the Fe(TMP)OH pyrrole protons at 81 ppm and of the meta hydrogens at 12 and 13 ppm.
(26) The epoxide **3** was prepared independently from **2** and basic H_2O_2. The ^{1}H NMR of this epoxide was identical to that obtained from the reaction of **1** with **2**.
(27) The reaction was carried out for only 1 h due to the instability of the epoxide product. Yield of product (14%) is based on [Fe(TMP)O_2]$^-$. The remaining 85% was unreacted starting material and several very minor side products.

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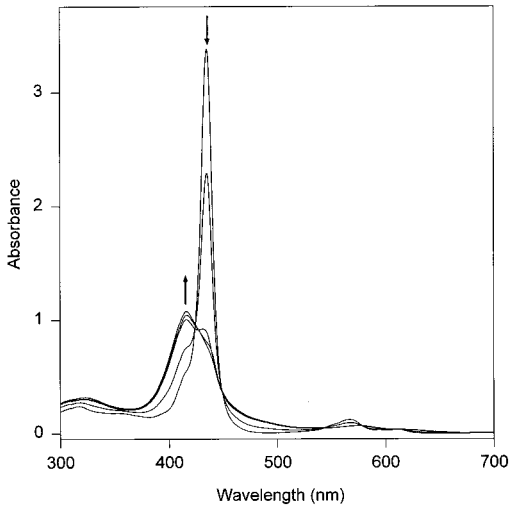
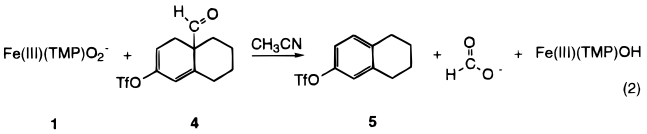


Figure 1. Change in the UV−vis spectrum of a 1 mM solution of $[Fe(TMP)O_2]^-$ after the addition of 1 equiv of aldehyde **4**. Aliquots were removed and measured over a 2.5-h period. The spectrum of the final scan is identical to that of Fe(TMP)OH. All reactions were carried out in CH_3CN at 25 °C under inert atmosphere.

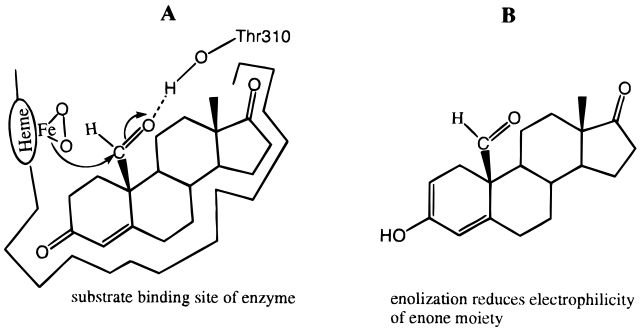
Asp309 in the active site of aromatase.[11] For this reason, we designed an analogue of the natural substrate that might more closely resemble the A and B rings of androstene-19-al-3,17-dione if and when it is enolized in the enzymatic binding site. We synthesized aldehyde **4**, incorporating a vinyl triflate as a stable mimic of the enolized A ring of the natural substrate. Addition of 1 equiv of aldehyde **4** to an acetonitrile solution of 1 equiv of $[Fe(TMP)O_2]^-$ [28] resulted in the direct and complete conversion of the characteristic UV−vis spectrum of the peroxo complex to that of Fe(TMP)OH (Figure 1). Analysis of the reaction mixture by GC/MS revealed complete conversion of aldehyde **4** to aryl triflate **5** (eq 2).[29] Anaerobic D_2O extraction



of the reaction mixture and analysis by ^1H NMR indicated the presence of formate (s, 8.2 ppm), thus demonstrating that carbon−carbon bond cleavage occurs without exposure to air. Thus, without the electrophilic enone moiety competing for the nucleophilic peroxo ligand, deformylation and aromatization are readily achieved with a nucleophilic ferric porphyrin peroxo complex.

Discussion. The unusual transformation achieved by aromatase in the third oxidative step of the synthesis of estrogens has been proposed to occur through a nucleophilic iron-heme bound peroxo.[7,30] We show in this report that a nucleophilic ferric porphyrin peroxo complex is able to achieve the aromatization of a steroid-like substrate if the enone moiety of the A ring is converted to an enol-like state. The time scale and yield for this reaction are impressive considering that this model system lacks many of the kinetic advantages available to the enzyme.

Scheme 2



The ability of the enzyme to effect the aromatization of androsten-19-al-3,17-dione despite the presence of an enone moiety suggests that several factors may be important in this selectivity. The orientation of the substrate in the binding site may be one potential aspect. Orienting the aldehyde moiety near the iron peroxo while shielding the enone moiety may be a selectivity strategy evolved by the enzyme (Scheme 2, A). Aromatase is also capable of increasing the electrophilicity of the aldehyde moiety by facilitating hydrogen bonding between the aldehyde oxygen and Thr310 in the active site.[11] Another selectivity strategy aromatase has been proposed to utilize is the enolization of the enone moiety prior to deformylation (Scheme 2B).[8,11,15,20] Our work is consistent with the possibility of enolization prior to aldehyde deformylation as we are able to achieve aromatization without a binding pocket or without increasing the electrophilicity of the aldehyde moiety.

In conclusion, the oxidative aromatization of aldehydes by $[Fe(TMP)O_2]^-$ reported here suggests that direct nucleophilic attack by a ferric peroxo heme enzymatic intermediate on an aldehydic substrate is feasible. Such a mechanism has been proposed by Akhtar et al.[7] for both cytochrome P-450 2B4 and aromatase and has been supported by recent mutagenesis experiments by Vaz et al.[10,12,13,19] Our results, taken together with the enzymatic selectivity exhibited by aromatase, suggest that enolization of the 3-keto group of the steroid may occur prior to deformylation by a ferric peroxo porphyrin intermediate.

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(28) $[Fe(TMP)O_2]^-$ is synthesized immediately prior to substrate addition (2.2 equiv of KO_2 and 2.2 equiv of K222). This method allows the peroxo species to be generated cleanly with no excess KO_2 present. Solvent must be rigorously dried according to the three-step method previously published.[21,23,24]

(29) In a typical reaction (1 mM), the aromatization of aldehyde **4** was complete in 2.5 h.

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