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A transition from ionic to free-radical mechanisms in chemistry and enzymology

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Abstract:

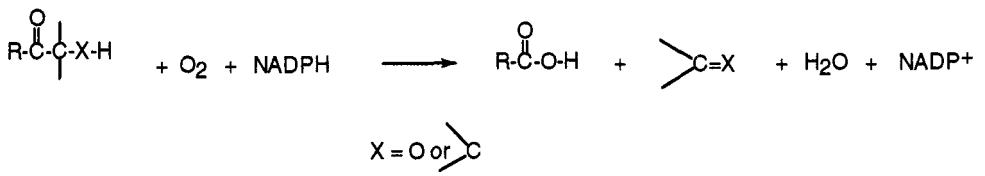
1) Examples were presented of enzymes which catalyse different generic reactions at the same active site. These enzymes which belong to the *P*-450 class are aromatase, 14α-demethylase, 17α-hydroxylase-17,20-lyase and nitric oxide synthase.

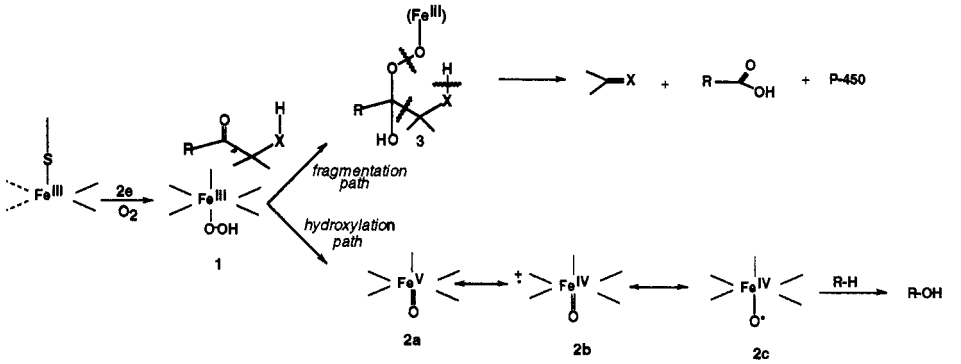
2) The multicatalysis is due to a selection process in which the functional group at the sensitive C-atom of the substrate chooses a compatible iron-oxygen species ($Fe^{IV}O\cdot$ or Fe^{III}-OOH) for further reaction.

3) The *P*-450 dependent hydroxylation and C-C bond cleavage reactions occur via a radical mechanism and the enzymes participating in these processes have evolved to deal with situations where the ionic processes are deemed energetically unfavourable.

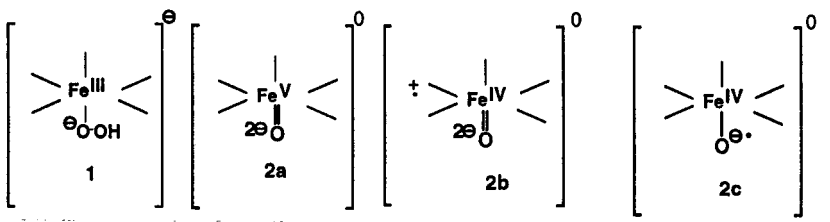
In this lecture, attention was focused on the transition from heterolytic to homolytic catalysis in chemical and enzymic reactions. It was argued that for C-H and C-C bond cleavage to occur via a carbanion the pKa of its conjugate acid may not be greater that 25; beyond this value free radical mechanisms are chosen in chemistry as well as enzymology (1) The most telling example of the latter class of reactions in chemistry are the Barton type of reactions developed during the Sixties at ETH (Zürich), RIMAC (Cambridge, USA) and Ciba (Basel) to functionalise the non-activated C-18 and C-19 methyl groups of steroids (pK_a of the carbon acid ≈ 50) through the use of suitably juxtapositioned alkoxy radicals (2). The functionalisation of these methyl groups also occurs during various biological transformations of steroids and is catalysed by cytochrome *P*-450 group of enzymes. The key event in these transformations is the hydroxylation reaction. Following a long period of uncertainty, consensus has now begun to emerge that the biological hydroxylation reaction also occurs by a radical mechanism and the crucial hydrogen abstraction step underpinning the process is catalysed by an iron-monooxygen species (referred to as the oxo-derivative)(3; for review see 1). The latter **2** is produced by a two-electron reduction of O_2 as shown in Scheme 1. Although several electronically equivalent structures for the species are possible, the formulation Fe^{IV}-$0^{\bullet}$ **2c**, which bears an uncanny resemblance to the chemists' alkoxy radical, can be most conveniently used to describe the hydroxylation reaction in a step-wise fashion (4).

In our laboratory, studies on aromatase (5) that is involved in the aromatisation of androgens and 14α-demethylase (6) that converts lanosterol into sterols revealed several new facets of *P*-450 dependent enzymes. These two multifunctional *P*-450 enzymes are involved not only in the hydroxylation reactions but also in the oxidation of alcohols into carbonyl compounds as well as in the cleavage of C-C bonds by an acyl-carbon fission as shown below.



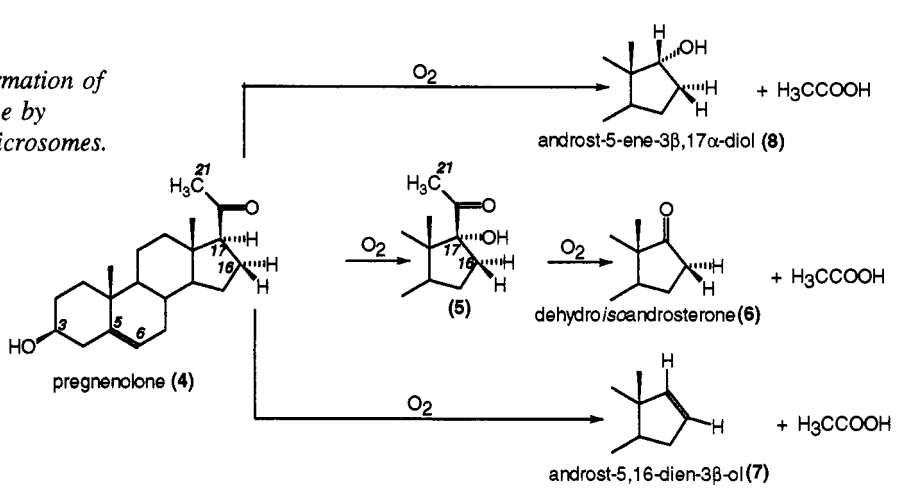


SCHEME 1. The branching of the peroxy species **1** *either towards the formation of the oxo-derivative* **2** *or adduct formation with a substrate containing an electrophilic substituent,* **1** → **3**. *The wavy lines in the adduct* **3** *show the bonds broken during the acyl-carbon cleavage. In accordance with the accepted convention of inorganic chemistry, while assigning charge to iron in the structures above, it is implicit that the pair of electrons forming the coordination bond resides with the ligand and not shared with the metal. It is to be noted that some workers prefer to make this feature explicit with respect to the oxygen ligand and also show the overall charge on the complex in which case, structures* **1**, **2a**, **2b** *and* **2c** *will need to be written as:*

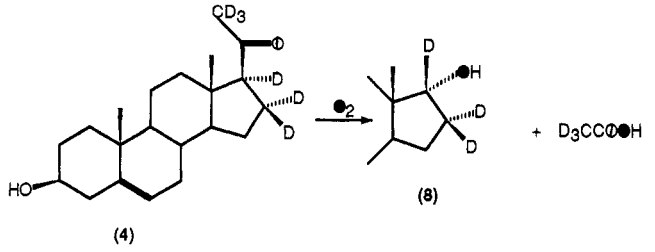


We have hypothesised that these different generic reactions are catalysed at a single active-site by two distinct iron-oxygen species; the oxo-derivative being involved in hydroxylation and alcohol oxidation while an Fe^{III}-OOH species in the acyl-carbon fission process, as shown in Scheme 1. The strongest evidence for the proposal of the Scheme has come from the study of androgen biosynthesis from pregnenolone catalysed by an enzyme preparation from pig testes (7). In the biosynthesis, pregnenolone **4** is first hydroxylated to **5** that then undergoes an acyl-carbon fission to produce **6**. A second acyl-carbon cleavage reaction accompanies the preceding process and leads to the formation of the 5,16-diene **7**. All these three reactions are catalysed by 17α-hydroxylase-17,20-lyase ($P\text{-}450_{17\alpha}$) **7**.

SCHEME 2. The transformation of pregnenolone by pig testes microsomes.

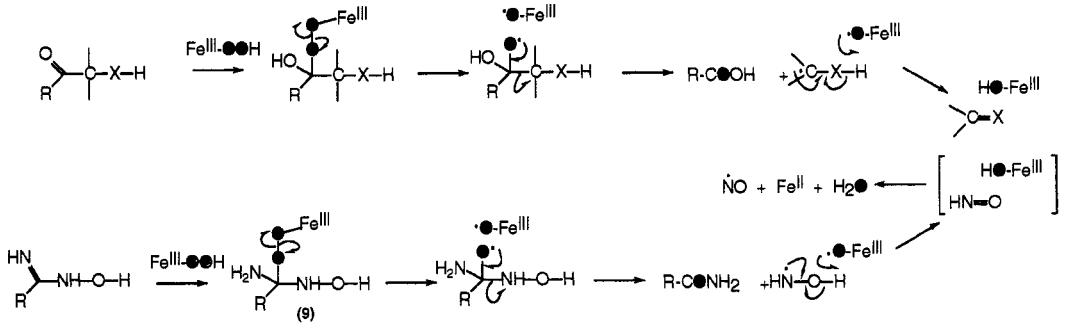


A further unusual cleavage **4** → **8**which is either the property of the same or a related *P*-450 enzyme has provided invaluable information on the mechanism of acyl-carbon bond cleavage (7b,8,9). It was found that the conversion **4** → **8** which occurs with the inversion of stereochemistry at C-17, is attended by the retention of all the hydrogen atoms of the precursor into the two products and involves the incorporation of an atom of oxygen into the steroid hydroxyl group as well as in the carboxyl group of released acetic acid (Scheme 3). All these findings are most readily accommodated by assuming that the acyl-carbon cleavage process is due to the participation of an Fe^{III}-OOH species and occurs through the intermediary of an hydroperoxy adduct of the type **3**. The inversion of stereochemistry at C-17 further proves that the fragmentation of the hydroperoxy adduct is not a concerted but a stepwise process.



SCHEME 3.
The status of hydrogen and oxygen atoms in the conversion of pre-gnenolone into **8**. *D* = ^2H

Further evidence for the fact that an Fe^{III}-OOH species is also involved in the formation of the 5,16-diene has been provided through the use of an aldehyde substrate that preferentially traps the Fe^{III}-OOH intermediate of Scheme 1, directing the reaction into the cleavage path (10). In the light of this and related observations, it is advocated that multicatalysis promoted by *P*-450 enzymes is due to a selection process in which the functional group at the sensitive C-atom of the substrate chooses a compatible iron-oxygen species (the oxo-derivative or Fe^{III}-OOH) for further reaction. That the generalisation embodied in Scheme 1 is not only applicable to the steroidal enzymes but may have wider implications is highlighted by the elegant isotopic results obtained on nitric oxide synthase (11). The enzyme catalyses first a hydroxylation reaction and then a cleavage process for which mechanistic alternatives involving either the oxo-derivative (12) or Fe^{III}-OOH are possible (13, 14). If the Fe^{III}-OOH species is involved in the nitric oxide syntheses reaction, then it may be used for a nucleophilic attack on an imino group, which is isoelectric with the carbonyl group, to produce an adduct **9** whose mode of decomposition may be modelled on the acyl-carbon cleavage until the last stage as shown in Scheme 4. With the acyl-carbon cleavage, the overall process requires a 4 electron reduction (2 from NADPH and 2 from within the scissile C-C bond) and the sequence is terminated by the regeneration of the Fe^{III} form of the enzyme. The formation of $\dot{N}O$, on the other hand, involves a 3 electron reduction and therefore the mechanism modelled on the acyl-carbon cleavage requires that at the final step of the first turn-over on the Fe^{II} form of the enzyme is regenerated and recycled. A variant on the theme in which the substrate is oxidised at an earlier stage is also available (14).



SCHEME 4. The mechanistic similarities between acyl-carbon cleavage (upper sequence) and the analogous conversion of N^G-hydroxy-L-arginine into L-citrulline and nitric oxide (lower sequence).

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