A Mono-oxygenase Model for Selective Aromatic Hydroxylation with Nickel(II)-Macrocyclic Polyamines

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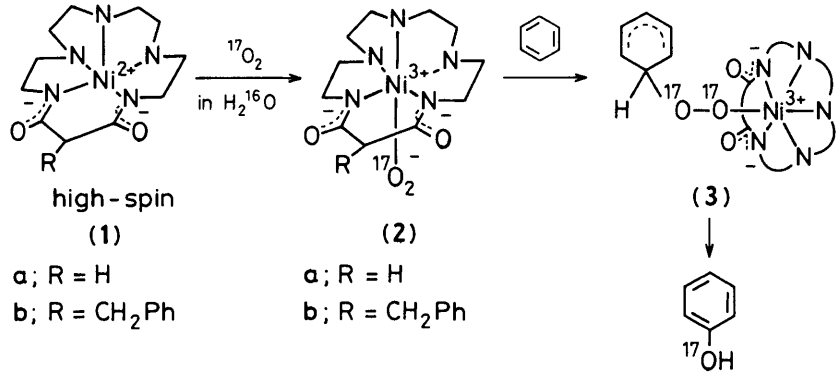
In a new model reaction for biological mono-oxygenases, a ^{17}O atom from $^{17}O_2$ is directly incorporated into an aromatic ring as an hydroxy group *via* the novel 1 : 1 Ni–O_2 complexes (**2**) formally existing as Ni^{3+}–O_2^-.

Since the first discovery of oxygenases by Hayaishi[1] and Mason,[2] much interest has been shown in their chemistry. Model complexes have been used in order to investigate their mechanisms and applications. However, the reaction pathways of O_2 with model complexes are very diversified and hence the enzyme mechanisms are far from completely understood; O_2 may initially be converted into the hydroxy radical[3] or oxenoid species,[4] and sometimes it may be used for substrate activation.[5] No model has ever offered clear-cut evidence for the direct incorporation of O_2 into substrates, as found for biological reactions.[6] In this communication we present a new mono-oxygenase model (**1**),[7] that activates co-ordinating O_2 in complex (**2**), which then selectively attacks aromatic substrates and results in hydroxylation (Scheme 1).

The mass spectrum of phenol resulting from the oxygenation of benzene in borate buffer ($H_2{}^{16}O$) at room temperature in the presence of complex (**1a**) in $^{17}O_2$ (50 atom % ^{17}O from Commissariat a l'Energic Atomique, France) shows molecular ion peaks (M^+) of equal intensity at *m/z* 94 and 95 clearly indicating that the phenol O atom is entirely and hence directly transferred from $^{17}O_2$ gas. This conclusion was reinforced by an experiment using a $^{16}O_2$ atmosphere and $H_2{}^{18}O$; the mass spectrum of the resulting phenol was identical to that formed using $H_2{}^{16}O$, thus proving that the O atom is not incorporated from H_2O.

The formation of the O_2 adduct is essential prior to aromatic oxygenation, as hydroxylation did not occur when excess of imidazole was present occupying the O_2 co-ordination site.† Evidence for the activation of O_2 by Ni-binding is provided by

† A kinetic study of O_2 adduct formation showed a preference for imidazole association (unpublished result).



Scheme 1. The N–H hydrogen atoms in the macrocyclic ligands are not shown.

magnetic susceptibility (Evans method)[8] and e.s.r. studies. It is noteworthy that while all of the model and natural Fe–O_2 complexes (including hemoglobin) are diamagnetic and e.s.r.-silent, owing to the electron spin couplings,[9] the Ni–O_2 adducts in aqueous solution are paramagnetic, $\mu_{eff} = 2.83\ \mu_B$. This leads to a formal bonding of Ni^{3+}–O_2^- with a weak spin interaction (due to weak Ni–O_2 bonding), which explains the similar visible and e.s.r. spectral behaviour to the Ni^{III} complexes.[7]‡ The e.s.r. spectrum of (**2b**) formed in $^{17}O_2$ exhibits three ill-defined lines of weak superhyperfine splitting (J = *ca.* 8.4 G, 1G = 10^{-4} T) which is interpreted as resulting from the weak interaction of Ni^{3+} with the nuclear spin of ^{17}O ($I = 5/2$).§ The Ni^{3+} spectrum of the $^{16}O_2$ ($I = 0$) adduct does not show such splitting.

The O_2 adduct (**2**) oxygenates non-activated aromatic compounds to the corresponding phenols. We propose that the strong superoxide (O_2^-) nature of the nickel complexes is responsible for such reactivity. With toluene, cresols are the only oxygenated products with no products from further oxygenation¶ or alkyl oxidation. This mechanistic feature separates the present oxygenation from previous hydroxylation methods.[10] We found the ratio of the cresol isomers to be *o*:*m*:*p* = 56:14:30 which differs from the ratios 71:5:24 for the Fenton system (hydroxy radical pathway)[11] and 46:27:27 for Udenfriend's system (oxenoid pathway).[4a] In addition, the latter two methods yield numerous unidentified products. Since the oxygenation reported here is not inhibited by superoxide dismutase and catalase, free O_2^- or O_2^{2-} is not thought to be involved. Also the fact that nitrobenzene is not oxygenated while anisole is (the main product is *o*-methoxyphenol) suggests that the activated O_2 species in the Ni–O_2 complex possesses electrophilic character.

In summary, we propose the aromatic hydroxylation mechanism shown in Scheme 1.** The existence of the intermediate (**3**), which corresponds to the Wheland intermediate in nitration, is supported by kinetic results; when a 1 : 1 mixture of C_6H_6 and C_6D_6 was used as the substrate in the aromatic hydroxylation, the resulting phenol consisted of C_6H_5OH and C_6D_5OH in a molar ratio of 1 : 1. No isotope effects could be found. In addition, electrophilic adducts similar to (**3**) were reported in the reactions of Co^{3+}-bound O_2^- in $[Co(CN)_5O_2]^3$ and [Co(Salpr)O_2] [Salpr=bis(3-salicylideneaminopropyl)amine] with the 2,4,6-tri-t-butylphenoxy radical.[5b,12] At present we are not certain whether the activated O_2 species in (**2**) attacks substrates *via* an oxenoid intermediate, as proposed for cytochrome P-450 oxygenation.[6]

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‡ The magnetic susceptibility of the Ni^{III} complex generated electrochemically or chemically from the high-spin ($\mu_{eff} = 2.83\ \mu_B$) Ni^{II} complex (**1**) is $\mu_{eff} = 1.71\ \mu_B$ ($S = 1/2$).

§ The interaction of Ni^{3+} with ^{17}O may theoretically be expected to create six lines of superhyperfine splitting probably with equal intensity. The observed three, very weak, ill-defined lines occur in the $g \parallel$ region where another set of three strong lines of superhyperfine splitting (due to ^{14}N–Ni^{III} coupling, $J = 22$ G) is present. Hence, some lines due to the ^{17}O–Ni^{III} coupling may be concealed. A more detailed e.s.r. analysis is underway.

¶ In a separate experiment we confirmed that phenol substrates are not oxygenated. The phenol oxygen atoms appear to occupy the O_2 binding site of the Ni^{II} complex (**1**), as judged by the colours (red) of the reaction mixtures.

** The yields of phenols produced are greatly increased by the addition of a reducing agent (*e.g.* $NaBH_4$ or Na_2SO_3). The NIH shifts of deuterium in [*o*-$^2H_1$]toluene and [*p*-$^2H_1$]toluene were not observed here.