Fe(NTA)-catalyzed Dioxygenation of 4-t-Butylcatechol and the Mechanism of Non-heme Ferric Dioxygenases

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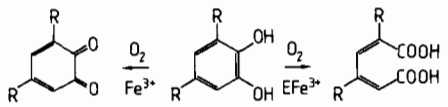
Abstract

Fe(NTA), activating the 'substrate' 4-t-butylcatechole, represents a functional active centre analogue of non-heme ferric dioxygenases. A Fe(NTA) catecholate complex with monodentate catecholate is the reactive species to undergo dioxygenation.

Introduction

Ferric ion catalyzed oxidation of catechols (I) with O_2 yields significantly different products in enzymic (*e.g.* catechol 1,2-dioxygenase) or non-enzymic reactions, respectively. In both mechanisms, catecholate is initially coordinated to iron(III). However, catecholate chelate ligands in ferric complexes, such as 3,5-di-t-butyl-catecholate ($DBcat^{2-}$ (1), R = t-Bu), undergo stepwise oxidation with oxygen via semiquinone to quinone (II) [1–3], whereas at the non-heme ferric-active centre of catechol 1,2-dioxygenase, catechol ((I), R = H) is oxidatively cleaved to muconic acid (III). Thus in the enzyme a 'simple' redox reaction is replaced by a dioxygenation, leading to dicarbonic acids which will fit into further metabolic pathways. This requires an as yet unknown activation of catecholate and/or O_2 at the non-heme ferric centre of the enzyme.



II **I** **III**

E = Catechol 1,2-Dioxygenase

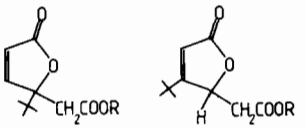
We have shown [2, 5] that $[Fe(NTA)(DBcat)]^{2-}$ (NTA = nitrilotriacetate; DBcat = 3,5-di-t-butyl-catecholate), as a first iron(III)–dioxygenase model system, reacts with O_2 effecting ring cleavage of the catecholate ligand. We now wish to demonstrate a similar reactivity of the analogous complex with the less oxidizable 4-t-butylcatechol ($BcatH_2$) (**VI**); a mechanism is presented for both the dioxygenase and the model system.

Experimental

All reactions with oxygen were carried out identically. A typical example is given below: to 10 mmol $BcatH_2$ in 100 ml ethanol, 115 ml 0.6 M borate buffer pH 8 and a solution of 10 mmol each $Fe(ClO_4)_3$ and $NTANa_3$ in 30 ml ethanol/water 1:1 were added. The deeply blue-green solution (showing a glass electrode reading of 'pH' 8.2) was stirred under O_2. After ten days, having consumed 10 mmol O_2, the solution turned reddish-brown. Evaporation of ethanol was followed by CH_2Cl_2-extraction of first the basic and then the acidified (H_2SO_4) aqueous phase. EPR spectra were run on a Varian E109 spectrometer; temperature, 77 K; microwave frequency, 9.24 GHz; modulation amplitude, 10 G; microwave power, 50 mW. The signals were observed both in the frozen reaction medium (H_2O/EtOH), and in frozen glasses (*e.g.* H_2O/propylene glycol). For interpretation of the EPR-data see [6].

Results and Discussion

Oxidation of $[Fe(NTA)(DBcat)]^{2-}$ by O_2 in a borate buffer at pH 8.2 yields the following products (relative to $BcatH_2$ as starting material, total yield 99%): 62% lactone ester (37% (**IVa**) and 25% (**Va**)), 28% lactonic acid (9% (**IVb**) and 19% (**Vb**)), and 10% unreacted $BcatH_2$. All lactones (**IV**) and (**V**) are



IVa: R = Et **Va**: R = Et
IVb: R = H **Vb**: R = H

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TABLE I. Oxidation of 4-t-Butylcatechol by O_2.[a]

No.	Molar ratio			Time [d]	Product, %[b]		
	Bcat	NTA	Fe		Bcat (VI)	Quinone (VII)	Lactones (IV) + (V)
1	1	1	1	10	10	–	89
2	10	1.3	1	20	18	3	71
3	100	1.3	1	20	35	15	45
4	3	–	1	5	30	60	–
5	1	–	–	20	80	10	5

[a]In EtOH/borate buffer 1:1, 'pH' 8.2 (see text). [b]Based on original $BcatH_2$.

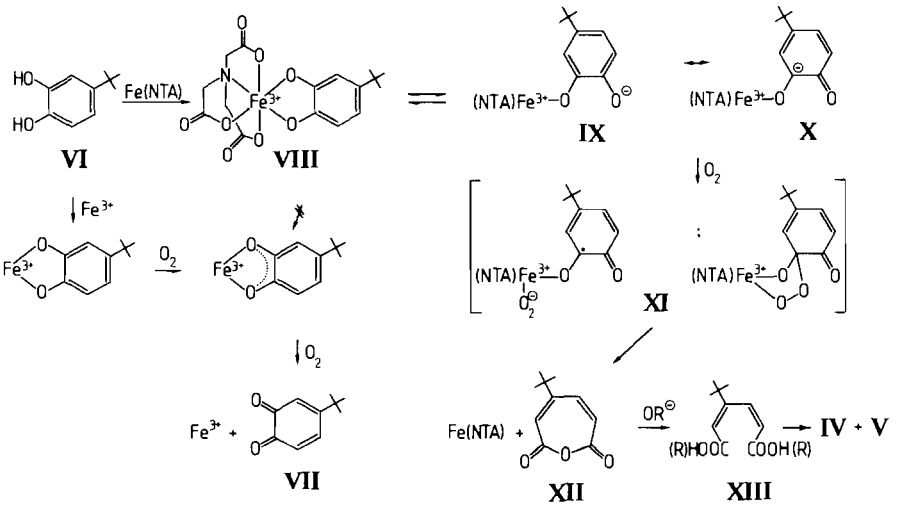
secondary products of muconic acid derivatives (**XIII**) [7], obtained from catecholate by dioxygenation. The total yield of ring cleavage products was 89% (Table I, No. 1). No alternative oxidation pathways were discernable, *e.g.* no quinone (**VII**) could be detected.

Thus, 4-t-butylcatechol in the ternary complex $[Fe(NTA)(DBcat)]^{2-}$ undergoes the same reaction as catecholate at the ferric active centre of catechol 1,2-dioxygenase. A 10- to 100-fold excess of catechol *vs.* Fe(NTA) (Table I, No. 2 and 3) gives rise to 71%, and 45% ring cleavage, respectively; *i.e.*, Fe(NTA) catalyzes, albeit slower than the enzyme, the catechol dioxygenation.

Control experiments demonstrate quinone formation instead of ring cleavage in the absence of NTA, *i.e.*, upon oxidation of simple ferric catecholate complexes (Table I, No. 4). Further, it is shown (Table I, No. 5) that 'free' catecholate in alkaline medium is protected from oxidation by boric acid via ester formation [8].

$[FeNTA)(DBcat)]^{2-}$ (**VIII**) was isolated as its tetrabutylammonium salt (dark blue–green crystals) and characterized as a catecholate chelate complex. The data are similar to those of the corresponding complex $[Fe(NTA)(DBcat)](pipH)_2$ [2] (M. G. Weller and C. Ruh, unpublished). However, catecholates chelated to ferric ions are known to be either non-oxidizable [4, 9–11] or to undergo oxidation to quinone (**VII**) via semiquinone ([12, 13]; *cf.* Table I, No. 4); they are not subject to dioxygenation. Some catecholate complexes of other transition metal ions undergo non-catalytic ring cleavage with O_2 [2, 4].

A dioxygenase mechanism was proposed [4] involving the attack of O_2 on catecholate bound to the ferric active centre as a monodentate ligand (see (**IX**)). This high density of negative groups around Fe^{3+} in the chelate complex (**VIII**) could, in solution, also give rise to an equilibrium with monodentate catecholate (**IX**). In fact, the EPR-spectrum of (**VIII**) contains signals of *two* high spin ferric complexes; one with essentially rhombic symmetry of the electronic environment of iron (E/D = 0.240; g_y = 3.65, g_z = 4.84, middle Kramer's doublet; g_y = 9.15, lower doublet), and a more axial species (E/D = 0.900–0.100; g_y = 7.85, g_z = 5.64). According to the broad, rhombic signal of solid $[Fe(NTA)(DBcat)](Bu_4N)_2$ (g_{eff} = 4.2), the 'rhombic' component corresponds to the chelate compound (**VIII**), and the 'axial' one to (**IX**), *i.e.*, with monodentate catecholate. Also a perfectly rhombic species (E/D = 1/3; $g_x = g_y = g_z = 4.29$, middle Kramer's doublet) appears with low intensity, corresponding to the eq. concentration of Fe(NTA)aq. (**IX**) is readily ketonized to (**X**), thus allowing O_2 to attack the aromatic ring, facilitated by an iron–oxygen interaction (**XI**). Iron catecholate complexes with



Scheme 1.

all coordination sites *cis* to catecholate occupied, thus preventing the formation of an additional Fe–O bond, *e.g.*, Fe(salen)(catH) [4], do *not* carry out dioxygenation. Insertion of oxygen to yield the anhydride (**XII**), ring opening to muconic acid (**XIII**) and lactonization finally leads to (**IV**) or (**V**) as products.

The ternary complex (**VIII**), unlike the binary catecholate complex (Table I, No. 4), is not subject to oxidation via semiquinone to quinone (**VII**), although the relative rates of reaction (see Table I) would favour oxidation against dioxygenation. Supposedly, 'hardening' of Fe^{3+} by the hard ligand NTA^{3-} disallows the electron shuttling through iron, *i.e.*, intermediate formation of Fe^{2+}, which is a prerequisite of semiquinone complex formation.

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