Oxidation at Carbon-1′ of DNA Deoxyriboses by the Mn-TMPyP/$KHSO_5$ System Results from a Cytochrome P-450-Type Hydroxylation Reaction

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Cationic porphyrin derivatives like *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin, TMPyP, have been shown to interact with DNA.[1] Moreover, the corresponding manganese derivative, Mn-TMPyP,[2,3] activated by a water-soluble oxygen atom donor like potassium monopersulfate,[4] provides an efficient DNA-cleaving system.[3a,5,6] From previous work, we evidenced that DNA cleavage by the Mn-TMPyP/$KHSO_5$ system was due to an oxidative attack at the C1′ and/or C5′ carbons of deoxyribose by isolation of 5-methylene-2-furanone (5-MF) and furfural (FUR), respectively, as deoxyribose residues (Schemes 1 and 2).[6,7] Since metalloporphyrins have been used as cytochrome P-450 models,[8] it was thought that the reactive metalloporphyrin species involved in DNA cleavage by Mn-TMPyP/$KHSO_5$ was a high-valent oxo intermediate. Such a hypothesis was supported by the observed clean breaks, excluding the possibility of a mechanism involving diffusible species. One way to verify that the metal–oxo was the cleaving agent was to determine the origin of the oxygen atom incorporated within the oxidized sugar residue. We reported recently that, for olefin epoxidations performed in aqueous medium with water-soluble metalloporphyrin catalysts, half of the oxygen atom incorporated in the epoxide came from the solvent and half from $KHSO_5$, the primary oxidant (it must be noted that $KHSO_5$ does not exchange oxygen atoms with water in experiments performed either with unlabeled monopersulfate and labeled water[9a] or with labeled monopersulfate and unlabeled water[9b]). A redox tautomerism mechanism involving the metal–oxo and the trans axial hydroxo ligand (equilibrium d in Scheme 1) was proposed to explain these results.[10] The same feature is expected for the origin of the oxygen atom incorporated in 5-MF and FUR during DNA cleavage by Mn-TMPyP/$KHSO_5$ if the hydroxylation is performed via a metal–oxo species.

In order to study the origin of the oxygen atom incorporated in 5-MF or FUR, we performed a DNA cleavage reaction with Mn-TMPyP using $KHS^{16}O_5$ and $H_2{}^{18}O$.[11] The sugar residues 5-MF and FUR were extracted and analyzed by GC–MS. Either $H_2{}^{16}O$ or $H_2{}^{18}O$ was used as solvent during the initial oxidative attack (a base is released during an H1′ attack, whereas a direct strand break is observed during an H5′ attack) and/or during the following heating step (strand break and release of 5-MF for H1′ attack, release of base and FUR for H5′ attack).[6] GC–MS analysis[12] of 5-MF and FUR (Table 1) allowed us to detect any possible incorporation of ^{18}O (the molecular peak M^+ is at 96 for both 5-MF and FUR, and it shifts at $M + 2 = 98$ when one ^{18}O is incorporated). The data can be interpreted as follows:

(i) The oxygen atom incorporated during the formation of FUR (Scheme 2 and Table 1) depends only on the nature of the solvent used during the heating step (either $H_2{}^{16}O$ or $H_2{}^{18}O$), indicating that the aldehyde oxygen atom from the aldehyde precursor or from FUR itself exchanged quickly with water in the reaction mixture after the initial oxidation step. In the case of DNA cleavage by the neocarzinostatin chromophore, Goldberg *et al.*[13] were able to determine the origin of the oxygen atom incorporated after the abstraction of H5′, because the aldehyde precursor was reduced *in situ* by the large excess of reducing agent used for drug activation. We tried to reduce *in situ* the aldehyde precursor of FUR by $NaBH_4$ just after the oxidation reaction and before the heating step and, after enzymatic release of the corresponding nucleosides and their derivatization, to check a possible ^{18}O incorporation.[14] Unfortunately, results indicated that exchange of the aldehyde oxygen atom with the solvent occurred quickly even at ambient temperature, making these experiments inconclusive.

(ii) As indicated in Table 1 (second entry), the mass spectrum of 5-MF released during the cleavage reaction shows two peaks, at $M^+ = 96$ and $(M + 2)^+ = 98$, in a ratio close to 1:1. This data suggests that the incorporated oxygen atom during the initial attack at C1′ leading to the 5-MF formation arose exclusively from the high-valent Mn–oxo species (Scheme 1, pathway b) which was previously shown, in oxygenation reactions per-

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(2) Mn-TMPyP stands for the pentaacetate of the diaquamanganese(III) derivative of *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin; see ref 3a for its preparation and ref 3b for its three-dimensional structure.

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(11) Cleavage experiments were performed in $H_2{}^{18}O$ and/or $H_2{}^{16}O$. $H_2{}^{18}O$ (98 atom %) was supplied by Eurisotop (Gif-sur-Yvette, France). All reactions were performed in duplicate. The reaction mixture (900 μL) containing 700 μM calf thymus DNA (in nucleotides), 35 μM Mn-TMPyP, 40 mM phosphate buffer pH 8, and 100 mM NaCl was lyophilized, redissolved in 1 g (890 μL) of $H_2{}^{18}O$, and then preincubated for 15 min at ambient temperature. The cleavage reaction was initiated by addition of 9 μL of 200 mM $KHS^{16}O_5$ (in $H_2{}^{16}O$). After 5 min of incubation, the reaction was quenched by pouring the reaction mixture onto a lyophilizate obtained from 100 μL of 1 M Hepes buffer pH 8 (this buffer was lyophilized in order to avoid dilution of the water label). Half of this reaction medium was extracted three times with 800 μL of butanol in order to remove all the $H_2{}^{18}O$ and then was redissolved in 450 μL of $H_2{}^{16}O$. The two aliquots (one in $H_2{}^{16}O$ and the other in $H_2{}^{18}O$) were heated for 15 min at 90 °C before cooling with an ice bath. The 5-MF and FUR were extracted by 3 × 500 μL of diethyl ether, and then the solvent was evaporated. Before reaching complete dryness, the samples were diluted in acetonitrile and analyzed. One similar experiment was also conducted in $H_2{}^{16}O$ exclusively. We checked that, in the absence of metalloporphyrin, no release of 5-MF and/or FUR was observed.

(12) GC–MS analyses were performed on a Hewlett-Packard 5890 instrument using electron-impact ionization at 70 eV. The carrier gas was He, and a nonpolar capillary column (12 m × 0.2 mm HL-1, cross-linked methylsilicone gum) was used. The injector temperature was 150 °C. Analyses were performed at 80 °C. The retention times were 0.97 and 1.17 min for FUR and 5-MF, respectively.

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(14) According to the protocol described in ref 13, a cleavage experiment performed in $H_2{}^{18}O$ on poly(dA)·poly(dT) (a polymer which presents a maximum of C5′ oxidation[6b]) produced, after $NaBH_4$ reduction and nuclease P_1 hydrolysis, the two nucleosides dA and dT always labeled at the 5′ position with oxygen arising from the solvent (98% ^{18}O).

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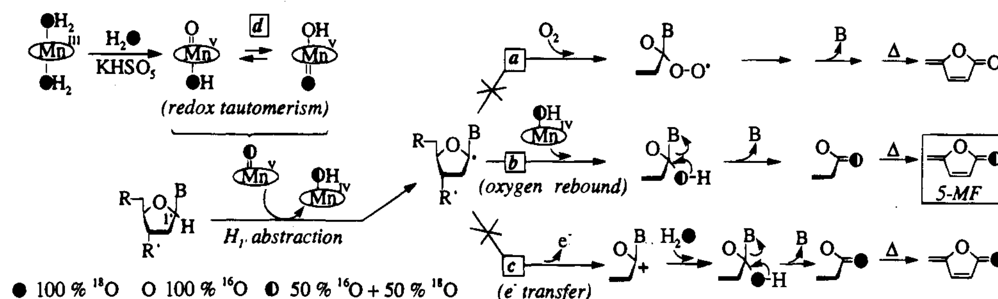
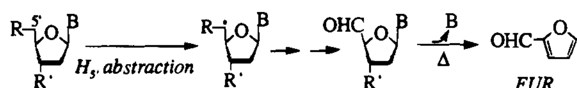
Scheme 1. Alternative Mechanisms To Account for the Possible Labeling Patterns at C1′ in Experiments Using $H_2{}^{18}O$ and $KHS^{16}O_5$[a]



[a] Δ = heating step; highlighted product (box) is the labeling effectively observed; R and R′ stand for phosphodiester residues of DNA.

Scheme 2. Formation of Furfural as Final Sugar Residue Resulting from Initial H5′ Abstraction[a]



[a] Δ = heating step; R and R′ stand for phosphodiester residues of DNA.

Table 1. Percentage of ^{18}O Incorporation in 5-MF and FUR after DNA Cleavage under Different Conditions as Described in the Experimental Section[a]

solvent		^{18}O incorporated (%)			
oxidative DNA cleavage	heating step	5-MF mass peak at 96	5-MF mass peak at 98	FUR mass peak at 96	FUR mass peak at 98
$H_2{}^{16}O$	$H_2{}^{16}O$	100	0	100	0
$H_2{}^{18}O$	$H_2{}^{18}O$	50	50	8	92
$H_2{}^{18}O$	$H_2{}^{16}O$	49	51	100	0

[a] Mean values from two independent experiments; $H_2{}^{18}O$ was 98 atom % in ^{18}O.

formed in $H_2{}^{18}O$, to incorporate 50% of oxygen coming from the primary oxidant (^{16}O from $KHSO_5$) and 50% from the solvent (^{18}O from $H_2{}^{18}O$). Replacing $H_2{}^{18}O$ by $H_2{}^{16}O$ during the heating step (Table 1, entry 3) did not significantly change the oxygen labeling of 5-MF. Compared to the case of FUR, the oxygen atom incorporated in 5-MF (or in its precursor) did not exchange with the solvent. These data strongly support the oxygen rebound mechanism[15] (Scheme 1, pathway b) and allow one to disregard, after the initial H1′ abstraction, the hypothesis of an electron transfer (pathway c) followed by addition of a water molecule on the cationic intermediate, which should give rise to 5-MF containing 100% of ^{18}O. An alternative hypothesis could be that dioxygen reacts with the C1′ radical (pathway a), but in this case the 5-MF formed should contain 0% of ^{18}O, which is not the case. Moreover, in parallel experiments performed either in air, in an oxygen-saturated atmosphere, or in an oxygen-deprived atmosphere,[16] we observed that the release of nucleobases, 5-MF and FUR did not differ significantly (data were the same within experimental errors), confirming that DNA cleavage by Mn-TMPyP/$KHSO_5$ was not oxygen-dependent.

In conclusion, the present observations indicate that, at least in the case of the oxidation of the C−H bond at the 1′ position of DNA sugars, only high-valent Mn-oxo-TMPyP was required, involving a *chemistry mimicking cytochrome P-450*. The Mn^V=O species abstracts an H1′ atom generating a carbon radical which quickly reacts with the intermediate Mn^{IV}−OH to form the C1′−OH precursor of 5-MF (oxygen rebound mechanism). At present, a similar mechanism at the C5′ site cannot be proposed due to the fast exchange of the oxygen atom of the aldehyde precursor generated in the first step of the reaction.

This mode of oxidation of DNA sugar C−H bonds by a metal−oxo porphyrin is clearly different from that observed in DNA cleavage by activated iron bleomycin. In this case, oxidation at C4′ involves a different mechanism called "diverted P-450 route"[18] where abstraction of H4′ is followed by either addition of dioxygen on the C4′ radical or abstraction of a second electron and addition of a water molecule to the C4′ cation.[19]

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