Nuclease Activity of a Water-soluble Manganese Porphyrin Associated with Potassium Hydrogen Persulphate: Oxidative Cleavage of DNA

Eric Fouquet, Geneviève Pratviel, Jean Bernadou, and Bernard Meunier*

Laboratoire de Chimie de Coordination du CNRS, 205, route de Narbonne, 31077 Toulouse Cedex, France

Oxidative cleavage of DNA is observed when the oxygen donor potassium hydrogen persulphate is associated with a water-soluble manganese porphyrin; the nuclease activity is obtained for low concentrations of both the manganese porphyrin (2—200 nM) and potassium hydrogen persulphate (1—25 μM), and the latter is more efficient than hydrogen peroxide in this metalloporphyrin-mediated cleavage of DNA.

Metalloporphyrins have been used extensively during the last seven years in cytochrome P-450 modelling studies (for recent reviews, see refs. 1 and 2). By association of single oxygen atom donors such as PhIO, NaOCl, ROOH, H_2O_2, and $KHSO_5$ with manganese and iron porphyrin complexes, biomimetic oxygenation reactions (epoxidation and hydroxy-

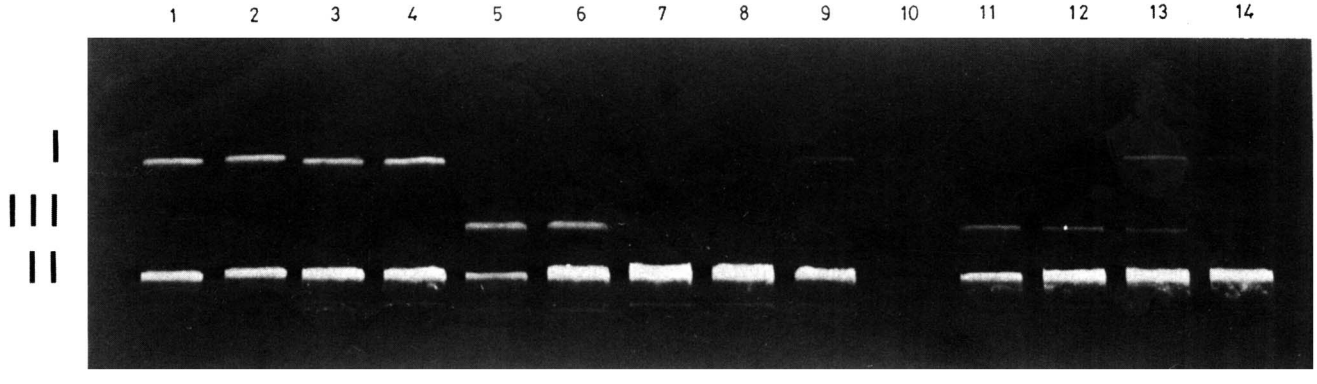

Figure 1. Strand scission of φ × 174 DNA by $Mn(Mepy)_4P$: effect of various concentrations of H_2O_2 or $KHSO_5$. The DNA was electrophoresed on an agarose gel and treated as described in the text. Porphyrin (250nM) to DNA-base pair (19μM) ratio was 1/76. Lane assignments: 1, DNA control; 2, $Mn(Mepy)_4$ *P* control; 3, H_2O_2 50mM control; 4, $KHSO_5$ 25μM control; 5 to 9, $Mn(Mepy)_4P$ + H_2O_2 50, 10, 2, 0.5, and 0.1mM, respectively; 10 to 14, $Mn(Mepy)_4P$ + $KHSO_5$ 25, 10, 3, 1, and 0.3μM, respectively. The various forms of φ × 174 DNA [covalently closed circular (form I), nicked circular (form II), and linear (form III)] are indicated.

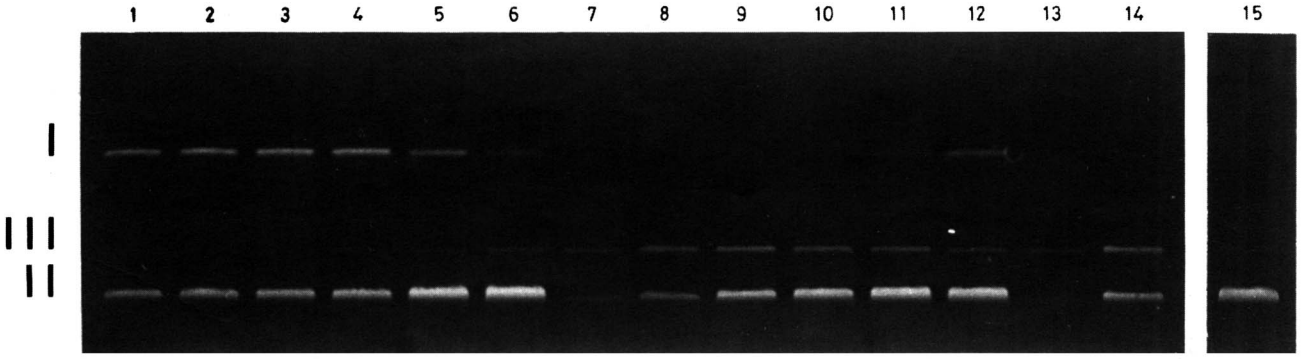

Figure 2. Strand scission of φ × 174 DNA (19μM in base pairs) by various concentrations of $Mn(Mepy)_4P$ in the presence of $KHSO_5$. Lane assignment: 1 and 2, DNA controls, 20 and 60 min digestion, respectively; 3 and 4, $Mn(Mepy)_4P$ 250nM control, 20 and 60 min digestion, respectively; 5 and 6, $KHSO_5$ 25μM, 20 and 60 min digestion respectively; 7 and 8, $Mn(Mepy)_4P$ 250nM + $KHSO_5$ 25 and 10μM, respectively, 20 min digestion; 9 and 10, $Mn(Mepy)_4P$ 25nM + $KHSO_5$ 25 and 10μM, respectively, 20 min digestion; 11 and 12, $Mn(Mepy)_4P$ 2.5nM + $KHSO_5$ 25 and 10μM, respectively, 20 min digestion; 13 and 14, $Mn(Mepy)_4P$ 25nM + $KHSO_5$ 25 and 10μM, respectively, 60 min digestion; 15, $Mn(Mepy)_4P$ 2.5nM + $KHSO_5$ 10μM, 60 min digestion.

lation) have been successfully developed. In these catalytic reactions the key role of an high-valent oxometal or oxometal-like species has been evident. However the exact nature of this active complex [M^{V}=O or M^{IV}–O·] is still under investigation.[3,4]

Of all the oxygen donors used in these oxygen-transfer reactions, only two are readily soluble in aqueous solution at physiological pH: H_2O_2, the natural oxygen donor for peroxidases, and $KHSO_5$ (potassium hydrogen persulphate, commercially available under the trademark Oxone®). With $KHSO_5$ as oxygen surrogate, it has been possible to study (i) cleavage of DNA by the bleomycin–iron complex[5] and (ii) the oxygenation of an olefin catalysed by manganese– or iron–bleomycin complexes.[6] In both cases, the data strongly support the existence of an active bleomycin–oxometal species able to cleave DNA or to epoxidise an olefin.

We report here the oxidative cleavage of DNA by a water-soluble manganese porphyrin complex in the presence of $KHSO_5$. The results contribute to the study of DNA breaks generated by metal complexes and oxygen species,[7–12] with a view to the use of these systems as artificial nucleases in DNA 'footprinting' experiments.[13–15]

Iodosylbenzene, another oxygen donor, which has to be solubilized in methanol–water, has also been used with metalloporphyrins in the oxidative cleavage of DNA.[16]

Our results show that the activating efficiency of $KHSO_5$ in association with $[Mn(Mepy)_4P](OAc)_5$† is greater than that of H_2O_2 under the same conditions. Figure 1 shows the activating role of a wide range of concentrations of $KHSO_5$ or H_2O_2 on the cleavage of φ × 174 DNA by Mn^{III}-porphyrin.‡ DNA with $Mn(Mepy)_4P$ does not exhibit significant cleavage when the ratio of $Mn(Mepy)_4P$ to base pairs is 1 : 76. In the presence of large doses of either H_2O_2 or $KHSO_5$ alone, only weak cleavage of form I (supercoiled) to form II (nicked circular) occurs; lower doses (not shown) do not lead to significant strand scission. When associated with H_2O_2 or $KHSO_5$, the Mn–porphyrin complex cleaves DNA in a dose-dependent way: as illustrated in Figure 1, the efficiency of cleavage increases for concentrations of H_2O_2 varying from 0.1 to 50mM (lanes 9 to 5), and of $KHSO_5$ varying from 0.3 to 25 μM (lanes 14 to 10). At the highest concentration of $KHSO_5$, linear DNA breaks into smaller fragments which migrate to the top of the gel (lane 10). The effectiveness of $KHSO_5$ is evident from these experiments; we estimate that the isoactive concentrations of $KHSO_5$ with respect to H_2O_2 are lowered by a factor of about 2000 to 4000 (we checked that the manganese complex has a very low catalase activity under these conditions; up to 85% of the initial dose of H_2O_2 is still present at the end of the incubation).

DNA strand scissions could be observed with concentrations of $[Mn(Mepy)_4P)](OAc)_5$ as low as 2.5 nM [ratio $Mn(Mepy)_4P$/base pairs = 1/7600]. Figure 2 shows experi-

† *meso*-Tetrakis-(*N*-methyl-4-pyridyl)porphyrin, $(Mepy_4)PH_2$, was obtained by methylation (MeI) of py_4PH_2 (Aldrich) as previously described.[17] Its Mn^{3+} complex was prepared as follows. An aqueous solution (15 ml) of $(Mepy)_4PH_2$ (100 mg) (iodide form) and $Mn(OAc)_2$ (ten-fold excess) was refluxed for 2 h. After cooling to room temperature the Mn^{III} complex was precipitated by addition of saturated KI solution and washed with cold water. The acetate form was obtained with ion-exchange resin (Amberlite IRN78, Prolabo) in MeOH solution. After filtration $[Mn(Mepy)_4P](OAc)_5$ [here abbreviated to $Mn(Mepy)_4P$] was obtained by precipitation in 1 : 4 MeOH–Et_2O (yield 80%). (The u.v.–visible spectrum is similar to that described by Harriman *et al.*[18])

‡ φ × 174 *DNA Digestion Conditions*. For all the experiments, DNA was diluted in phosphate buffer (5 mM; pH 7.4). The reaction involved 5 μl of φ × 174 DNA (50 μg ml^{-1}), 10 μl of metalloporphyrin solution in 50 mM-phosphate pH 7.4 buffer, and 5 μl of $KHSO_5$ or H_2O_2 diluted in the same buffer. Digestion time was for 20 min (or 60 min when mentioned), at 20 °C.

Electrophoresis. Metalloporphyrin-mediated DNA cleavage was monitored by agarose gel electrophoresis. Reactions were quenched by 5 μl of a 'stopping reagent' and samples were kept on ice. The stopping reagent consisted of 250mM-Tris-HCl pH 7.2 buffer containing 75% glycerol and 0.05% Bromophenol Blue. We have checked that 50mM-Tris-HCl pH 7.2 buffer (final concentration in the quenched reaction samples) degrades more than 90% of the $KHSO_5$ in 2 min; control experiments show no DNA strand scission by the degradation products. Reaction mixtures were then run in 0.8% agarose slab horizontal gel containing ethidium bromide 1 μg ml^{-1}, at constant current (25 mA for 15 h), in 89mM-Tris-borate pH 8.3 buffer. Bands were located by u.v. light and photographed.

ments with decreasing amounts of $Mn(Mepy)_4P$ from 250 (1 porphyrin to 76 base pairs) to 2.5 nM (1 porphyrin to 7600 base pairs; $\phi \times 174$ DNA = 5386 base pairs) in the presence of either 25 or 10 μM$KHSO_5$; DNA digestion time was 20 or 60 min.

Comparing the DNA bands in the presence of $Mn(Mepy)_4P$ and $KHSO_5$ (25 μM), we note that after 20 min incubation, for all the concentrations of porphyrin tested (250 to 2.5 nM), form I completely disappears (lanes 7, 9, and 11); the intensity of nicked circular and linear DNA bands gradually decreases as the concentration of $Mn(Mepy)_4P$ increases. After 60 min DNA digestion in the presence of 25nM-$Mn(Mepy)_4P$, the cleavage is almost complete (lane 13) and quite different from the corresponding system without metalloporphyrin, although in this last case noticeable conversion of DNA form I into forms II and III occurs.

For 10μM-$KHSO_5$, in the absence of $Mn(Mepy)_4P$, no degradation is detected after 20 and 60 min incubations (not shown), but in the presence of 250, 25, or 2.5 nM-$Mn(Mepy)_4P$ (lanes, 8, 10, and 12), DNA breaks are evidenced; almost complete disappearance of form I is noted at 25nM-$Mn(Mepy)_4P$ with 20 min digestion (lane 10), or 2.5 nM-$Mn(Mepy)_4)P$ with 60 min digestion (lane 15).

In conclusion, $KHSO_5$, tested as activating agent in DNA cleavage experiments in the presence of $Mn(Mepy)_4P$, appears at least 1000 times more efficient than H_2O_2 (this work) or iodosylbenzene[16] in inducing DNA breaks. The 'nuclease activity' of this association could be observed with concentrations of reactants as low as 2.5—25nM in metalloporphyrin and 10—25μM in oxygen donor ($KHSO_5$).

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