G-Quadruplex Ligands

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Interaction of Cationic Manganese Porphyrin with G-Quadruplex Nucleic Acids Probed by Differential Labeling of the Two Faces of the Porphyrin**

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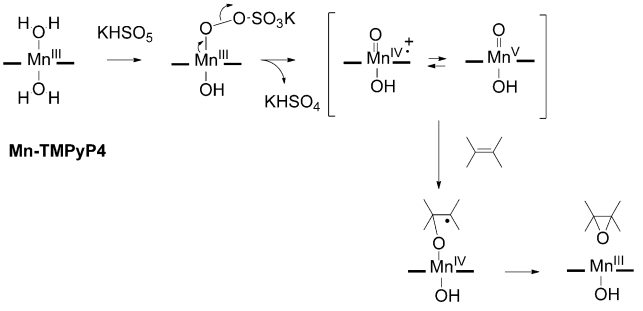
G-quadruplex nucleic acids have attracted much interest in recent years because they appear to be involved in the regulation of fundamental processes of life, in particular, those connected to the development of cancer.[1–9] Several in vitro structures are now available.[10–15] G-quadruplex nucleic acids consist of four strands of nucleic acids that form in guanine-rich DNA/RNA sequences with stretches of tandem guanine residues. The four strands are held together by a hydrogen-bonded arrangement of four guanines (the G-quartet unit). Stacking of planar consecutive G-quartets further stabilizes the G-quadruplex structures. G-quadruplex nucleic acids have four external grooves, and for those formed by intermolecular folding, they have also single-stranded loops connecting the guanine tracts. A central ionic channel with K^+ (and/or Na^+) ions exists in the core of the superimposed G-quartets. Their presence in human cells has recently been shown.[16]

With the aim of interfering with their biological functions, a number of small ligands targeting G-quadruplex nucleic acids have emerged.[17–20] *meso*-Tetrakis(4-*N*-methylpyridiniumyl)porphyrin (H_2-TMPyP4) is a long known G-quadruplex ligand[21] and is often used as a reference compound in the field. H_2-TMPyP4, like other large aromatic ligands, should exhibit π–π stacking interactions with an external guanine quartet of the G-quadruplex structure. However, this type of binding is still under debate. Two structures of the complex between H_2-TMPyP4 and G-quadruplex DNA were reported.[22,23] The first structure (*c-myc* oncogene promoter sequence) shows the porphyrin interacting with the top G-quartet, although at a relatively long distance, not in perfect accordance with known π–π stacking interactions. The second structure (telomeric sequence) showed a porphyrin involved in a stacking interaction with a base pair in the loop at the top of the G-quardruplex but not with the uppermost G-quartet itself. Porphyrin H_2-TMPyP4 interacts with telomeric G-quadruplex models with a relatively high binding constant, on the order of $K_a \approx 10^6$–10^8 M^{-1}.[24–27] The binding of H_2-TMPyP4 to the c-myc promoter parallel quadruplex is tighter with a binding constant of $K_a \approx 10^8$–10^9 M^{-1}.[28] The variations in the binding constant arise from different ionic strengths and experimental conditions.

The introduction of a metal ion such as Mn^{III} or Co^{III} into the center of the porphyrin macrocycle gives two molecules of water as axial ligands. This does not preclude the interaction of the porphyrin with the G-quadruplex but does lower the affinity compared to the non-metalated porphyrin.[27] These metalated porphyrins could in principle interact with the G-quadruplex by external π–π stacking with the top G-quartet, provided the axial water ligand fits within the central ionic channel of the quadruplex, or alternatively, interact with the grooves (or loops) of the quadruplex. These two binding modes should differ by the accessibility of the two faces of the porphyrin macrocycle. External π–π stacking would have only one accessible face from the bulk solvent, while binding to the quadruplex grooves would leave two faces of the porphyrin accessible. In the absence of any structural data on the interaction of the metalated porphyrins containing axial water ligands with G-quadruplexes, and considering that the mode of interaction of H_2-TMPyP4 with the top G-quartet of these structures is unknown, we determined the accessibility of the two faces of the manganese derivative of H_2-TMPyP4, Mn-TMPyP4, while bound to a G-quadruplex using labeling experiments.

The water-soluble and dissymmetric peroxide $KHSO_5$ can coordinate with the manganese ion of the porphyrin by displacing the water molecule from one face of the porphyrin (Scheme 1). Heterolytic cleavage of the O–O bond leads to the formation of a high-valent metal-oxo species.[29] It is an oxomanganese(IV) porphyrin radical cation, $(P^{\cdot+})Mn^{IV}{=}O$, formally referred to as $Mn^{V}{=}O$,[30] which is able to transfer its "oxo" oxygen atom onto a substrate by a mechanism similar to the cytochrome P450 enzymes.[31] The activation of Mn-



Scheme 1. Activation of manganese porphyrin (Mn-TMPyP4) by $KHSO_5$ and oxygen atom transfer in olefin epoxidation. The porphyrin macrocycle is simplified as two bold lines.

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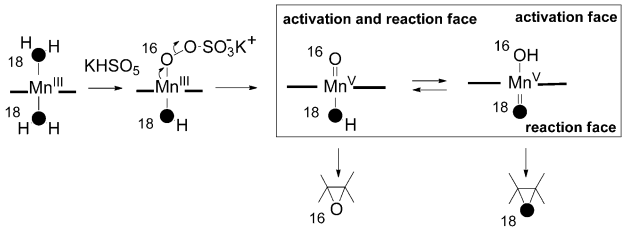
TMPyP4 with $KHSO_5$ in aqueous buffer is extremely rapid and efficient. The face of the porphyrin where peroxide coordination triggers the formation of the metal-oxo species must be accessible from the solvent.

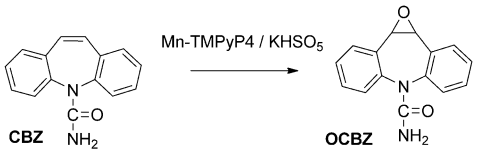
In the case of water-soluble metallo porphyrins, it has been shown that the oxygen atom incorporated in the substrate does not originate exclusively from the peroxide, owing to an oxo–hydroxo tautomerism mechanism (Scheme 2).[31–33] This oxygen atom can also originate from the *trans* water molecule on the opposite face of the



Scheme 2. Oxo–hydroxo tautomerism of high-valent metal-oxo manganese porphyrin in water and subsequent labeling of the product of the reaction of olefin epoxidation.

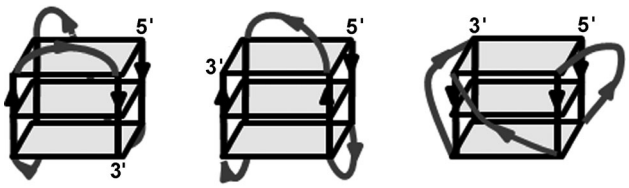
porphyrin. The reaction face of the porphyrin, where the oxo entity reacts with the substrate may be different from the activation face, where the oxo species was originally formed. Consequently, measurement of the oxo–hydroxo tautomerism provides a way to tell whether the two faces of the porphyrin are accessible to the solvent. If the reaction face where the metal-oxo entity locates is the same as the activation face, the oxygen atom incorporated into the substrate originates from the peroxide. If the reaction face is opposite to the activation face, the oxygen atom incorporated into the substrate originates from water. When the two faces of the metalloporphyrin are equally accessible, the oxygen atom incorporated into the substrate will originate equally from the peroxide and the water molecule. It has been shown that the ratio is 50% from $KHSO_5$ and 50% from water under normal reaction conditions in buffered solutions.[32–34] Herein, we tested the oxo–hydroxo tautomerism of the metal-oxo form of Mn-TMPyP4 porphyrin in complex with G-quadruplex nucleic acids by analyzing the oxygen atom incorporated into the epoxidation product of carbamazepine (CBZ; Scheme 3).

We used two different G-quadruplex forming oligonucleotides: first, 5′-d[TT(GGGTTA)$_3$GGGA] (TTG4A), corresponds to a variable form of the human telomere sequence previously shown to adopt a hybrid type 1 structure (95%) in KCl containing medium.[35] In the presence of NaCl, TTG4A



Scheme 3. Mn-TMPyP4/$KHSO_5$ catalyst converts carbamazepine (CBZ) into carbamazepine-10,11-oxide (OCBZ).

folds into an antiparallel G-quadruplex. Second, 5′-d(TGAGGGTGGGGAGGGTGGGGAA) oligonucleotide (c-myc), corresponds to the human oncogene c-myc promoter sequence (myc2345), which adopts a propeller-type parallel-stranded G-quadruplex structure in KCl medium.[36] Scheme 4 shows a schematic drawing of the three types of folding.



Scheme 4. Scheme showing the different G-quadruplex structures. The left and middle structures correspond to a hybrid type 1 and antiparallel orientation of the TTG4A sequence in K^+ and Na^+ buffers, respectively. The right structure shows the propeller-type parallel-stranded orientation of the c-myc sequence in K^+ buffer.

The oxidation reaction of CBZ by Mn-TMPyP4/$KHSO_5$ was previously described in phosphate buffer at pH 5 in the presence of 10% methanol.[32] Because the optimal pH value for this oxidation reaction is pH 5, the structure of the G-quadruplexes was determined by circular dichroism under these experimental conditions (Supporting Information, Figures S1–S4). The folding of the quadruplexes appeared identical to the folding observed at neutral pH (Supporting Information, Figures S1,S2). The addition of 10% methanol to the buffer also did not alter the structure of the G-quadruplexes (Supporting Information, Figures S3,S4). In phosphate buffer (50 mM) containing 10 mM KCl, the TTG4A oligonucleotide showed a typical CD spectrum of the classic hybrid type 1 structure of telomeric quadruplex DNA. In phosphate buffer (pH 5) containing NaCl, TTG4A folded into a typical antiparallel G-quadruplex structure. Thus, the folding of these G-quadruplexes is not affected by the lower pH value of the buffer. In addition, the G-quadruplex structure of the TTG4A oligonucleotide proved stable following the lyophilization and redissolution steps that were necessary for experiments in labeled water. Furthermore, we have previously shown that Mn-TMPyP4 interaction with TTG4A does not change its CD spectrum.[27]

Oxidation of CBZ by Mn-TMPyP4/$KHSO_5$ was first carried out in the absence of G-quadruplex DNA. CBZ (500 μM) was reacted with Mn-TMPyP4 (2 μM) for one minute at 25 °C in phosphate buffer (pH 5) containing KCl (or NaCl). At the end of the reaction, unreacted CBZ and the epoxide OCBZ were extracted using dichloromethane (extraction was quantitative) and the organic phase was analyzed by HPLC coupled to electrospray mass spectrometry (Supporting Information, Figure S5). OCBZ epoxide (Table 1) was the only oxidation product detected. The low yield (approximately 7% with respect to CBZ) is due to the short reaction time. The oxo–hydroxo tautomerism on the Mn^V=O was measured by performing the same reaction in $H_2{}^{18}O$. The incorporation of ^{18}O atoms from the labeled water into OCBZ

Table 1: Oxygen atom transfer during the oxidation of CBZ.[a]

Quadruplex DNA	Mn-TMPyP4 [μM]	OCBZ[b] [μM]	^{18}O in OCBZ[c] [%]
– (K)	2	33 ± 10[d]	41 ± 3
– (Na)	2	32 ± 2[d]	39 ± 2
TTG4A (K)	2	1.2 ± 0.5	12 ± 1
TTG4A (Na)	2	1.0 ± 0.4	12 ± 1
c-myc (K)	2	0.7[e]	13[e]
TTG4A (K)	0	0.02 ± 0.01	0

[a] CBZ (500 μM) was mixed with with Mn-TMPyP4 (2 μM)/$KHSO_5$ (500 μM) in 50 mM phosphate buffer (pH 5), 10 mM KCl (K) or 10 mM NaCl (Na), for 1 min at 25 °C in the absence (–) or presence of DNA (40 μM): hybrid type 1 folded G-quadruplex TTG4A (K), propeller-type parallel G-quadruplex folded c-myc (K) oligonucleotides, and antiparallel folded G-quadruplex TTG4A (Na). [b] Measured by integration of the OCBZ peak area in the HPLC UV- or extracted ionic current (EIC)-trace compared to standard curves. [c] The percentage of CBZ ^{18}O-oxide was calculated from the ratio of peak intensities at $m/z = 255.1$ versus $m/z = 253.1$, as well as from the ratio of the sodium adduct peak intensities at $m/z = 277.1$ versus $m/z = 275.1$. The maximum possible incorporation of ^{18}O from oxo–hydroxo tautomerism is 50%. [d] CBZ conversion $< 10\%$. [e] Two measurements.

was 41% in KCl buffer (39% in NaCl buffer), showing that the oxo–hydroxo tautomerism was effective although not complete (Table 1, Figure 1 A,B; Supporting Information, Figure S6 A,B for NaCl conditions). With the present experimental conditions, epoxide formation may compete with oxo-aqua exchange, as was the case of 35% ^{18}O-incorporation that was previously observed.[30] A short reaction time was necessary in the presence of G-quadruplex DNA to avoid oxidative damage to the DNA. Indeed, we chose to measure the incorporation of the ^{18}O-atom in OCBZ at the very start

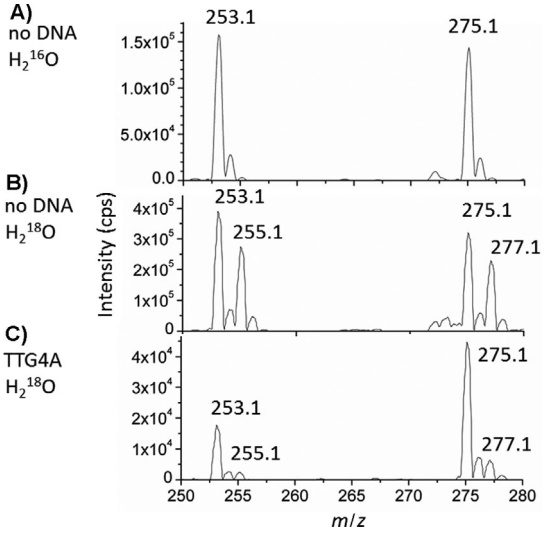
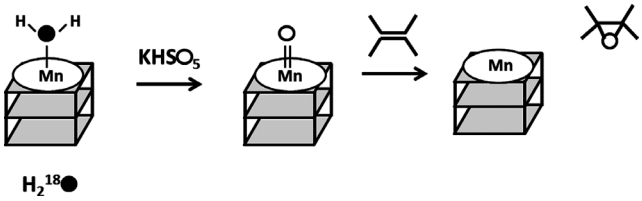


Figure 1. In-line mass analysis of OCBZ labeling in KCl-containing buffer. A) In the absence of DNA and in $H_2{}^{16}O$, the $(M+H)^+$ ion of OCBZ is observed at $m/z = 253.1$ and the sodium adduct at $m/z = 275.1$. B) In the absence of DNA and in $H_2{}^{18}O$, isolated OCBZ consists of 59% with one ^{16}O-atom ($m/z = 253.1$ and $m/z = 275.1$) and 41% with one ^{18}O-atom ($m/z = 255.1$ and $m/z = 277.1$). C) In the presence of G-quadruplex DNA TTG4A, the incorporation of an ^{18}O atom into OCBZ ($m/z = 255.1$ and $m/z = 277.1$) drops to 12%.

of the reaction to analyze the reactivity of porphyrin in complex with DNA before any oxidative DNA damage could occur (Supporting Information, Figures S7,S8), which could release free porphyrin into the bulk solvent. In the same way, to avoid any free porphyrin in the reaction medium, the concentration of the G-quadruplex oligonucleotide (40 μM) was high compared to that of Mn-TMPyP4 (2 μM), and the two components were preincubated for 15 minutes to establish an association equilibrium before starting the reaction by addition of CBZ and $KHSO_5$.

In the presence of G-quadruplex DNA (TTG4A and c-myc), the yield of the epoxidation reaction dropped dramatically (Table 1). This was probably due to reduced access of CBZ to the manganese porphyrin in complex with DNA. The incorporation of ^{18}O from $H_2{}^{18}O$ into OCBZ was extremely low (12–13%; Figure 1 C). The almost exclusive incorporation of an ^{16}O atom, originating from the peroxide, into the epoxide is an indicator of the oxidation of CBZ being due to a quadruplex-bound manganese-oxo porphyrin reacting through the same face as that of the incoming peroxide. This deficiency in the oxo–hydroxo tautomerism when the manganese porphyrin is in complex with DNA is in accordance with only one face of the porphyrin being accessible to solvent during the reaction and thus, to the porphyrin interacting with the quadruplex DNA through π–π stacking on the top G-quartet (Scheme 5). We found similar evidence of porphyrin binding for each of the three types of quadruplexes tested (antiparallel, parallel, and hybrid), which also confirms the non-specific binding of this porphyrin to different quadruplexes.



Scheme 5. Interaction of Mn-TMPyP4 with the top face of the quadruplex DNA.

The formation of OCBZ and subsequent labeling with ^{16}O owing to a direct reaction of $KHSO_5$ with CBZ can be disregarded. In the absence of Mn-TMPyP4, no significant substrate conversion occurred (Table 1). The residual 12–13% of ^{18}O-incorporation for the bound porphyrin may be attributed to a minor fraction of the porphyrin still being able to undergo oxo–hydroxo tautomerism while interacting with the DNA, for instance through interaction with the loop or groove, giving access to both faces.[33]

In conclusion, the symmetric (oxo–hydroxo tautomerism) or dissymmetric (lack of oxo–hydroxo tautomerism) labeling of the two faces of a high-valent manganese-oxo porphyrin allows us to ascertain the solvent accessibility of these two faces and consequently, gives us information about the interaction of the porphyrin with G-quadruplexes. This study is possible because of a strong interaction between the porphyrin and the target DNA, because the solution needs to

be free of any unbound porphyrin. A complex of a porphyrin and G-quadruplex DNA was previously used as a "DNAzyme", with heme serving as the porphyrin.[37–40] The same concept was used in the present work but for the purposes of investigating the structure of the complex. This concept could be extended to any non-covalent complex between a metalloporphyrin and a biological or material partner or to any non-heme metal complex able to undergo an oxo–hydroxo tautomerism within its coordination sphere.

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[1] N. Maizels, *Nat. Struct. Mol. Biol.* **2006**, *13*, 1055.
[2] D. J. Patel, A. T. Phan, V. Kuryavyi, *Nucleic Acids Res.* **2007**, *35*, 7429.
[3] S. Balasubramanian, S. Neidle, *Curr. Opin. Chem. Biol.* **2009**, *13*, 345.
[4] S. Neidle, *FEBS J.* **2010**, *277*, 1118.
[5] K. M. Miller, R. Rodriguez, *Expert Rev. Clin. Pharmacol.* **2011**, *4*, 139.
[6] A. Bugaut, S. Balasubramanian, *Nucleic Acids Res.* **2012**, *40*, 4727.
[7] D. Gomez, A. Guedin, J. L. Mergny, B. Salles, J. F. Riou, M. P. Teulade-Fichou, P. Calsou, *Nucleic Acids Res.* **2010**, *38*, 7187.
[8] A. Decorsiere, A. Cayrel, S. Vagner, S. Millevoi, *Genes Dev.* **2011**, *25*, 220.
[9] J. F. Fisette, D. R. Montagna, M. R. Mihailescu, M. S. Wolfe, *J. Neurochem.* **2012**, *121*, 763.
[10] J. Dai, M. Carver, D. Yang, *Biochimie* **2008**, *90*, 1172.
[11] Y. Qin, L. H. Hurley, *Biochimie* **2008**, *90*, 1149.
[12] S. Neidle, *Curr. Opin. Struct. Biol.* **2009**, *19*, 239.
[13] A. T. Phan, *FEBS J.* **2010**, *277*, 1107.
[14] S. M. Haider, S. Neidle, G. N. Parkinson, *Biochimie* **2011**, *93*, 1239.
[15] S. Amrane, M. Adrian, B. Heddi, A. Serero, A. Nicolas, J. L. Mergny, A. T. Phan, *J. Am. Chem. Soc.* **2012**, *134*, 5807.
[16] R. Rodriguez, K. M. Miller, J. V. Forment, C. R. Bradshaw, M. Nikan, S. Britton, T. Oelschlaegel, B. Xhemalce, S. Balasubramanian, S. P. Jackson, *Nat. Chem. Biol.* **2012**, *8*, 301.
[17] T. M. Ou, Y. J. Lu, J. H. Tan, Z. S. Huang, K. Y. Wong, L. Q. Gu, *ChemMedChem* **2008**, *3*, 690.
[18] D. Monchaud, A. Granzhan, N. Saettel, A. Guedin, J. L. Mergny, M. P. Teulade-Fichou, *J. Nucleic Acids* **2010**, 525862.
[19] A. Granzhan, D. Monchaud, N. Saettel, A. Guedin, J. L. Mergny, M. P. Teulade-Fichou, *J. Nucleic Acids* **2010**, 0460561.
[20] S. N. Georgiades, N. H. Abd Karim, K. Suntharalingam, R. Vilar, *Angew. Chem.* **2010**, *122*, 4114; *Angew. Chem. Int. Ed.* **2010**, *49*, 4020.
[21] R. T. Wheelhouse, D. Sun, H. Han, F. Xiaoguang Han, L. H. Hurley, *J. Am. Chem. Soc.* **1998**, *120*, 3261.
[22] A. T. Phan, V. Kuryavyi, H. Y. Gaw, D. J. Patel, *Nat. Chem. Biol.* **2005**, *1*, 167.
[23] G. N. Parkinson, R. Ghosh, S. Neidle, *Biochemistry* **2007**, *46*, 2390.
[24] C. Wei, G. Jia, J. Zhou, G. Han, C. Li, *Phys. Chem. Chem. Phys.* **2009**, *11*, 4025.
[25] L. Martino, B. Pagano, I. Fotticchia, S. Neidle, C. Giancola, *J. Phys. Chem. B* **2009**, *113*, 14779.
[26] P. Murat, R. Bonnet, A. Van der Heyden, N. Spinelli, P. Labbe, D. Monchaud, M. P. Teulade-Fichou, P. Dumy, E. Defrancq, *Chem. Eur. J.* **2010**, *16*, 6106.
[27] C. Romera, O. Bombarde, R. Bonnet, D. Gomez, P. Dumy, P. Calsou, J. F. Gwan, J. H. Lin, E. Defrancq, G. Pratviel, *Biochimie* **2011**, *93*, 1310.
[28] J. M. Dettler, R. Buscaglia, V. H. Le, E. A. Lewis, *Biophys. J.* **2011**, *100*, 1517.
[29] M. Pitié, G. Pratviel, *Chem. Rev.* **2010**, *110*, 1018.
[30] J. T. Groves, J. Lee, S. S. Marla, *J. Am. Chem. Soc.* **1997**, *119*, 6269.
[31] B. Meunier, S. P. de Visser, S. Shaik, *Chem. Rev.* **2004**, *104*, 3947.
[32] J. Bernadou, A.-S. Fabiano, A. Robert, B. Meunier, *J. Am. Chem. Soc.* **1994**, *116*, 9375.
[33] M. Pitié, J. Bernadou, B. Meunier, *J. Am. Chem. Soc.* **1995**, *117*, 2935.
[34] J. Bernadou, B. Meunier, *Chem. Commun.* **1998**, 2167.
[35] K. N. Luu, A. T. Phan, V. Kuryavyi, L. Lacroix, D. J. Patel, *J. Am. Chem. Soc.* **2006**, *128*, 9963.
[36] A. T. Phan, Y. S. Modi, D. J. Patel, *J. Am. Chem. Soc.* **2004**, *126*, 8710.
[37] S. Nakayama, H. O. Sintim, *J. Am. Chem. Soc.* **2009**, *131*, 10320.
[38] Y. He, Y. Tian, C. Mao, *Mol. Biosyst.* **2009**, *5*, 238.
[39] R. Freeman, E. Sharon, C. Teller, A. Henning, Y. Tzfati, I. Willner, *ChemBioChem* **2010**, *11*, 2362.
[40] L. C. Poon, S. P. Methot, W. Morabi-Pazooki, F. Pio, A. J. Bennet, D. Sen, *J. Am. Chem. Soc.* **2011**, *133*, 1877.

