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A G-Quadruplex Ligand with 10000-Fold Selectivity over Duplex DNA

Isabelle M. Dixon,[#] Frédéric Lopez,[§] Agueda M. Tejera,[†] Jean-Pierre Estève,[‡] Maria A. Blasco,[†] Geneviève Pratviel,*[,#] and Bernard Meunier[||]

Laboratoire de Chimie de Coordination, CNRS, 205 route de Narbonne, 31077 Toulouse Cedex 4, France, INSERM IFR 31, Institut Louis Bugnard, Hôpital Rangueil, TSA 50032, 31059 Toulouse Cedex 9, France, Spanish National Cancer Center, Melchor Fernández Almagro 3, 28029 Madrid, Spain, INSERM U 531, Institut Louis Bugnard, Hôpital Rangueil, TSA 50032, 31059 Toulouse Cedex 9, France, and Palumed, BP 28262, 31682 Labège Cedex, France

Received August 2, 2006; E-mail: pratviel@lcc-toulouse.fr

There is considerable interest in the design of small molecules that selectively target telomeric DNA sequences and G-quadruplexes owing to their possible role as antitumor chemotherapeutic agents. The single-stranded overhang of the human G-rich telomeric DNA sequence, made of TTAGGG repeats, is able to fold into a quadruplex DNA structure in vitro.[1] Quadruplex DNA structures have also been detected in vivo.[2] Since each cell division is accompanied by an erosion of the telomeres, critical telomere shortening induces replicative senescence and apoptose, whereas maintaining the telomeres above a certain length confers a cell the capacity to divide a large number of times. Telomerase, a ribonucleoprotein that maintains telomere length,[3] is active in more than 85% of cancer cells and this is one of the important features of their malignant character. Consequently, the inhibition of telomerase has been identified as an attractive target for cancer therapy.[4,5] Stabilization of G-quadruplex structures by small molecules prevents telomerase elongation of telomeres by disrupting the interaction between the enzyme and its substrate, the unfolded G-rich single strand. In addition, in view of testing these molecules as potential antitumor drugs, their toxicity toward healthy cells has to be as low as possible and this requires at least a good selectivity for quadruplex over duplex DNA.

Most of the reported compounds able to bind and stabilize G-quadruplex DNA are based on heteroaromatic structures in general associated with positive charges.[5,6] Potent telomerase inhibitors with submicromolar IC_{50} values for in vitro telomerase inhibition assays have been reported.[7] Up to now, the natural compound telomestatin is the most efficient in vitro telomerase inhibitor with an $IC_{50} = 5$ nM.[8]

A significant challenge in the field is to find a molecule with sufficient affinity and specificity that it could ultimately be used against cancer cells. The best reported compounds in this respect include trisubstituted acridines (BRACO19),[7a,e,9] peptide-hemicyanine conjugates,[10] and a square planar nickel(II) complex based on a Schiff-base ligand.[7d] They show a quadruplex over duplex binding constant ratio in the range of 40−50. In addition BRACO19 has been shown to be effective in xenograft tumor models.[9] Another class of potent discriminating agents consists of engineered zinc finger proteins which show a ratio between the quadruplex and duplex DNA equilibrium binding constants ≥300.[11] We report in the present work molecule **1** (Figure 1), a manganese(III) porphyrin combining a central aromatic core and four flexible cationic arms,

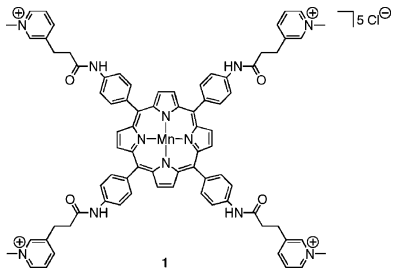

Figure 1. Pentacationic manganese(III) porphyrin **1**.

which is able to discriminate between quadruplex and duplex DNA by 4 orders of magnitude.

The noncovalent equilibrium binding constants of porphyrin **1** with duplex and quadruplex DNA were measured by surface plasmon resonance (SPR) under previously described experimental conditions.[12] SPR grants access to the ratio of the equilibrium binding constants, which is a measure of the G-quadruplex binding selectivity. The chosen quadruplex-forming sequence was the human telomeric sequence, 5′-$AG_3TTAG_3TTAG_3TTAG_3$. The sensorgrams show an obvious selectivity of compound **1** for quadruplex DNA (Figure 2). The binding of **1** to GC and AT duplex DNA gives affinity constants in the 10^4 M^{-1} range, whereas it reaches 10^8 M^{-1} with quadruplex DNA (Table S1). However, because of the very strong affinity of **1** for the bound quadruplex DNA, the interaction is biased by phenomena such as mass transport limitation or steric crowding. These artifacts can be minimized by decreasing the surface loading. Therefore, to obtain a more precise evaluation of the binding constant of **1** with quadruplex DNA, the SPR binding conditions were optimized (lower DNA loading and lower concentrations of analyte) (Figure 3). After a systematic Scatchard analysis, the interaction of **1** with quadruplex DNA was fitted with a nonequivalent 2-site model, the set of values in boldface type (Table 1) corresponding to the site of lower affinity.

The bulky cationic substituents surrounding the aromatic core of **1**, which preclude a close interaction with the double-stranded DNA structures, could be responsible for its poor affinity for duplex DNA. Besides we can hypothesize that the very high affinity for the four-stranded DNA structure originates from a combination of interactions between the G-quartet and the porphyrin core on the one hand, and between the grooves and/or loops and the flexible cationic arms on the other. Having two axial ligands, a manganese(III) porphyrin does not intercalate between G quartets. However, it might be envisaged that stacking interactions could take place with the last tetrad of quadruplex DNA, provided the loss of one axial ligand (and electrostatic interactions between the central Mn^{III} ion and the carbonyl oxygens of G bases) or the fitting of an axial

[#] LCC CNRS.
[§] INSERM IFR 31, Institut Louis Bugnard.
[†] Spanish National Cancer Center.
[‡] INSERM U 531, Institut Louis Bugnard.
[||] Palumed.

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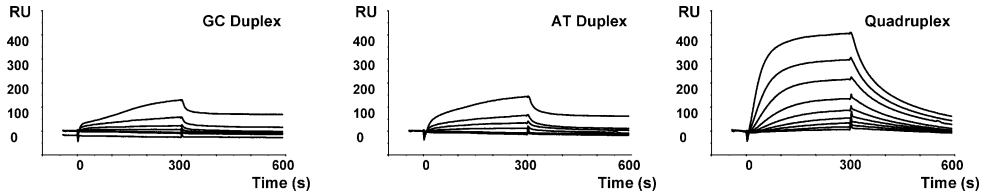

Figure 2. Sensorgrams (resonance units vs time) for the binding of porphyrin **1** on duplex and quadruplex DNA at high chip loading (See Supporting Information).

Table 1. Kinetic and Equilibrium Constants for the Interaction of **1** with Quadruplex DNA[a]

k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	K_a (M^{-1})
$\mathbf{0.5 \times 10^6}$	$\mathbf{5 \times 10^{-4}}$	$\mathbf{1 \times 10^9}$
3.7×10^5	1×10^{-2}	3.7×10^7

[a] Values in bold correspond to a site of higher affinity. Values are from the data of Figure 3.

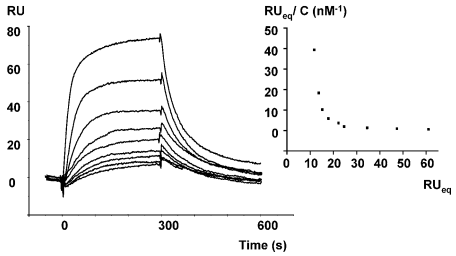

Figure 3. Sensorgrams (resonance units vs time) for the binding of porphyrin **1** (0.3 to 100 nM) on quadruplex DNA at low chip loading, with the corresponding Scatchard plot in the inset.

water ligand within the central ion channel (with possible hydrogen bonds between the protons of H_2O and the carbonyl oxygens). It is noteworthy that cationic tetra(*N*-methylpyridiniumyl) porphyrins have been among the first compounds reported to be able to target quadruplex DNA, either as the free base H_2-TMPyP[13] or its manganese analogue Mn-TMPyP.[14] However, this first generation of porphyrins did not discriminate between quadruplex and duplex DNA[12,14] owing to a strong binding to duplex DNA itself.[12,14,15]

The very high affinity of **1** for quadruplex DNA is associated with a good capacity to inhibit telomerase. The cell-free enzyme-based telomeric repeat amplification protocol (TRAP) assay showed that **1** caused inhibition of telomerase at submicromolar concentration, with $IC_{50} = 580$ nM.

Combining a central aromatic core and four relatively sterically demanding flexible arms carrying cationic end groups, both a very high affinity and an excellent selectivity for G-quadruplex DNA over GC-rich or AT-rich duplex DNA were achieved with a metalloporphyrin. Further studies are necessary to better understand the binding mode of **1** and to test it in vitro on tumor cell lines and possibly observe telomere shortening.

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Supporting Information Available: Synthesis and characterization of **1**, surface plasmon resonance analysis, Scatchard plots corresponding to Figure 2 and TRAP assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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