Published in final edited form as:

Chem Commun (Camb). 2006 December 7; (45): 4685–4687. doi:10.1039/b611731g.

Tetramethylpyridiniumporphyrazines—a new class of G-quadruplex inducing and stabilising ligands†

Diana P. N. Gonçalves, Raphaël Rodriguez, Shankar Balasubramanian, and Jeremy K. M. Sanders*

Abstract

3,4-Tetramethylpyridiniumporphyrazines bind strongly and selectively to human telomeric G-quadruplex DNA, inducing the formation of an antiparallel quadruplex in a process that mimics molecular chaperones.

Telomerase is a reverse transcriptase which is responsible for the synthesis of telomeres, and is upregulated in about 80–85% of human cancer cells.¹ Human telomeric DNA (*Htelo*) is composed of tandem repeats of the TTAGGG sequence with a single stranded 3'-end overhang, where the guanine-rich strand can fold into a four-stranded G-quadruplex structure. Telomeric DNA quadruplexes can form at the chromosome extremities.² The formation and stabilisation of intramolecular telomeric G-quadruplex structures by quadruplex binding molecules can inhibit telomerase activity *in vitro*. Thus, there is considerable interest in ligand-mediated strategies for the interference of telomere maintenance that can induce cell death.^{3–5} As telomerase activity is low in human somatic cells, it is a very promising target for anticancer drug development. Furthermore, it has been demonstrated that G-quadruplex motifs are prevalent throughout the genome.⁶

Cationic porphyrins, in particular tetramethylpyridiniumporphyrin (TMPyP4), are well known for their ability to bind to different types of G-quadruplexes and, in some cases, to facilitate G-quadruplex formation.^{7–11} We now report that tetramethylpyridiniumporphyrazines (TMPyPz) bind strongly to quadruplexes, selectively inducing the antiparallel conformer.

Tetrapyridinoporphyrazines (TPyPz) are non-symmetrical phthalocyanine azo-analogues in which four pyridine moieties substitute the four benzene groups in the macrocycle periphery. They differ from porphyrins by having nitrogen atoms in the *meso* positions linking the individual pyrrole units. The pyridyl groups of the 3,4-TPyPz compounds can readily be methylated to give 3,4-TMPyPz which is water soluble. The syntheses of the 3,4-TPyPz, 3,4-TPyPz zinc(II), 3,4-TMPyPz and 3,4-TMPyPz zinc(II) porphyrazines (Fig. 1) have already been reported,^{12,13} but, to the best of our knowledge, no TMPyPz-G-quadruplex binding properties have been reported.

The UV-Vis titration spectra of both 3,4-TMPyPz and 3,4-TMPyPz zinc(II) porphyrazines with annealed *Htelo* in a 50 mM TRIS-HCl (pH 7.4), 150 mM KCl buffer (Fig. 2) show a red-shift of the respective Soret bands and a decrease in the hypochromicity; these

†Electronic supplementary information (ESI) available: Binding plots and CD data.

© The Royal Society of Chemistry 2006

* University Chemical Laboratory, Lensfield Road, Cambridge, UK CB2 1EW. E-mail: jkms@cam.ac.uk; Fax: +44 (0) 1223 336017; Tel: +44 (0) 1223 336411.

observations are often taken to indicate a specific mode of binding and strong stacking interactions with G-quadruplex DNA.^{14,15}

The UV-Vis titration results were converted into Scatchard plots (*vide in ESI[†]*) and dissociation constants were determined by linear fitting. 3,4-TMPyPz binds strongly to the *Htelo* G-quadruplex, showing a dissociation constant (K_D) of $0.2 \pm 0.02 \mu\text{M}$, whilst 3,4-TMPyPz zinc(II) porphyrine presents weaker binding and a K_D of $1.0 \pm 0.7 \mu\text{M}$ (Table 1). The binding stoichiometry extracted from the Scatchard plot and confirmed by a Job plot (*vide in ESI[†]*) is 1 : 1 for 3,4-TMPyPz; however we find that the *Htelo* G-quadruplex binds four molecules of 3,4-TMPyPz zinc(II). TMPyP4 also shows high binding stoichiometries but, to the best of our knowledge, no structural rationalisation has yet been presented.¹⁶ According to results obtained by Haq *et al.*, using similar experimental conditions, the TMPyP4 porphyrin shows a K_D of $14 \mu\text{M}$,¹⁶ which is almost two orders of magnitude weaker than the observed binding for the porphyrines.

The binding of the two cationic porphyrines to duplex (5-biotin-[GGCATAGTGGCGTGGCGTTAGC]-3 hybridised with its complementary sequence) and *Htelo* G-quadruplex (5-biotin-[GTTA(GGGTTA)₄GG]-3) was also investigated using surface plasmon resonance (SPR). An example of a SPR sensogram is shown in Fig. 3.

The dissociation constants obtained from the SPR experiments with the *Htelo* quadruplex are similar to those obtained by UV-vis spectroscopy (Table 1). The 3,4-TMPyPz shows a K_D of $0.17 \pm 0.02 \mu\text{M}$, while the 3,4-TMPyPz zinc(II) presents a K_D of $0.40 \pm 0.2 \mu\text{M}$. The porphyrines do not show any significant binding affinities against duplex DNA at a concentration up to $5 \mu\text{M}$, which represents a lower limit of 30 for the binding specificity for quadruplex over duplex.[‡] This is a very important improvement in relation to TMPyP4, which does not present any quadruplex/duplex selectivity.

The stronger binding of the 3,4-TMPyPz, relative to TMPyP4, with intramolecular G-quadruplexes, may be attributed to the more extended porphyrine π -system which might completely overlap the four guanines of the G-tetrad.

The presence of K^+ ions induces and stabilises the parallel and antiparallel conformations of the human telomeric G-quadruplex.¹⁷⁻²⁰ CD spectroscopic titrations on $10 \mu\text{M}$ of annealed DNA indicate that both 3,4-TMPyPz and 3,4-TMPyPz zinc(II) porphyrines preferentially induce the formation of the antiparallel G-quadruplex conformation (Fig. 4a); the characteristic antiparallel positive peak at around 295 nm is amplified,¹⁷ while the peak at 265 nm, characteristic of the parallel conformation, is suppressed. The binding of both porphyrines to the G-quadruplex also increases its thermal stability (*vide in ESI[†]*).

This strong binding and selectivity raised the possibility that the porphyrines could induce the formation of a G-quadruplex from non-annealed *Htelo* DNA in a K^+ -free buffer. Indeed, we found that the addition of 3,4-TMPyPz or 3,4-TMPyPz zinc(II) induces the development of a positive peak near 295 nm (antiparallel conformation), at the expense of the 255 nm peak (Fig. 4b) and that this appears to be a fast transition (less than min). A clear isoselliptic point at 272 nm (Fig. 5) suggests a clean transition to the antiparallel quadruplex upon addition of the porphyrines.

It has been demonstrated that 3,4-TMPyPz binds strongly to the G-quadruplex in a 1 : 1 ratio, whilst the 3,4-TMPyPz zinc(II) shows weaker binding and different stoichiometry,

[†]Electronic supplementary information (ESI) available: Binding plots and CD data.

[‡]We were unable to assess any binding experiments for concentrations above $5 \mu\text{M}$, since this ligand shows non-specific binding with duplex DNA at higher concentrations.

probably due to aggregation. As these porphyrazines can be prepared in good yields,^{12,13} bind strongly to G-quadruplex DNA and are able to recognise and induce a particular G-quadruplex conformation, they are promising candidates currently being investigated in quadruplex-related chemical binding studies.

Acknowledgments

The Portuguese Science Foundation (Fundação para a Ciência e a Tecnologia-FCT-Portugal) SFRH/BD/12414/2003 (D. P. N. Gonçalves), the CRUK (R. Rodriguez), are gratefully acknowledged for financial support. S. Balasubramanian is a BBSRC Cancer Development Research Fellow.

Notes and references

1. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW. *Science*. 1994; 266:2011. [PubMed: 7605428]
2. Schaffitzel C, Berger I, Postberg J, Hanes J, Lipps HJ, Plückthun A. *Proc. Natl. Acad. Sci. U. S. A.* 2001; 98:8572. [PubMed: 11438689]
3. Damm K, Hemmann U, Garin-Chesa P, Hael N, Hauffmann I, Priepke H, Niestroj C, Daiber C, Enenkel B, Guilliard B, Lauritsch I, Müller E, Pascolo E, Sauter G, Pantic M, Martens UM, Wenz C, Lingner J, Kraut N, Retting WJ, Schnapp A. *EMBO J.* 2001; 20:6958. [PubMed: 11742973]
4. Teulade-Fichou M-P, Carrasco C, Guittat L, Bailly C, Alberti P, Mergny J-L, David A, Lehn J-M, Wilson WD. *J. Am. Chem. Soc.* 2003; 125:4732. [PubMed: 12696891]
5. Zhou J-L, Lu Y-J, Ou T-M, Zhou J-M, Huang Z-S, Zhu X-F, Du C-J, Bu X-Z, Ma L, Gu L-Q, Li Y-M, Chan AS-C. *J. Med. Chem.* 2005; 48:7315. [PubMed: 16279791]
6. Huppert JL, Balasubramanian S. *Nucleic Acids Res.* 2005; 33:2908. [PubMed: 15914667]
7. Izbicka E, Wheelhouse RT, Raymond E, Davidson KK, Lawrence RA, Sun D, Windle BE, Hurley LH, Von Hoff DD. *Cancer Res.* 1999; 59:639. [PubMed: 9973212]
8. Han FX, Wheelhouse RT, Hurley LH. *J. Am. Chem. Soc.* 1999; 121:3561.
9. Han H, Cliff CL, Hurley LH. *Biochemistry*. 1999; 38:6981. [PubMed: 10353809]
10. Siddiqui-Jain A, Grand CL, Bearss DJ, Hurley LH. *Proc. Natl. Acad. Sci. U. S. A.* 2002; 99:11593. [PubMed: 12195017]
11. Gonçalves DPN, Ladame S, Balasubramanian S, Sanders JKM. *Org. Biomol. Chem.* 2006; 4:3337. [PubMed: 17036123]
12. Martí C, Nonell S, Nicolau M, Torres T. *Photochem. Photobiol.* 2000; 71:53. [PubMed: 10649889]
13. Ramirez C, Antonacci C, Ferreira J, Sheardy RD. *Synth. Commun.* 2004; 34:3373.
14. Pasternack RF, Gibbs EJ, Villafranca JJ. *Biochemistry*. 1983; 22:5409. [PubMed: 6652071]
15. Wheelhouse RT, Sun D, Han H, Han FX, Hurley LH. *J. Am. Chem. Soc.* 1998; 120:3261.
16. Haq I, Trent JO, Chowdhry BZ, Jenkins TC. *J. Am. Chem. Soc.* 1999; 121:1768.
17. Balagurumoorthy P, Brahmachari SK. *J. Biol. Chem.* 1994; 269:21858. [PubMed: 8063830]
18. Li J, Correia JC, Wang L, Trent JO, Chaires JB. *Nucleic Acids Res.* 2005; 33:4649. [PubMed: 16106044]
19. Chang C-C, Chu J-F, Kau F-J, Chiu Y-C, Lou P-J, Chen H-C, Chang T-C. *Anal. Chem.* 2006; 78:2810. [PubMed: 16615797]
20. Ying L, Green JJ, Li H, Klenerman D, Balasubramanian S. *Proc. Natl. Acad. Sci. U. S. A.* 2003; 100:14629. [PubMed: 14645716]
21. Schouten JA, Ladame S, Mason SJ, Cooper MA, Balasubramanian S. *J. Am. Chem. Soc.* 2003; 125:5594. [PubMed: 12733873]

Fig. 1.
Chemical structures of the 3,4-TPyPz, 3,4-TPyPz zinc(II), 3,4-TMPyPz and 3,4-TMPyPz zinc(II) porphyrazines.

Fig. 2. (a) UV-Vis titration spectra of *Htelo* into a solution of TMPyPz; (b) UV-Vis titration spectra of *Htelo* into a solution of TMPyPz zinc(II). Both experiments were performed at 20 °C and in a 50 mM TRIS-HCl (pH 7.4), 150 mM KCl buffer.

Fig. 3.

(a) Sensogram overlay obtained for 6 different concentrations of 3,4-TMPyPz (313, 156, 78, 39, 19, 9.8 nM, top to bottom) binding to the *Htelo* quadruplex; (b) 3,4-TMPyPz binding curve with G-quadruplex obtained using the BIAeval V3.0.2 software (BIAcore AB, Sweden, 1994). The units of RU are seconds. The SPR experiments were carried out in 50 mM TRIS-HCl pH 7.4, 100 mM KCl using a streptavidin functionalised chip on a BIAcore 3000 SPR biosensor, as described previously.²¹

Fig. 4.

(a) CD spectra of 10 μ M of annealed *Htelo* DNA in a 50 mM TRIS (pH 7.4), 150 mM KCl buffer (black), in the presence of 12 equiv. of 3,4-TMPyPz (red) and of 12 equiv. of 3,4-TMPyPz zinc(II) (blue), at 20 °C. (b) CD spectra of 10 μ M of non-annealed *Htelo* DNA in a 50 mM TRIS-HCl (pH 7.4) buffer (black), in the presence of 9 equiv. of 3,4-TMPyPz (red) and of 9 equiv. of 3,4-TMPyPz zinc(II) (blue), at 20 °C.

Fig. 5. CD spectra of 10 μM of non-annealed *Htelo* in a K^+ -free buffer (black), in the presence of 1.2 equiv. (red), 5 equiv. (green) and 9 equiv. (blue) of the 3,4-TMPyPz zinc(II) porphyrazine.

Table 1Dissociation constants (K_D) of 3,4-TMPyPz and 3,4-TMPyPz zinc(II) to quadruplex DNA

Porphyrazines	K_D <i>Htelo</i> /μM	
	UV-Vis	SPR
3,4-TMPyPz	0.20 ± 0.02	0.17 ± 0.02
3,4-TMPyPz Zn	1.0 ± 0.7	0.40 ± 0.2