Cationic Porphyrins as Telomerase Inhibitors: the Interaction of Tetra-(*N*-methyl-4-pyridyl)porphine with Quadruplex DNA

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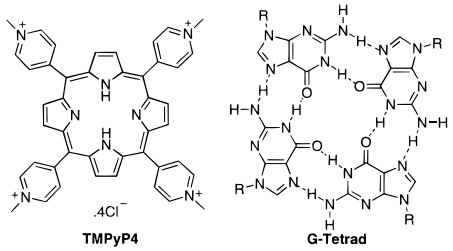
Telomerase presents a potentially selective target for the design of new antitumor drugs. The structure of the telomerase protein remains elusive (although recently shown to be related to other reverse transcriptases[1]), and in consequence, the design of telomerase inhibitors has hitherto been restricted to antisense strategies directed toward binding or cleaving the template sequence of the telomerase RNA[2] or established strategies for inhibiting reverse transcriptases.[3] Several unique nucleic acid structures are associated with the telomerase reaction cycle,[4,5] and a notable achievement has been the targeting of quadruplex DNA and its validation as a receptor for the structure-based design of nonnucleotide telomerase inhibitors.[6] We supposed that 5,10,-15,20-tetra-(*N*-methyl-4-pyridyl)porphine (TMPyP4) was of appropriate size to stack with the G-tetrads that stabilize quadruplex DNA. Herein we report how this porphyrin interacts with human telomeric quadruplexes, stabilizes quadruplex DNA to thermal denaturation, and inhibits human telomerase in a cell-free system.



TMPyP4 G-Tetrad

The interaction of TMPyP4 with quadruplexes based on human telomeric sequences was investigated by a variety of techniques. In a series of one-dimensional high-field NMR experiments, TMPyP4 was titrated into solutions of parallel-stranded quadruplexes[7] formed by the sequences d(TTAGGG), d(TTAGGGTT), and d(TTAGGGTTA). Line broadening and chemical shift changes in the DNA resonances indicated that TMPyP4 was associated with the TTAG portion of the sequences. However, the resultant broad resonances of both the porphyrin and DNA protons rendered two-dimensional experiments uninformative. UV titration experiments with the intramolecular quadruplex[8] d($AG_3[T_2AG_3]_3$) showed sharp isosbestic points, hypochromicity, and a marked red-shift. These data indicated a discrete mode of binding, strong stacking interactions, and a stoichiometry of two porphyrins per quadruplex. The latter was confirmed by a Job plot.[9] The UV and NMR data together strongly suggest that TMPyP4 binds to quadruplex DNA by stacking externally on the G-tetrads rather than by intercalating between them.

The inherent photosensitizing activity of porphyrins was used to probe the binding site of TMPyP4 on quadruplex DNA. A single-stranded sequence bearing four human telomeric repeats was treated with TMPyP4 and then exposed to light. The cleavage patterns are shown in Figure 1A. In the absence of potassium ions (lanes 1−4), uniform cleavage at purines was observed. However, under conditions that promote quadruplex formation (100 mM KCl, lanes 5−8), a clear selectivity for cleavage at G1, G6, G7, and G12 was observed. This could be related to the secondary structure of the quadruplex: whatever the folding topology of the sequence, these four guanines are necessarily members of the same tetrad (an example fold is shown in Figure 1B). Thus, these data implied that the porphyrin was stacked externally on the G1, G6, G7, and G12 tetrad. This result was consistent with the UV and NMR results, although the different sequences and conditions employed resulted in different stoichiometries.

The ability of potassium ions and quadruplex-interactive compounds to inhibit telomerase has been ascribed to stabilization of DNA quadruplexes.[5,6] The potassium-dependent block to DNA polymerase has been shown to be a selective and sensitive indicator of the formation of intramolecular quadruplexes in a DNA template,[10] and this assay has been adapted to demonstrate the stabilization of quadruplex by small molecules (Figure 2). An 18-base primer, complementary to the 5′-end of a single-stranded DNA template, which included four telomeric repeats, was extended using Taq DNA polymerase. In the absence of K^+ or Na^+ (Figure 2A, lane 1), there was only a weak stop at the site corresponding to the start of the putative quadruplex, but on addition of TMPyP4, these bands became more intense, with increasing TMPyP4 concentration reaching a plateau at 0.3 μM (see lanes 2−10).

The stabilization of quadruplex by TMPyP4 was further demonstrated by examining the temperature dependence of the block to Taq DNA polymerase. The pause may be relieved by thermal denaturation of the quadruplex; thus, the intensity of bands at the pause site decreased with increasing temperature, with a significant loss of intensity by 55 °C (Figure 2B, lanes 1−5). In the presence of TMPyP4, however, the intense bands at the pause site persisted up to 65 °C (Figure 2B, lanes 6−10). Thus, the binding of TMPyP4 to quadruplex exaggerated the block posed to Taq DNA polymerase and increased the melting temperature of the quadruplex.

Finally, we have found TMPyP4 to be a potent inhibitor of human telomerase. Figure 3A shows the concentration-dependent effect of TMPyP4 on the ladder produced by telomerase extension of an 18-base primer in a quantitative, cell-free primer-extension assay using [α-^{32}P]dGTP.[11] Quantitation of the bands in each ladder allowed calculation of the telomerase activity relative to the control, and the IC_{50} was determined from the graph (Figure 3B). TMPyP4 showed an IC_{50} of 6.5 ± 1.4 μM. The pattern of

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(1) Lingner, J.; Hughes, T. R.; Shevchenko, A.; Mann, M.; Lundblad, V.; Cech, T. R. *Science* **1997**, *276*, 561−567.

(2) (a) Feng, J.; Funk, W. D.; Wang, S. S.; Weinrich, S. L.; Avillon, A. A.; Chiu, C. P.; Adams, R. R.; Chang, E.; Allsop, R. C.; Yu, J. *Science* **1995**, *269*, 1236−1241. (b) Norton, J. C.; Piatyszek, M. A.; Wright, W. E.; Shay, J. W.; Corey, D. R. *Nat. Biotechnol.* **1996**, *14*, 615−619. (c) Mata, J. E.; Joshi, S. S.; Palen, B.; Pirruccello, S. J.; Jackson, J. D.; Elias, N.; Paige, T. J.; Medlin K. L.; Iveson P. L. *Toxicol. Appl. Pharmacol.* **1997**, *144*, 189−197.

(3) (a) Strahl. C.; Blackburn, E. H. *Nucleic Acids Res.* **1994**, *22*, 893−900. (b) Fletcher, T. M.; Salazar, M.; Chen, S.-F. *Biochemistry* **1996**, *35*, 15611−15617.

(4) (a) Blackburn, E. H. *Nature* **1991**, *350*, 569−573. (b) Salazar, M.; Thompson, B. D.; Kerwin, S. M.; Hurley, L. H. *Biochemistry* **1996**, *35*, 16110−16115.

(5) Zahler, A. M.; Williamson, J. R.; Cech, T. R.; Prescott, D. M. *Nature* **1991**, *350*, 718−720.

(6) Sun, D.; Thompson, B.; Cathers, B. E.; Salazar, M.; Kerwin, S. M.; Trent, J. O.; Jenkins, T. C.; Neidle, S.; Hurley, L. H. *J. Med. Chem.* **1997**, *40*, 2113−2116.

(7) Wang, Y.; Patel, D. J. *Biochemistry* **1992**, *32*, 8112−8119.

(8) Wang, Y.; Patel, D. J. *Structure* **1993**, *1*, 263−282.

(9) Likussar, W.; Blotz, D. F. *Anal. Chem.* **1971**, *43*, 1265−1272.

(10) Weitzmann, N. M.; Woodford, K.; Usdin, K. *J. Biol. Chem.* **1996**, *34*, 20958−20964.

(11) Sun, D.; Hurley, L. H.; Von Hoff, D. D. Submitted for publication.

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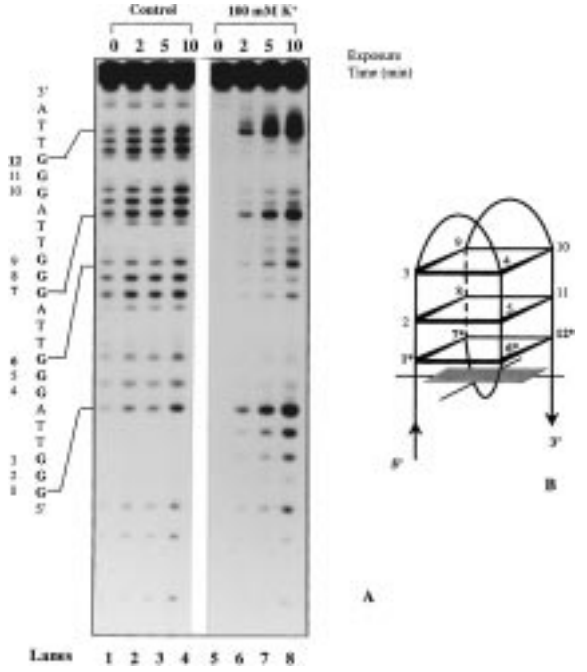

Figure 1. TMPyP4-induced photocleavage of single-stranded DNA bearing a 5′-d(TTAGGG)$_4$-3′ sequence. (A) Gel showing nonspecific cleavage at purines in the absence of potassium (lanes 1−4) and selective cleavage at G1, G6, G7, and G12 under conditions that favor quadruplex formation (lanes 5−8). (B) An example of folding topology for the quadruplex showing that the four most cleaved sites (*) are all on the same tetrad and the site at which the porphyrin was stacked.

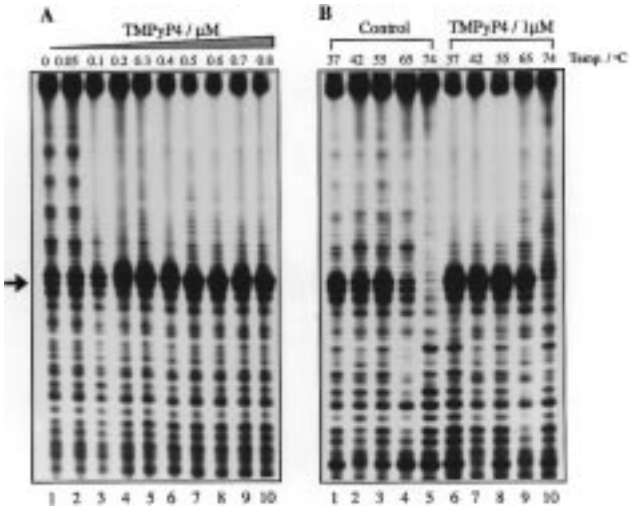

Figure 2. DNA synthesis arrest by intramolecular quadruplex formation in a single-stranded template bearing four telomeric repeats. (A) Effect of increasing the concentration of TMPyP4. (B) Effect of increasing temperature in the absence and presence of TMPyP4. The arrow marks the 5′-end of the telomeric sequence.

ladders formed by telomerase extension of the 18-base primer in the presence of TMPyP4 was different from that seen in the control reaction. In the presence of TMPyP4 (at all but the highest concentration used), the amounts of the first and second extension products were little affected, but a significant reduction in the formation of products corresponding to more than four extensions was seen (see Figure 3A). Since a quadruplex structure cannot be formed until after at least two rounds of extension, this result supports the contention that TMPyP4 interacts mainly with quadruplex structures formed during the telomerase reaction. Previously, we observed that an anthraquinone inhibited telomerase by targeting G-quadruplex structures generated during the telomerase reaction and produced a change in the pattern of ladder formation that is very similar to that observed in the presence of TMPyP4.[6] A wide variety of analogues of TMPyP4 has been evaluated in this assay, and a rough SAR for these analogues has been determined, which is consistent with a mechanism of action involving stacking.[12]

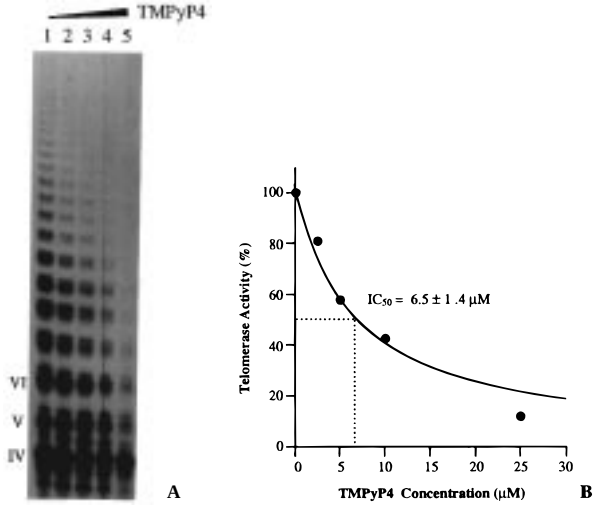

Figure 3. Inhibition of human telomerase by TMPyP4. (A) Effect of TMPyP4 on the ladders produced by telomerase extension of an 18-base primer. Lanes 1−5 contained 0, 2.5, 5, 10, and 25 μM of TMPyP4, respectively; the roman numerals to the left of the gel indicate the number of T_2AG_3 repeats. (B) Graphical determination of the IC_{50}.

In summary, we have shown that TMPyP4 binds strongly to DNA quadruplexes relevant to the functioning of telomerase by stacking on the G-tetrads at the core of the quadruplex. This result is consistent with other work using extended parallel quadruplex structures.[13] The binding results in stabilization of the quadruplex, a phenomenon that has previously been linked to inhibition of telomerase.[5,6] Accordingly, the lead porphyrin TMPyP4 was found to be an effective inhibitor of human telomerase in HeLa cell extract.

The validity of telomerase as a target for chemotherapy has recently been questioned. In mice deficient in the gene for telomerase RNA, it was found that the absence of telomerase activity and consequent shortening of telomeres did not reduce the tumorigenicity of murine cells.[14] However, we have found that cellular effects following administration of TMPyP4 may not be related only to telomerase inhibition but also to disruption of other G-quadruplex structures, leading to chromosomal instability and cell senescence (E. Izbicka et al., unpublished results).

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Supporting Information Available: Spectra of UV and NMR titration experiments; UV job plot; experimental details of the polymerase arrest, photocleavage, and telomerase assays including DNA sequence listings; graphs of polymerase arrest data in Figure 2 (9 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(12) Wheelhouse, R. T.; Sun, D.; Hurley, L. H. *Proc. Am. Assoc. Cancer Res.* **1997**, *38*, 6637. Unpublished results.
(13) Anatha, A. V.; Sheardy, R. J. *Biophys. J.* **1997**, *72*, A422.
(14) Blasco, M. A.; Lee, H.-W.; Hande, M. P.; Samper, E.; Lansdorp, P. M.; DePinho, R. A.; Greider, C. W. *Cell* **1997**, *91*, 25−34.