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Telomestatin, a Potent Telomerase Inhibitor That Interacts Quite Specifically with the Human Telomeric Intramolecular G-Quadruplex

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Telomestatin is a natural product isolated from *Streptomyces anulatus* 3533-SV4 and has been shown to be a very potent telomerase inhibitor.[1] The structural similarity between telomestatin and a G-tetrad (see Figure 1) suggested to us that the telomerase inhibition might be due to its ability either to facilitate the formation of or trap out preformed G-quadruplex structures, and thereby sequester single-stranded $d[T_2AG_3]_n$ primer molecules required for telomerase activity.[2] Indeed a number of other G-quadruplex-interactive compounds, including the anthraquinones,[3a−d] cationic porphyrins,[3e−h] perylenes,[3i−k] ethidium derivatives,[3l] quinolones,[3m] piperazines,[3n] pentacyclicacridinium salts,[3o] and fluoroquinophenoxazines,[3p] have been shown to inhibit telomerase, most probably by this telomeric primer sequestration mechanism. Significantly, telomestatin appears to be a more potent inhibitor of telomerase (5 nM) than any of the previously described G-quadruplex-interactive molecules.[1] In this communication we provide the first experimental evidence that telomestatin selectively facilitates the formation of or stabilizes intramolecular G-quadruplexes, in particular, that produced from the human telomeric sequence $d[T_2AG_3]_4$.

In the first experiment various concentrations of an oligomer with four repeats of the human telomeric sequence $d[T_2AG_3]_4$ were each incubated with increasing concentrations of telomestatin at 20 °C for 30 min. At the higher concentrations of telomestatin the intensity of a new band with high mobility that corresponds to the intramolecular basket-type G-quadruplex[4] was significantly increased, indicating that telomestatin promotes or stabilizes the formation of the intramolecular G-quadruplex (Figure 2A,B). At DNA concentrations of 0.005 and 0.2 μM, EC_{50} values of 0.03 and 0.53 μM telomestatin were found. In a parallel experiment with the mutated oligonucleotide $d[T_2AGAG]_4$, there was no conversion of the mutated sequence to a G-quadruplex structure by telomestatin (Supporting Information).

To investigate the ability of telomestatin to facilitate or stabilize *intermolecular* G-quadruplex structures, a 29-mer DNA strand containing six consecutive guanines, which has been demonstrated to form the interstrand G-quadruplex,[5] was incubated with increasing concentrations of telomestatin at 20 °C for 30 min. The results show that telomestatin facilitates or stabilizes the formation of a modest amount of an intermolecular G-quadruplex structure that migrates more slowly than single-stranded linear DNA (Supporting Information). A comparison of formation of intra- versus intermolecular G-quadruplex structures shows that telomestatin was much less efficient (>10×) at converting linear DNA into the intermolecular rather than the intramolecular species at 20 μM (Supporting Information).

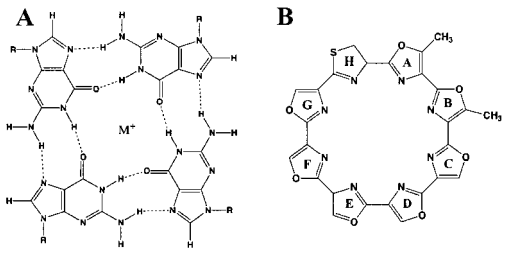

Figure 1. Structures of a G-tetrad (A) and telomestatin (B).

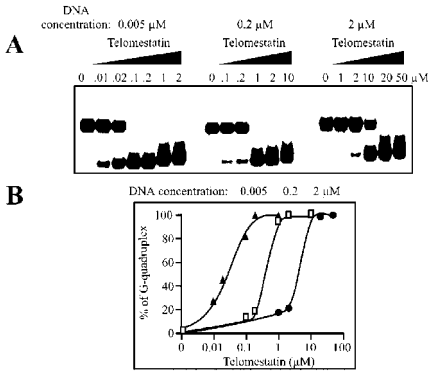

Figure 2. Effects of telomestatin on the formation of intramolecular G-quadruplex. (A) End-labeled oligonucleotide ($d[T_2AG_3]_4$) with three different concentrations was incubated for 30 min with various concentrations of telomestatin in buffer, as previously described.[5] After incubation, samples were mixed with glycerol (final 5%) and run on a 12% native polyacrylamide gel with 1× TBE. (B) G-quadruplex and linear DNA in (A) were quantified using ImageQuant 5.1 software from Molecular Dynamics.

A simulated annealing (SA) docking approach was used to study the binding interactions of telomestatin with the intramolecular antiparallel G-quadruplex structure.[5] Each intramolecular G-quadruplex molecule was found to bind two telomestatin molecules (unpublished results). A 2:1 model for the telomestatin bound in the external stacking mode in an energy minimized complex with the human telomeric basket-type G-quadruplex is shown in Figure 3.[6] Four different telomestatin G-quadruplex structures were modeled. Each of the two possible 1:1 end-stacked complexes and then the 2:1 complex were evaluated together with a 1:1 complex in which the telomestatin was intercalated between two tetrads (Supporting Information). The binding energies were −186.2 (1:1 diagonal loop), −93.6 (1:1 lateral loop), −191.6 (2:1 diagonal and lateral loops), and −63.1 kcal/mol (1:1 intercalated). During the SA docking of the 1:1 diagonal loop complex, the telomestatin−G-quadruplex complex underwent a large conformational rear-

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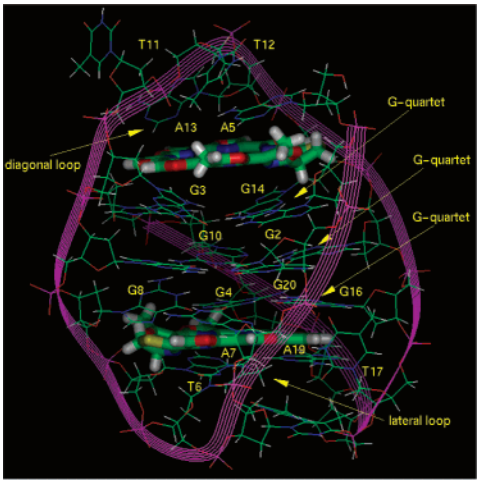

Figure 3. Structure of the most stable docking model from the single-frame snapshot of a last run of MD simulations of telomestatin (stick model in color coded by atom type) bound to the diagonal and lateral loops of the human telomeric repeat $d[AG_3(T_2AG_3)_3]$ G-quadruplex (the phosphate backbone is shown in magenta). PDB 143D.

rangement that led to significant changes in the relative position, orientation, and potential energy of both the telomestatin and the G-quadruplex−DNA complex.[6] In the final minimized structure, the two methyl-substituted oxazole rings (A and B) of telomestatin (Figure 1B) are oriented toward one of the guanine bases (G2 in Figure 3) and positioned within a distance of 2.5−4.0 Å. The methyl oxazole ring (A) attached to the thiazole ring (H) showed strong favorable steric interactions with the sugar phosphate backbone of G2. In contrast, the thiazole ring retained its initial position relative to the G-quadruplex structure. During the simulations the thiazole ring retained significant interactions with the phosphate backbone and exhibited strong electrostatic interactions with G14. The oxygen acceptor group of the oxazole ring (C) takes part in a strong hydrogen bonding interaction with nitrogen atoms of G10 (N9−G10···O 2.37 Å; N3−G10···O 2.92 Å), and these interactions form a well-defined site for a stable complex structure. This was observed in all the final conformers of the structures generated during SA docking. The oxazole ring (D) adjacent to ring C involved in H-bonding interactions with G10 showed both stacking and electrostatic interactions with G3. In the case of the remaining oxazole rings (E−G), the absence of hydrogen-bonding interactions with A5 and A13 is due to the overriding stabilization of the stacking interactions with the G-tetrad, and the telomestatin oxazole rings are oriented away from A5 and A13 by a distance of 7 Å. The eight centrally positioned ring nitrogens of telomestatin point into the central carbonyl channel of the G-tetrad and were observed to form strong electrostatic interactions with the guanines of this tetrad. Telomestatin-induced rearrangements corresponding to local changes in G-quadruplex structure were observed in the diagonal loop corresponding to the T11 and T12 base regions.[4b]

We have also studied the binding mode of the second telomestatin molecule located in the lateral loop region of the 1:1 telomestatin $d[AG_3(T_2AG_3)_3]$ G-quadruplex using SA docking. The presence of the first telomestatin molecule in the diagonal loop region favored change in the lateral loop binding and sugar−phosphate backbone region; thus only a few translations/rotations were sufficient to dock the second molecule. In the final minimized complex structure, the two methyl-substituted oxazole rings (A and B) of telomestatin are oriented into the minor groove and exhibit steric interactions with the phosphate backbone of G20. The oxazole rings E−G exhibit $\pi-\pi$ stacking interactions and are sandwiched between bases G4 and A19. Analysis of dynamic trajectories reveals that the presence of a second telomestatin molecule further increases the overall binding energy of the 1:1 complex from −186.2 to −191.6 kcal/mol in the 2:1 complex (Supporting Information).

In conclusion, telomestatin is the first natural product shown to be a telomerase inhibitor by virtue of its ability to facilitate the formation of or stabilize G-quadruplex structures. The results do not allow us to differentiate between facilitation and/or stabilization of G-quadruplex structures at this time. Our observation that a G-quadruplex-interactive molecule without significant groove interactions is able to reorient in a G-quadruplex structure points to the importance of core interaction with an asymmetric G-quadruplex structure in producing selective binding. Furthermore, the G-quadruplex interactions of telomestatin are more selective for the intramolecular structure in contrast to other G-quadruplex-interactive agents, such as TMPyP4.

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Supporting Information Available: Figures showing the effects of DNA sequence, telomestatin, and TMPyP4 on the formation of G-quadruplexes and a table of interaction energies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) The structural changes induced by telomestatin were identified from the comparative analysis of dynamic trajectories of complex structures using cluster analysis and RMS difference of the phosphate backbone positions in each cluster. The pattern of RMS difference produced by telomestatin binding in the complex is similar for all four structures employed in the cluster analysis and are in the range of 0.8−1.3 Å. The large difference in RMS deviation (2.1−2.6 Å) was observed in the T_2A loop and the G-tetrad region. The observed difference in the RMS deviation in the intercalation site reveals that the telomestatin induces significant conformational changes during its binding mode into the intercalation site and thereby forms a stable complex model in subsequent runs of SA docking. Similarly, the RMS differences were computed for the 1:1 and 2:1 complex models and are in the range of 0.8−1.1 Å.

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