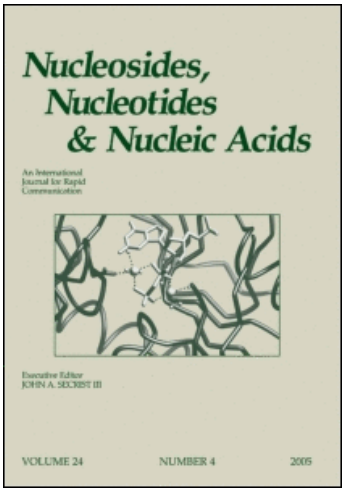

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INTERACTION OF PORPHYRIN WITH G-QUADRUPLEX STRUCTURES

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▫ *Isothermal titration calorimetry (ITC) is a sensitive technique for probing bimolecular processes and can provide direct information about the binding affinity and stoichiometry and the key thermodynamic parameters involved. ITC has been used to investigate the interaction of the ligand H_2TMPyP to the two DNA quadruplexes, $[d(AGGGT)]_4$ and $[d(TGGGGT)]_4$. Analysis of the ITC data reveals that porphyrin/quadruplex binding stoichiometry under saturating conditions is 1:2 for $[d(AGGGT)]_4$ and 2:1 for $[d(TGGGGT)]_4$, respectively.*

Keywords G-Quadruplex, Porphyrin, Drug-DNA Interactions, Isothermal Titration Calorimetry

INTRODUCTION

DNA sequences with multiple guanine stretches can form quadruplex structures that are important for a number of biological processes and disease-related mechanisms. In particular G-quadruplex formed from telomeric sequence repeats may be important for telomere maintenance, and DNA replication and is a potential target for novel anticancer drugs.[1]

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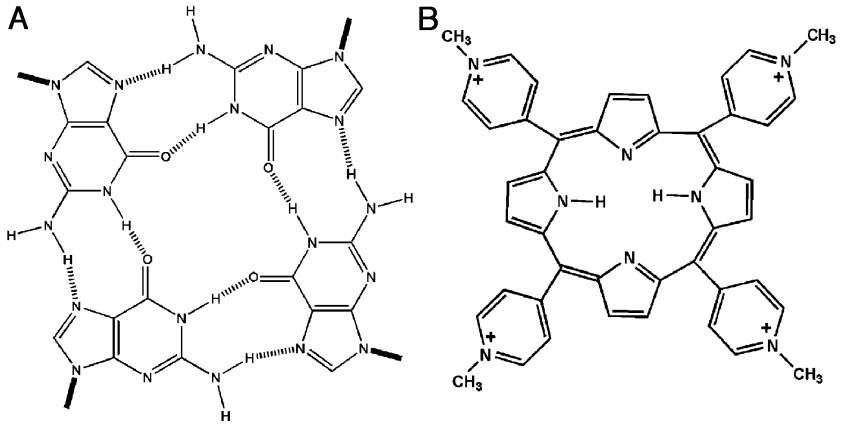

FIGURE 1

Many anticancer, antibiotic, and antiviral drugs exert their primary biological effects by reversibly interacting with quadruplex structures.[2] Therefore, these biomolecules represent a major target in drug development strategies designed to produce next generation therapeutics for diseases such as cancer.[3] Several small molecules have been shown to interact with quadruplex DNA and are considered credible selective inhibitors of telomerase, a potential target for anticancer therapy. The metal-free tetracation porphyrin (H_2TMPyP) was shown to be an effective

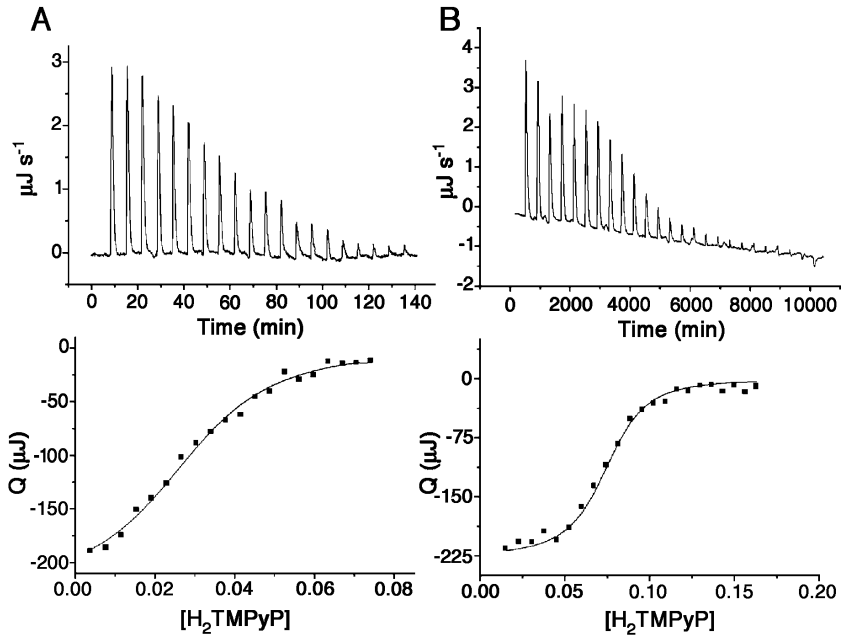

FIGURE 2

TABLE 1 Thermodynamic Parameters for the Porphyrin/Quadruplex Binding at 25°C

	n	K_b ($\times 10^5$ M^{-1})	$\Delta_bH°$ (kJ mol^{-1})	$\Delta_bG°$ (kJ mol^{-1})	$T\,\Delta_bS°$ (kJ mol^{-1})
$[d(TGGGGT)]_4$	2 ± 0.1	2.5 ± 0.2	− 23.9 ± 2.8	− 30.8 ± 0.2	6.9 ± 2.8
$[d(AGGGT)]_4$	0.5 ± 0.03	1.7 ± 0.4	− 40.5 ± 3.3	− 30.0 ± 0.6	− 10.6 ± 3.2

inhibitor of human telomerase by an in vitro assay. Therefore, a molecule such as cationic porphyrin provides an appropriate choice to probe the quadruplex binding, for the following reasons: 1) The dimensions of H_2TMPyP are roughly similar to those of a G-tetrad (Figure 1), and 2) It has constituents that can hydrophobically and electrostatically interact with a G-tetrad.[4]

The aim of this work was to investigate the H_2TMPyP binding proprieties with two DNA quadruplexes, $[d(AGGGT)]_4$ and $[d(TGGGGT)]_4$, by isothermal titration calorimetry (ITC). These systems represent the DNA quadruplex structures formed by truncated human and *Oxytricha* telomeric sequences, respectively.

The quadruplexes were formed by dissolving the oligonucleotides in the appropriate buffer and heating the solution at 90°C for 5 min. The solution was slowly cooled to room temperature, and then equilibrated for 1 day at 4°C. The buffer used was 10 mM NaH_2PO_4, 200 mM NaCl and 0.1 mM EDTA at pH=7.0.

Figure 2 shows the results of calorimetric titration for the interaction of H_2TMPyP with $[d(AGGGT)]_4$ and $[d(TGGGGT)]_4$, respectively. Sample of DNA quadruplex and porphyrin were prepared in the same buffer at pH 7.0. For measuring the parameters of ligand to DNA interaction, the cell (2.0 mL volume) was loaded with a 50–100 μM solution of the quadruplex DNA. The porphyrin concentration in the injection syringe was 1 mM. A titration experiment typically consisted of 20 to 25 injections, each of 5 μL or 10 μL volume and 15 s duration, respectively, with a 400 second interval between injections. The data were corrected for small heat effects observed in control injections of porphyrin into buffer. The correct binding isotherms were fitted using a model that assumes a single set of identical binding sites to determine the stoichiometry n, binding constant K_b, and interaction enthalpy $\Delta_bH°$ parameters. The thermodynamic parameters determined from fit are collected in Table 1.

The binding data obtained by ITC reveal that the saturating porphyrin/quadruplex binding stoichiometries is 1:2 for $[d(AGGGT)]_4$ and 2:1 for $[d(TGGGGT)]_4$, respectively.

Binding to each DNA sequence is characterized by affinities of about 2×10^5 M^{-1} per binding site. These values agree with a 7.7×10^4 M^{-1} value reported for the porphyrin with $[d(TTTTGGGG)]_4$ quadruplex.[4] The porphyrin/quadruplex stoichiometry 2:1 for $[d(TGGGGT)]_4$ is consistent with ligand interaction between the two external pairs of G-tetrads planes. On the other hand, the stoichiometry 1:2 for $[d(AGGGT)]_4$ suggests that one porphyrin molecule bridges two quadruplex molecules.

In summary, the present calorimetric studies indicate that the binding stoichiometry is strongly dependent on quadruplex base composition.

REFERENCES

1. Hurley, L.H.; Wheelhouse, R.T.; Sun, D.; Kerwin, S.M.; Salazar, M.; Fedoroff, O.Y.; Han, F.X.; Han, H.; Izbicka, E.; Von Hoff, D.D. G-quadruplexes as targets for drug design. Pharmacol. Ther. **2000**, *85*, 141–158.
2. Haq, I. Thermodynamics of drug-DNA interactions. Arch. Biochem. Biophys. **2002**, *403*, 1–15.
3. Haq, I.; Ladbury, J. Drug DNA recognition: energetics and implications for design. J. Mol. Recognit. **2000**, *13*, 188–197.
4. Haq, I.; Trent, J.O.; Chowdhry, B.Z.; Jenkins, T.C. Intercalative G-tetraplex stabilization of telomeric DNA by a cationic porphyrin. J. Am. Chem. Soc. **1999**, *121*, 1768–1779.