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Shedding light on the interactions of guanine quadruplexes with tricationic metalloporphyrins

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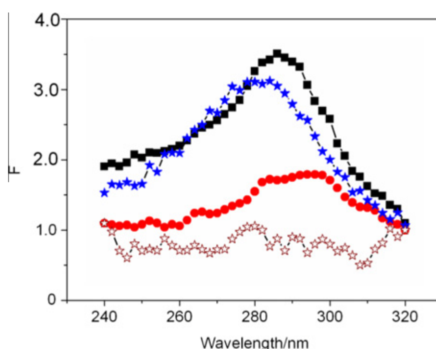
HIGHLIGHTS

- ▶ The interactions of antiparallel G4 with metalloporphyrins were spectrally researched.
- ▶ Three new mechanisms were proposed in the metalloporphyrin-G4 binding nature.
- ▶ SERS and FRET spectral were combined in the metalloporphyrin-G4 binding research.
- ▶ Cu(II) porphyrin was able to stabilize the quadruplex structure to a greater extent.

GRAPHICAL ABSTRACT

Porphyrin metallation allowed the conclusion that the presence of one axial ligand perpendicular to the aromatic plane did not hamper π–π stacking interactions between quadruplex and the aromatic parts of porphyrin on the other face while porphyrin with two axial ligands was unable to undergo such interaction due to geometrical factors. Free base porphyrin and porphyrin without axial ligands are able to stabilize the quadruplex structure to a greater extent than the other metal complexes and thus may be potential anti-cancer drugs.

FRET spectra of 10 μM porphyrins in the presence of $AG_3(T_2AG_3)_3$ in NaCl buffer at a molar ratio of [G4]/[Por] = 10:1.



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ABSTRACT

G-quadruplex DNA presents a potential target for the design and development of novel anticancer drugs. The porphyrin TMPyP4 was early reported to be a suitable motif for G-quadruplex DNA interaction. We inserted various metal ions such as Zn(II), Cu(II), Co(III) in the center of the aromatic core of tricationic TMPyP4-like porphyrin and examined their interactions with an antiparallel G-quadruplex DNA by a combination of spectroscopy and Job plot methods. Porphyrin metallation allowed the conclusion that the presence of one axial ligand perpendicular to the aromatic plane did not hamper π–π stacking interactions between quadruplex and the aromatic parts of porphyrin on the other face while porphyrin with two axial ligands was unable to undergo such interaction due to geometrical factors. Free base porphyrin and porphyrin without axial ligands are able to stabilize the quadruplex structure to a greater extent than the other metal complexes and thus may be potential anti-cancer drugs.

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Introduction

The single-stranded telomere segment at the 3′ ends of chromosomal DNA are able to form a variety of four-stranded structures known as G-quadruplexes (G4) in vitro, based on a G-tetrad structure of four Hoogsteen-paired, coplanar guanines (Fig. S1a in Supplementary materials). Such structures have been implicated in single-stranded telomeric DNA, which is a noncoding DNA found at the ends of linear eukaryotic chromosomes with a general sequence of tandemly repeated segments of between 6 and 8 bases possessing guanine clusters. When the single-stranded 3′ end of a telomere folds into a G4 structure, it is no longer available to act as a primer for the enzyme telomerase [1]. Telomerase is deactivated in most somatic cells at birth, but in 85–90% of human tumors, it acts to extend telomere length, thus rescuing cells from crisis [2]. The potential application of G-quadruplexes to cancer treatment has been the driving force behind the investigation of ligands that stabilize G-quadruplexes and/or induce their formation (G4-ligands) [3–5].

During the last decade an intensive research was focused on the synthesis of G4-ligands and a number of G-quadruplex-binding compounds have been identified [6–8]. Porphyrins with their large aromatic structure and the four meso-positions available for substitution are interesting scaffold for the targeting of G-quadruplex DNA [9–11]. In particular, the free base porphyrin (5, 10, 15, 20-tetrakis(1-methyl-4-pyridyl)-21H, 23H-porphine) (TMPyP4) has been intensively researched as a good G-quadruplex-binding model because of its unique symmetric structure and high positive charge [12–14].

However, although TMPyP4 is widely studied in G-quadruplex-binding research, its practical application in clinic is often restricted by its stable methylpyridinium substituents. Thus, TMPyP4-like porphyrins appending active substituents attract a great deal of interests since they are expected to have not only similar G-quadruplex-binding behaviors to TMPyP4 but also wider practical application through coupling with other bioactive molecules. Recently we have successively investigated the G-quadruplex-binding behaviors of tricationic pyridium porphyrins bearing an anthraquinone chromophore [15]. However, to the best of our knowledge, despite various reports on the interaction of free base porphyrin with G4 structures, studies on the G4-interaction of metal porphyrins, especially for the metal complexes of TMPyP4-like pyridium porphyrins, is limited [16]. We suppose that different metal porphyrins may have different G4-binding behaviors and bring new surprise for us.

The present work investigates the interactions of an unsymmetrical tricationic pyridium porphyrin (1) and its zinc(II) (2), copper(II) (3), cobalt(III) (4) complexes to an antiparallel-stranded guanine quadruplex $AG_3(T_2AG_3)_3$ (see structure in Fig. S1b in Supplementary materials) using absorption titration spectroscopy, surface-enhanced Raman spectroscopy (SERS), fluorescence resonance energy transfer (FRET), induced circular dichroism (ICD) spectroscopy as well as Job plot method; three mechanisms are proposed to describe the binding nature of the porphyrins with the quadruplex structure. The abilities to stabilize the quadruplex structure of these porphyrins were also comparatively studied.

Experimental

Materials

The cationic porphyrins, **1**, **2**, **3** and **4** (see structures in Fig. S2 in Supplementary materials) were formerly synthesized and characterized in our laboratory [17].

The DNA oligonucleotides were purchased from the Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China) in an HPLC-purified form. Single-strand extinction coefficients were calculated from mono- and dinucleotide data by a nearest-neighbor approximation method [18], using extinction coefficients at 260 nm of 228 500 M^{-1} cm^{-1}. The formation of intra- and inter-molecular G-quadruplexes was carried out as follows: the oligonucleotide sample 5′-$AG_3(T_2AG_3)_3$-3′ (AG22), dissolved in a NaCl buffer solution (consisting of 10 mM Tris–HCl, 1 mM Na_2EDTA, and 100 mM NaCl) was heated to 90 °C for 5 min, gently cooled to room temperature, and then incubated at 4 °C overnight. The formation of $AG_3(T_2AG_3)_3$ was affirmed by the appearance of positive peak near 295 nm and negative trough near 260 nm in CD spectra (Fig. S3 in Supplementary materials), which is characteristic of antiparallel-stranded G-quadruplexes [19]. The CD peaks of G4 DNA were almost unaffected by the addition of the porphyrins, indicating that the antiparallel conformation was maintained throughout the spectroscopic measurements under our conditions.

Instruments and methods

UV–Vis spectra were recorded on a Perkin–Elmer-Lambda-850 spectrophotometer. SERS spectra were carried out on an inVia Laser Micro-Raman Spectrometer of Renishaw, with a power of 20 mW at the samples. FRET spectra were recorded on a Perkin–Elmer Ls55 spectrofluorophotometer. CD spectra were recorded on a JASCO-J810 spectrometer.

Absorption spectra were recorded in the 300–700 nm range at room temperature. The titration was terminated when the wavelength and intensity of the absorption band for porphyrins did not change any more upon three successive additions of G-quadruplexes. To ensure that the porphyrin/G4 DNA system was at equilibrium, an absorbance titration was performed by collecting two spectra after each DNA addition: the first spectrum was collected immediately after adding DNA, and the second was collected 10 min after adding DNA. No difference was observed for the red shift or hypochromicity of the porphyrins' Soret band (data not shown) with and without the wait time, demonstrating that the system reached equilibrium during the 2–3 min between DNA addition, mixing, and spectral collection.

In the Scatchard equation, $r/C_f = K(n - r)$, where r is the number of moles of porphyrin bound to 1 mol of G-quadruplex (C_b/C_{DNA}), n is the number of equivalent binding sites, and K is the affinities of ligands for those sites [20]. The concentrations of free porphyrin (C_f) and bound porphyrin (C_b) are calculated using $C_f = C(1 - \alpha)$ and $C_b = C - C_f$, respectively, where C is the total porphyrin concentration (5 μM). The fraction of bound porphyrin (α) was calculated using the equation, $\alpha = (A_f - A)/(A_f - A_b)$ [14], where A_f and A_b are the absorbance of the free and fully bound porphyrin at the Soret maximum of porphyrin, respectively, and A is the absorbance at any given point during the titration. The percent hypochromicity of the Soret band of porphyrin can be calculated using the equation $\Delta H = [(\varepsilon_f - \varepsilon_b)/\varepsilon_f] \times 100\%$, where $\varepsilon_b = A_b/C_b$ [14,19].

For SERS experiment, Ag colloids were prepared by reducing $AgNO_3$ with EDTA according to the reported method [21]. The Ag colloid/porphyrin (or Ag colloid/G4) SERS-active systems were prepared by mixing equal volume of the porphyrins (or G4) solution with the Ag colloid in Tris buffer to obtain the desired porphyrin or G4 concentrations. In the porphyrin/G4 complex experiments, the solution of G4 was mixed with the porphyrin solution at a G4/porphyrin ratio of 30:1, then an equal volume of the mixed solution was fully mixed with the Ag colloid, and the spectrum was immediately measured at room temperature. The final concentrations of porphyrins and G4 in all of the SERS-active systems were 5 and 150 μM, respectively.

As to the FRET experiment, excitation wavelength was scanned between 240 and 320 nm with selected concentrations of G4 DNA,

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