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Spectroscopic investigation on the interaction of copper porphyrazines and phthalocyanine with human telomeric G-quadruplex DNA

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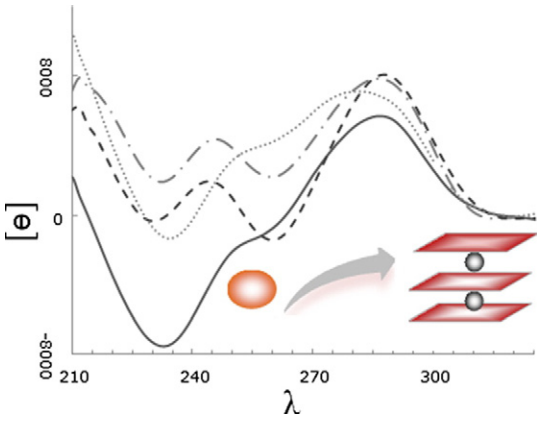
HIGHLIGHTS

- Interaction of Cu(PcTs), [Cu(2,3-tmtppa)]$^{4+}$ and [Cu(3,4-tmtppa)]$^{4+}$ with telomeric G-quadruplex DNA is investigated.
- Interaction of the porphyrazines is stronger than the phthalocyanine.
- K^+ form of G_4 converges to the Na^+ form after binding to the porphyrazines.
- Porphyrazines could be suitable candidates for investigations about inhibition of telomerase.

GRAPHICAL ABSTRACT



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ABSTRACT

The G-quadruplex DNA is a novel target for anticancer drug discovery and many scientific groups are investigating interaction of small molecules with G-quadruplex DNA to discover therapeutic agents for cancer. Here, interaction of a phthalocyanine (Cu(PcTs)) and two tetrapyridinoporphyrazines ([Cu(2,3-tmtppa)]$^{4+}$ and [Cu(3,4-tmtppa)]$^{4+}$) with Na^+ and K^+ forms of human telomeric G-quadruplex DNA has been investigated by spectroscopic techniques. The results indicated that interaction of the cationic porphyrazines is remarkably stronger than the anionic phthalocyanine and they presumably bind to the G-quadruplex DNA through end-stacking. Fluorescent intercalator displacement assay implied the displacement ability of the complexes with thiazole orange. In addition, circular dichroism spectra of both quadruplex forms converge to the Na^+ isoform after binding to the porphyrazines. In conclusion, the porphyrazines as the complexes that bind to the G-quadruplex DNA, could be suitable candidates for further investigations about inhibition of telomerase enzyme.

1. Introduction

DNA is often regarded as a duplex molecule, however duplex DNA is not the only structure of DNA; single-stranded guanosine-rich oligodeoxyribonucleotides (GROs) can form quadruplex structures through Hoogsteen hydrogen bonding [1]. G-quadruplexes are built from the stacking of successive G–G–G–G tetrads (G-tetrads) and stabilized by bound monovalent Na^+ and K^+ cations [1–3]. G-quadruplexes are found in biologically important regions such as several important proto-ancogene promoter regions, immunoglobulin switch regions and telomeres [4–6]. Telomere is the only region that guanine-rich DNA is single strand. The guanine-rich 3′-overhangs of telomeres equilibrate between single-stranded and G-quadruplex folds. G-quadruplex structure in telomere causes to inhibit the activity of telomerase.

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Telomerase as a DNA polymerase adds the telomeric repeated sequences onto the ends of telomere. This enzyme is active in about 85–90% of human cancer cells, while most of the somatic cells lack detectable levels of this enzyme [7–9]. Therefore, inhibition of telomerase is a promising strategy for anti-cancer drug development. It was reported that formation and stabilization of telomeric G-quadruplex structures by quadruplex binding molecules, inhibit telomerase activity *in vitro* [10]. Accordingly, interaction of selective ligands with G-quadruplex structure has been recently considered as an approach to design and develop new anticancer drugs [10–13]. Interaction of porphyrins and their derivatives with G-rich telomere sequence and inhibition of human telomerase been reported in the several published studies [14–18]. Phthalocyanines are tetrapyrrolic macrocycles, which unlike the porphyrins have nitrogen atoms linking the individual pyrrole units instead of methine bridges. The aza analogs of the phthalocyanines (azaPcs) such as tetramethylmetalloporphyrazines are heterocyclic Pc analogs, which have been studied over the past decades. These complexes are phthalocyanines in which the outer aromatic benzene rings are replaced with electron-withdrawing pyridine rings. The N,N,N,N-tetramethylatedquaternized forms of tetrapyridinoporphyrazines (tmtppa) are tetra-positively charged and hence water soluble. According to the literature reports, these complexes do not aggregate in aqueous solutions. This property makes them ideal candidates for use in medical applications [19]. There has been considerable research into the binding of porphyrines to double helix and quadruplex DNA, but to our best knowledge, only a few reports have been published on the binding of porphyrazines to DNA. Interaction of metal tetrapyridinoporphyrazine complexes with double helix DNA has previously reported by our group [19–21]. There is also a report on the interaction of metal-free porphyrazines and zinc(II) porphyrazines with Htelo G-quadruplex [22]. Here, an investigation on the interaction of two cationic tetrapyridinoporphyrazines including $[Cu(2,3\text{-tmtppa})]^{4+}$ and $[Cu(3,4\text{-tmtppa})]^{4+}$ complexes and an anionic phthalocyanine (Cu(PcTs)) with human telomere G-quadruplex DNA has been carried out using absorption and fluorescence spectroscopy as well as circular dichroism technique. As is well known, G-quadruplex DNA adopts different G-quadruplex conformations in presence of Na^+ and K^+ ions. Hybrid type intramolecular G-quadruplexes appear to be the predominant conformation of human telomeric sequences in K^+ solution, while the NMR structure in sodium solution is an anti-parallel basket type quadruplex [23,24]. Here, the interaction between both structures of telomeric G-rich quadruplex DNA formed in presence of K^+ and Na^+ ions with the copper porphyrazines and phthalocyanine has been investigated by the spectroscopic techniques.

2. Material and methods

2.1. Material

Cu (PcTs) was purchased from Merck. Cu(2,3-tmtppa) and Cu(3,4-tmtppa) were prepared and purified by the method described previously [19]. The G-rich HPLC purified oligonucleotide, d(AGGGTTAGGGTTAGGGTTAGGG), was purchased from Takapouzist (Iran). All other material was purchased from Sigma.

2.2. Methods

2.2.1. Preparation of G-quadruplex

The oligonucleotide Tel22, d(AGGGTTAGGGTTAGGGTTAGGG), was dissolved in 1 mM phosphate buffer containing KCl or NaCl and 0.1 mM EDTA, pH 7. The concentration of KCl and NaCl in the buffer was 5 mM in the absorption and fluorescence measurements and 150 mM in the circular dichroism experiments. After dissolving the lyophilized oligonucleotide samples in desired buffer, they were heated to 90 °C for 5 min, gently cooled to room temperature, and then incubated at 4 °C overnight.

2.2.2. UV–vis absorption spectroscopy

UV–vis spectroscopic titrations were performed using Pharmacia Ultraspec 4000 at 25 °C using a 1 cm path length quartz cuvette. Solution of the complexes was freshly prepared before performing the experiment and their concentrations were determined using molar extinction coefficient values of 1.67×10^5, 1.24×10^5 and $1.29 \times 10^4\ M^{-1}\ cm^{-1}$ respectively for Cu(2,3-tmtppa), Cu(3,4-tmtppa) and Cu(PcTs) [19,25]. The titration experiments were done by addition of stock solution of G-quadruplex DNA (60 μM) to the cuvette containing the complex solution; the complex was dissolved in the same buffer used for preparation of the oligonucleotide. The titration was finished when intensity of the complex Q band was fixed. UV–vis absorption spectra were analyzed for determination of binding constant, percentage of hypochromicity or hyperchromicity, binding stoichiometry and red shift.

The binding constant (K_b) values were calculated by the following equation [26,27]:

$$\frac{[DNA]}{|\varepsilon_a - \varepsilon_f|} = \frac{[DNA]}{|\varepsilon_b - \varepsilon_f|} + \frac{1}{K_b(|\varepsilon_b - \varepsilon_f|)} \quad (1)$$

where [DNA] is the concentration of DNA in base pairs, ε_a, ε_f and ε_b correspond to the $A_{observed}$/[the complex], the extinction coefficient of the free complex and that of the complex in the fully bound form, respectively, and K_b is the intrinsic binding constant. A plot of [DNA]/($|\varepsilon_a - \varepsilon_f|$) versus [DNA] will have a slope of $1/(|\varepsilon_b - \varepsilon_f|)$ and an intercept equal to $1/(K_b(|\varepsilon_b - \varepsilon_f|))$. K_b is then given by the ratio of the slope to the intercept.

Percentage of hypochromicity was determined using the following equation [28]:

$$\text{Hypochromicity } (\%) = \frac{|\varepsilon_f - \varepsilon_{fb}|}{\varepsilon_f} \times 100. \quad (2)$$

In addition, the difference spectra were used to generate the Job plots for determination of the stoichiometry of interactions between the complexes and the quadruplex DNA [29].

2.2.3. Fluorescence spectroscopy

Fluorescent spectra were taken on a Perkin-Elmer (Varian, Australia) equipped with a Xenon lamp pulsed at 80 Hz. To record the emission spectra of thiazole orange, the experiments were performed at an excitation wavelength of 501 nm. The measurements were done using a 1 cm quartz cell and a bandwidth of 5 nm for both excitation and emission monochromators. For fluorescence quenching experiments, the molar ratio of 2:1 thiazole orange (0.5 μM) to the oligonucleotide (0.25 μM) was prepared and titrated with the complexes until the emission spectra were fixed. To determine the quenching constant, the modified form of Stern–Volmer equation (Eq. (3)) was applied [30,31].

$$\frac{F_0}{F} = (1 + K_{SV}[Q])\exp(V[Q]) \quad (3)$$

$$K_{SV} = K_q \times \tau_0 \quad (4)$$

where F_0 and F are the fluorescence intensity in the absence and presence of the quencher respectively. K_{SV} is the dynamic quenching constant, [Q] is the molar concentration of the quencher and V is the static quenching constant. The Stern–Volmer constant is the product of the rate constant for quenching (k_q) and life time of the luminescent in the absence of quencher (τ_0) (Eq. (4)).

2.2.4. Circular dichroism measurements

Circular dichroism (CD) measurements were performed on an Aviv Model 215 Circular dichroism Spectrometer (Lakewood, NJ) at 25 °C using quartz cell with a path length of 1 mm. Concentration of the oligonucleotide was 10 μM. The scans were recorded using a bandwidth of 1 nm. The molar ellipticity was determined as $[\theta] = (\theta \times 100MRW)/cl$,

where c is the oligonucleotide concentration in mg/mL, l is the light path length in centimeters, MRW is mean residue weight of Tel22, and θ is the measured ellipticity in degrees at wavelength [29].

3. Results and discussion

3.1. Absorption spectroscopy

UV–vis spectroscopy is an informative technique for finding the extent of drug interaction with DNA and drug–DNA binding mode. Figs. 1 and 2 show the change in the absorption spectra of the porphyrazines after titration with the oligonucleotide in presence of Na^+ and K^+ ions respectively. The absorption spectra of the phthalocyanine in presence of Na^+ and K^+ ions are respectively shown in Fig. 1S and Fig. 2S (supplementary data). Obviously, the electronic absorption spectral features of Cu(2,3-tmtppa) and Cu(3,4-tmtppa) consist of Q and B-bands. The B-band position locates in the UV–visible region at about 340 nm and the Q-band of Cu(2,3-tmtppa), Cu(3,4-tmtppa) and Cu(PcTs) appears at 638, 678 and 610 nm respectively. As shown in Figs. 1 and 2, the difference among the spectra in Q-band is more obvious than the B band and therefore all analyses were carried out at the Q bands. Fig. 1A shows the change in the absorption spectra of Cu(2,3-tmtppa) in Na^+ ion. As observed, the intensity of soret band decreases with addition of the G_4 DNA, and then red-shift appears with further increase in the concentration of G_4. Similar changes are observed in the absorption spectrum of Cu(2,3-tmtppa) in K^+ (Fig. 2A). The values of red shift and hypochromicity (%) are presented in Table 1. Obviously, hypochromicity of Cu(2,3-tmtppa) is 62% and 65% in presence of K^+ and Na^+ respectively and the bathochromic shift of both cases is identical to 7 nm indicating no remarkable difference between absorption spectra of Cu(2,3-tmtppa) due to the interaction with Na^+ and K^+ forms of the telomeric G-quadruplex. In the case of Cu(3,4-tmtppa), the other copper porphyrazine, the red shift is 27 nm after binding of the complex to both forms of quadruplex DNA and maximum hypochromicity is 58% and 51% in presence of K^+ and Na^+ forms of G_4 respectively (Figs. 1B, 2B and Table 1). As a result, red shift and hypochromicity are observed upon addition of both forms of telomeric G-quadruplex (Na^+ and K^+ forms) to both copper porphyrazines, but the values of hypochromicity and red shift for Cu(2,3-tmtppa) and Cu(3,4-tmtppa) are not the same. The change in the absorption spectrum of the copper phthalocyanine upon addition of both forms of DNA is observed in Fig. 1S and Fig. 2S (supplementary data). Obviously, neither hyperchromosity nor red shift, changes upon addition of Na^+ and K^+ forms of telomeric G-quadruplex to the phthalocyanine. In other words, unlike the porphyrazine complexes, the spectra of Cu(PcTs) remain unchanged upon addition of the G_4 and therefore no quantitative parameters were obtained from the spectra of this complex.

As is well known, the absorption spectra are the most common means to determine the metal complex binding mode to DNA. In the case of duplex DNA, intercalation of the porphyrin between the base pairs results in about 15 nm red shift and a near 35% hypochromicity of the porphyrin soret band. Outside binding without stacking is demonstrated by small red shift (about 6–8 nm) and hypochromicity (10%) or even hyperchromicity of the soret band. Outside binding with stacking is indicated by variable effects on the soret band [32,33].

Obviously, for titrations of Cu(2,3-tmtppa) with K^+ and Na^+ forms of the quadruplex, the red shift is in the range of outside binding, but the large hypochromicity is in accordance with intercalation. In the case of Cu(3,4-tmtppa) not only the maximum red shift but also the maximum value of hypochromicity falls in the range of intercalation. If the spectra (Figs. 1B and 2B) are shown more accurately, the change in the spectra of Cu(3,4-tmtppa) can be divided into two stages; in the first stage, a large hypochromicity and in the second stage a large red shift with very small hyperchromicity is shown. The changes of both stages are intermediate between those expected for intercalative and outside binding modes. It is important to note, the values of red shift and hypochromicity given above for different binding modes were determined for long pieces of duplex DNA [32,33] and additional data are required to determine binding mode of the complexes to the quadruplex DNA. Asadi et al. [21] previously evaluated the interaction of the two copper porphyrazines with calf thymus DNA as genomic double helix DNA and demonstrated the coexistence of intercalation and outside binding modes for Cu(2,3-tmtppa) and an intercalation binding mode for Cu(3,4-tmtppa). Although it is not possible to discriminate end-stacking from intercalation using absorption spectroscopy, we guess one of the binding modes of the porphyrazines to both forms of the quadruplex is end-stacking. End stacking is an interaction mode that could only occur on quadruplex DNA. Undoubtedly, end stacking is insignificant for long duplex DNA. Probably, the changes of the spectra are related to neither outside binding nor intercalation; it appears that the changes result from the end stacking. Previous studies indicated that end stacking is a predominant interaction for the majority of ligands and G-quadruplex investigated [34–38].

The quantitative data including the binding constant and the stoichiometry of interaction between the porphyrazines and the quadruplex DNA were obtained by analysis of the absorption spectra; the results are listed in Table 1. As noted, Job plot (supplementary data, Fig. 3S) and Eq. (1) were respectively used to determine the number of complexes attached to per molecule of the DNA and binding constant. Obviously, binding stoichiometry of Cu(2,3-tmtppa) and Cu(3,4-tmtppa) to

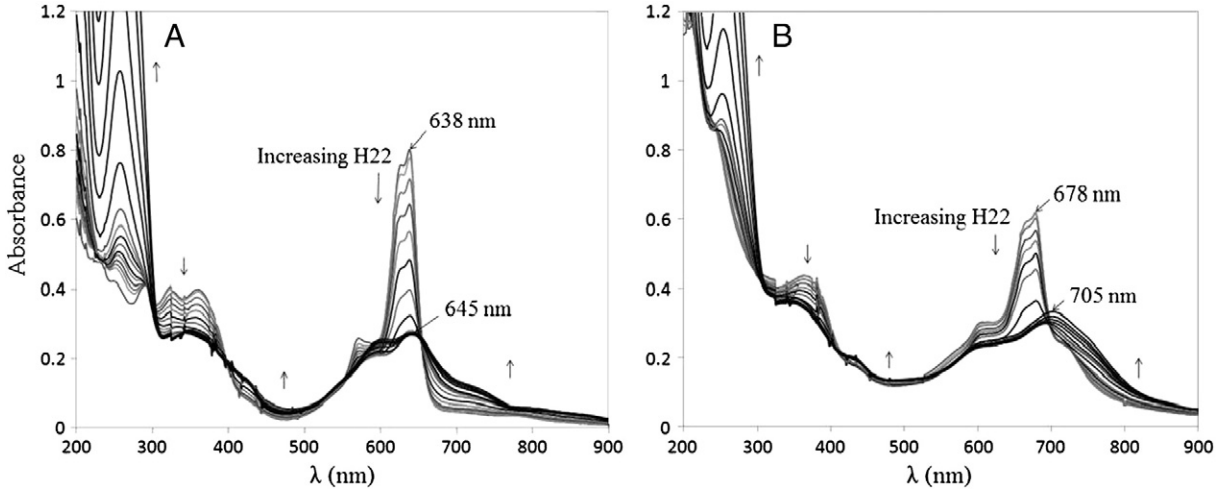


Fig. 1. Absorption spectra of A: 4.8 μM $[Cu(3,4\text{-tmtppa})]^{4+}$ and B: 5.3 μM $[Cu(2,3\text{-tmtppa})]^{4+}$ titrated with H22 in 1 mM phosphate buffer containing 5 mM NaCl and 0.1 mM EDTA, pH 7.0.

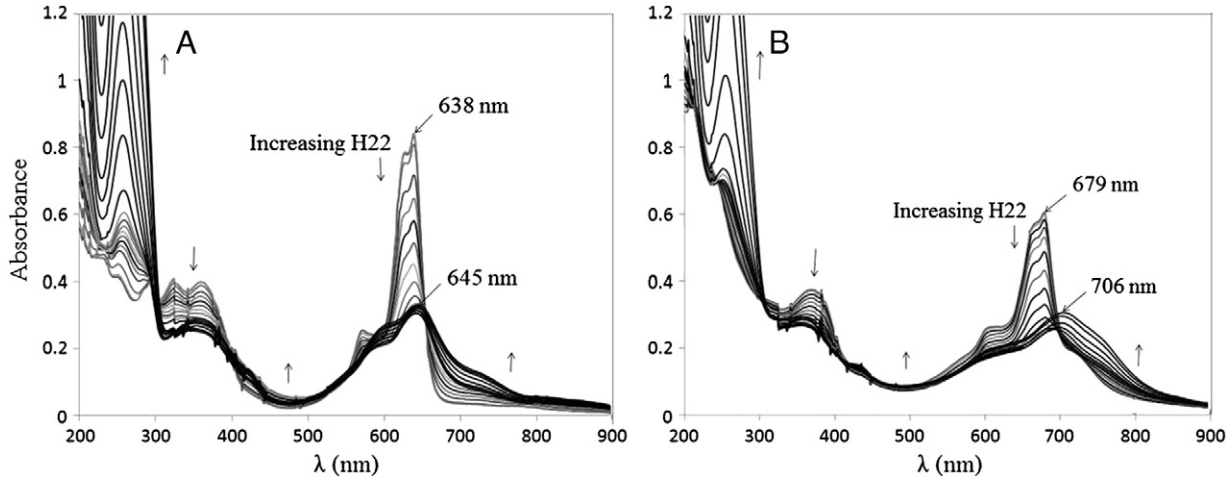


Fig. 2. Absorption spectra of A: 4.8 μM $[Cu(3,4\text{-tmtppa})]^{4+}$ and B: 5.3 μM $[Cu(2,3\text{-tmtppa})]^{4+}$ titrated with H22 in 1 mM phosphate buffer containing 5 mM KCl and 0.1 mM EDTA, pH 7.0.

Na^+ form of the telomeric G-quadruplex is respectively 1:5.9 and 1:5 and these values for the K^+ form are respectively 1:4.5 and 1:5.5 indicating small difference between the number of Cu(2,3-tmtppa) and Cu(3,4-tmtppa) molecules that bind to one molecule of both forms of DNA. The values of equilibrium DNA binding constants, K_b, change in the range of 6.8×10^5 M^{-1} to 15.3×10^5 M^{-1}. The minimum and maximum values of K_b are respectively observed for Cu(2,3-tmtppa) in presence of sodium ion and Cu(3,4-tmtppa) in presence of potassium ion.

3.2. Fluorescent intercalator displacement

Fluorescent intercalator displacement (FID) is a well-known assay based on the loss of fluorescence of a DNA-bound intercalator or minor-groove binder upon displacement by a DNA-binding molecule. Ethidium bromide or thiazole orange (TO) is generally used as DNA-bound intercalator. Preliminary studies have demonstrated that thiazole orange binds to the quadruplex-forming oligonucleotide with high affinity, but ethidium bromide weakly binds to the quadruplex [39,40]. In this investigation, TO was used for fluorescent intercalator displacement assay. The spectra are shown in Fig. 4S (supplementary data). Fluorescence intensity of thiazole orange (1 μM) in presence of 0.5 μM of K^+ and Na^+ forms of G_4-DNA was obtained at maximum wavelength of emission before (F_0) and after (F) titration with the solution of the three complexes. In Fig. 3, F_0/F was plotted against the molar ratio of the complex to DNA. Stern–Volmer plots have been also presented in the supplementary data (Fig. 5S). Positive slope of the plots results from quenching of thiazole orange due to addition of the phthalocyanine and both porphyrazines. The quenching indicates the displacement ability of the complexes with TO. Stern–Volmer plots of the porphyrazines (Fig. 4SA) also exhibit a slight positive deviation from a straight line suggesting that both static quenching and dynamic quenching are involved. In addition, the quenching effect of the two porphyrazines is noticeably higher than the phthalocyanine (Fig. 3) implying that binding of Cu(PcTs) to both forms of the quadruplex is weaker than Cu(2,3-tmtppa) and Cu(3,4-tmtppa). Furthermore, the result indicates that Cu(2,3-tmtppa) is a more effective quencher than Cu(3,4-tmtppa).

Table 1
Binding constant (K_b), binding stoichiometry (n), red-shift and hypochromicity for binding of $[Cu(2,3\text{-tmtppa})]^{4+}$ (cu2,3) and $[Cu(3,4\text{-tmtppa})]^{4+}$ (cu3,4) to H22 in the buffer containing 5 mM KCl (K^+) and 5 mM NaCl (Na^+).

	K_b (M^{-1}) ($\times 10^5$)	n (complex/H22)	Red-shift (nm)	Hypochromicity (%)
Cu2,3 (K^+)	14.27	4.54	7	62.12
Cu2,3 (Na^+)	6.88	5.98	7	65.57
Cu3,4 (K^+)	15.32	5.56	27	58.56
Cu3,4 (Na^+)	12.71	5.00	27	51.35

3.3. Circular dichroism measurements

Structure of the G-rich sequence in presence and absence of the complexes was verified by circular dichroism spectroscopy. Circular dichroism (CD) is a very sensitive technique in detecting changes in structures of polynucleotides and useful for the characterization of G-quadruplex-forming oligonucleotides [41–43]. Circular dichroism spectra of K^+ form and Na^+ form of Tel22 are respectively presented in Figs. 4 and 5. A positive signal at 288 nm and a negative one at 234 nm (Fig. 4, spectrum1) implying Tel22 exhibit typical CD signals assigned to hybrid G-quadruplex in the buffer containing K^+ ion. CD spectrum of Tel22 in presence of Na^+ ion has positive peak at 286 and negative peak near 264 nm indicating basket-type G-quadruplex. The effect of the three complexes on CD spectra of K^+ and Na^+ forms of Tel22 is respectively shown in Figs. 4 and 5, too. Obviously, the location of positive and negative CD signals of K^+ form of Tel22 changes in presence of the two cationic porphyrazines; while in presence of the anionic phthalocyanine, the location of CD bands is almost identical (Fig. 4). The CD spectra of the K^+ form of quadruplex in presence of the two porphyrazines become similar to the spectrum of the Na^+ form (Fig. 5, spectrum 1), indicating similarities in structural characteristics. In

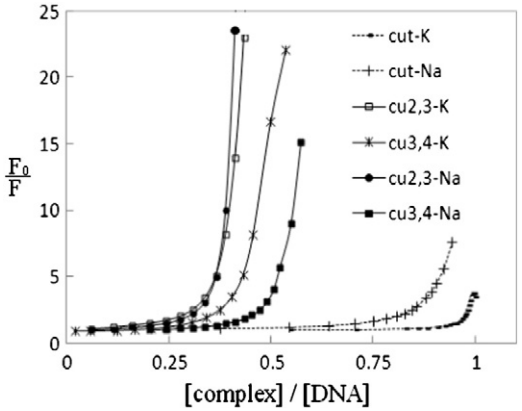


Fig. 3. Fluorescence quenching of TO-H22 ([TO] = 0.5 μM and [H22] = 0.25 μM) by $[Cu(2,3\text{-tmtppa})]^{4+}$ (cu 3,4), $[Cu(3,4\text{-tmtppa})]^{4+}$ (cu 2,3) and the copper(II) phthalocyanine (cut) in 1 mM phosphate buffer containing 0.1 mM EDTA, pH 7.0 and 5 mM NaCl (Na) or 5 mM KCl (K). F_0 and F are emission of TO-H22 in absence and presence of the three complexes.

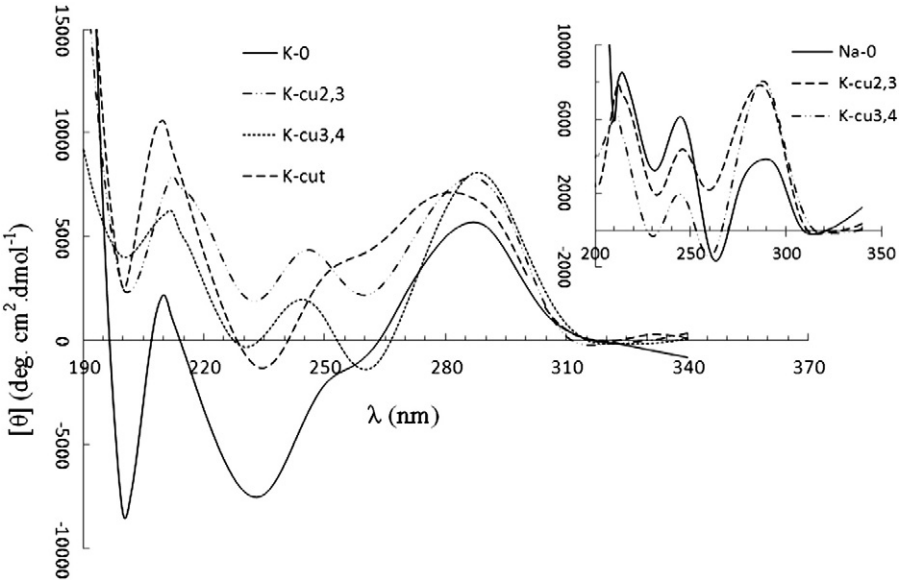


Fig. 4. Circular dichroism spectra of the H22 in absence (——K-0) and presence of $[Cu(2,3\text{-tmtppa})]^{4+}$ (–··–K-cu2,3), $[Cu(3,4\text{-tmtppa})]^{4+}$ (·····K-cu3,4), and the copper(II) phthalocyanine (– – –K-cut) in phosphate buffer 1 mM containing 150 mM KCl and EDTA 0.1 mM, pH 7. (——Na-0) shown in inserted figure is circular dichroism spectrum of the H22 in absence of the complex in phosphate buffer 1 mM containing 150 mM NaCl and EDTA 0.1 mM, pH 7.

order to compare the spectra more directly, the spectrum of Na^+ form of DNA, the K^+ form in presence of Cu(2,3-tmtppa) and the K^+ form in presence of Cu(2,3-tmtppa) are illustrated in inset of Fig. 4. As shown, the CD spectra of K^+ form of DNA in presence of both complexes have positive peak at 286 nm and negative peak near 264 nm. Obviously, these features were observed in the spectrum of Na^+ form of Tel22 in absence of the complexes. This result implies that the hybrid G-quadruplex converges into basket type G-quadruplex upon interaction with the porphyrazines. Certainly, the basket type G-quadruplex formed in presence of K^+ ion and the copper porphyrazines doesn't exactly resemble to that formed in presence of Na^+, because the intensity of their maximum and minimum peaks noticeably differs (Fig. 4, inset). Unlike the porphyrazines, the phthalocyanine affects only the intensity of spectrum and it has no meaningful effect on the position of negative and positive peaks of the K^+ form of DNA.

As noted, the effect of complexes was also evaluated on Na^+ form of Tel22. Fig. 5 presents the spectra of Na^+ form of Tel22 in absence and presence of the complexes. Obviously, neither the porphyrazines nor the phthalocyanine changes the position of the typical CD signals assigned to basket-type G-quadruplex of Tel22 formed in presence of Na^+ ion and no new band appears upon interaction of the complexes with Na^+ form of Tel 22. Obviously, only intensity of the spectrum of Na^+ form of Tel22 changes upon interaction with the complexes (Fig. 5). This result implies that the basket type DNA doesn't convert to the other conformation, although the complexes affect the features of this type of G_4. As previously reported, rigidity of the structure and distance between the chromospheres affect the intensity of CD signal [44]. Accordingly, it seems that rigidity and/or the distance between G-tetrads changes due to the interaction with the complexes.

In summary, the results indicate that, although the Na^+ and K^+ forms of the quadruplex DNA have distinctive CD spectral characteristics, the CD spectra of both quadruplex forms converge to very similar CD spectra, when the quadruplexes interact with the porphyrazines. In fact, binding of the complexes to the G-quadruplex structure induces

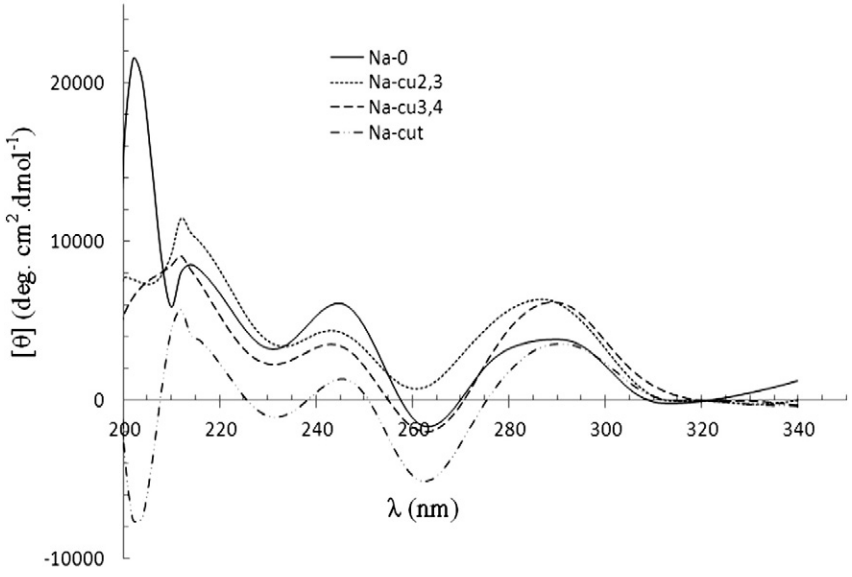


Fig. 5. Circular dichroism spectra of the H22 in absence (——Na-0) and presence of $[Cu(2,3\text{-tmtppa})]^{4+}$ (·····Na-cu2,3), $[Cu(3,4\text{-tmtppa})]^{4+}$ (– – –Na-cu3,4), and the copper(II) phthalocyanine (–··–Na-cut) in phosphate buffer 1 mM containing 150 mM NaCl and EDTA 0.1 mM, pH 7.

a structural change to the quadruplex DNA whose CD spectrum more closely resembles that of the Na^+ isoform.

As previously reported, potassium cations can better stabilize the G-quadruplexes compared with sodium ions [45–47], although binding of both porphyrazines facilitates a conformational switch from the K^+ hybrid form to Na^+ antiparallel form. In the K^+ form, potassium cations fit into the cavity between two guanine tetrads, while in the Na^+ form, sodium cations are placed in the middle of tetrad planes [45–48]. It had been previously reported that, ligands with positively charged center could act as "pseudo" potassium ion and locate above the center of the G-quartets in the region of high negatively-charge density [48], hence, it is reasonable to note that two porphyrazines probably compete with K^+ located between the G-quartets and changed the structure of Tel22 from common hybrid G-quadruplex to antiparallel structure. Presumably, the porphyrazines don't replace with sodium ion because this cation places in the middle of tetrad planes in the quadruplex structure. This can explain why Na^+ form of the quadruplex don't change essentially upon binding to the complexes.

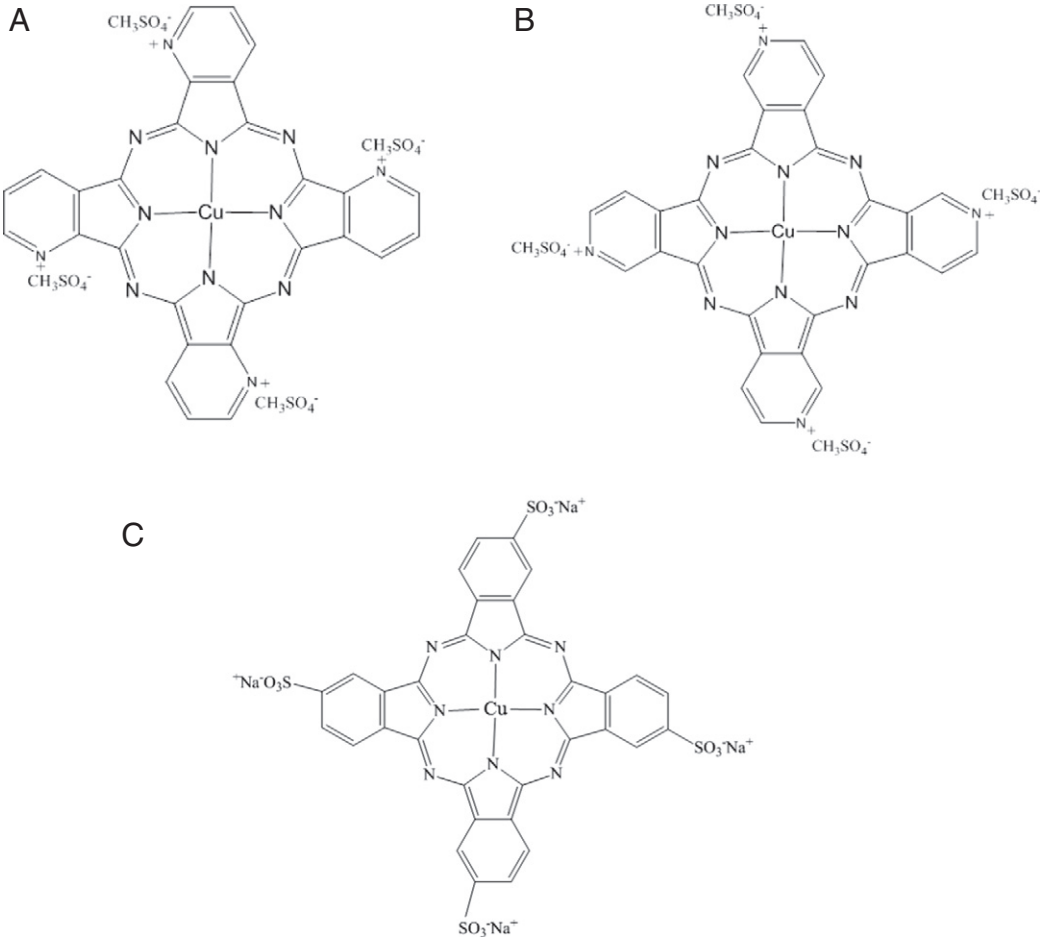
As noted in the results of absorption spectroscopy, binding constants were obtained by Eq. (1) and presented in Table 1. As shown, the values of K_b for binding of both porphyrazines to K^+ form of quadruplex are larger than that to the Na^+ form indicating that both porphyrazine complexes bind to the K^+ form more tightly than the Na^+ form. This result can be related to the structural changes observed by circular dichroism; as mentioned, structural transition occurs in the K^+ form of quadruplex but not in the Na^+ form. Probably, the change in the structure of mixed type quadruplex causes the complexes to bind more tightly to the quadruplex DNA.

4. Conclusion

As noted, we evaluate the binding of the three copper complexes including $[Cu(2,3\text{-tmtppa})]^{4+}$ and $[Cu(3,4\text{-tmtppa})]^{4+}$ as cationic porphyrazines and Cu(PcTs) as anionic phthalocyanine to the structurally distinct Na^+ and K^+ forms of human telomeric quadruplex DNA. The results demonstrate that the effect of the two porphyrazines on both forms of G_4 is very pronounced compared with the phthalocyanine. As mentioned, absorption spectrum of the phthalocyanine is invariant upon addition of G_4, so the displacement ability of the complex was monitored by the decrease of TO emission. The fluorescence decrement was noticeably lower with the phthalocyanine as compared to that with the two porphyrazines. This suggests that binding of Cu(PcTs) to the quadruplex is weaker compared to Cu(2,3-tmtppa) and Cu(3,4-tmtppa). Furthermore, CD result shows that Cu(PcTs) doesn't disrupt the structure of K^+ and Na^+ forms of the quadruplex.

As noted the phthalocyanine is negatively charged and the two porphyrazines are positively charged. G-quadruplexes, like all nucleic acids, have negative charge, and therefore cationic ligands generally bind more tightly to them. That is why the effect of Cu(PcTs) as anionic phthalocyanine would be weaker than the cationic porphyrazines.

Comparing the spectroscopic results of the two porphyrazines implies that the binding of porphyrazines to the quadruplex DNA is affected by location of the substituent groups on the periphery of these complexes. As noted in the results, there is difference between the effects of Cu(2,3-tmtppa) and Cu(3,4-tmtppa) on the quadruplex DNA. As is shown in Scheme 1, except for the different positions of the cationic charges on the two porphyrazine complexes, there is no



Scheme 1. Structures of A: N,N′,N″,N‴-tetramethyltetra-2,3-pyridinoporphyrazinatocopper(II), B: N,N′,N″,N‴-tetramethyltetra-3,4-pyridinoporphyrazinatocopper(II) and C: copper(II) phthalocyanine 3,4,4″,4‴-tetrasulfonic acid, tetrasodium salt.

difference between them. Consequently, location of the substituent groups on the periphery of porphyrazines is an important factor for the interaction of the porphyrazines with quadruplex. This result could stimulate future research in the area of synthesis of new porphyrazines for application in medicine.

On the whole, this investigation implies that porphyrazines could be suitable candidates for further *in vitro* and *in vivo* investigation about inhibition of telomerase that is a promising strategy for anti-cancer drug development.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bpc.2013.11.009.

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