A spectroscopic investigation of the interaction between c-MYC DNA and tetrapyridinoporphyratinatozinc(II)

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Abstract The c-MYC gene plays an important role in the regulation of cell proliferation and growth and it is overexpressed in a wide variety of human cancers. Around 90% of c-MYC transcription is controlled by the nuclease-hypersensitive element III1 (NHE III1), whose 27-nt purine-rich strand has the ability to form a G-quadruplex structure under physiological conditions. Therefore, c-MYC DNA is an attractive target for drug design, especially for cancer chemotherapy. Here, the interaction of water-soluble tetrapyridinoporphyratinatozinc(II) with 27-nt G-rich strand (G/c-MYC), its equimolar mixture with the complementary sequence (GC/c-MYC) and related C-rich oligonucleotide (C/c-MYC) is investigated. Circular dichroism (CD) measurements of the G-rich 27-mer oligonucleotide in 150 mM KCl, pH 7 demonstrate a spectral signature consistent with parallel G-quadruplex DNA. Furthermore, the CD spectrum of the GC rich oligonucleotide shows characteristics of both duplex and quadruplex structures. Absorption spectroscopy implies that the complex binding of G/c-MYC and GC/c-MYC is a two-step process; in the first step, a very small red shift and hypochromicity and in the second step, a large red shift

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and hyperchromicity are observed in the Q band. Emission spectra of zinc porphyrazine are quenched upon addition of three types of DNA. According to the results of spectroscopy, it can be concluded the dominant binding mode is probably, outside binding and end stacking.

Keywords Zinc porphyrazine · c-MYC promoter · DNA binding · Spectroscopy

1 Introduction

c-Myc plays an important role in the regulation of cell proliferation and growth [1–3]. It is tightly regulated in normal cells but cancer cells express c-Myc uncontrollably. The c-Myc gene is overexpressed in a wide variety of human cancers including 80% of breast cancers, 70% of colon cancer, 90% of gynecological cancers, 50% of hepatocellular carcinomas and a variety of hematological tumors [4–6]. Direct evidence supports the involvement of G-quadruplexes in transcriptional control of the c-Myc oncogene. ~90% of c-Myc transcription is controlled by the nuclease-hypersensitive element III₁ (NHE III₁), whose 27-nt purine-rich strand has the ability to form a G-quadruplex structure under physiological conditions. The C-rich strand in this region can form an i-motif DNA secondary structure. Single stranded G-quadruplex and i-motif structures in NHE III₁ sequence can energetically compete with the double-stranded form of DNA in this region [7–11].

c-Myc DNA is an attractive target for drug design, especially for cancer chemotherapy and interaction of ligands with G-quadruplex DNA in NHE III₁ sequence has received considerable interest in recent literature [11, 12]. A number of G-quadruplex-interactive agents like porphyrins and their derivatives have been shown to bind to the quadruplex DNA structure [13, 14]. Phthalocyanines differ from porphyrins by having nitrogen atoms link the individual pyrrol units. The aza analogues of the phthalocyanines (aza-Pcs) such as tetramethylmetalloporyphrazines are heterocyclic Pc analogues, 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 (tmtpa) are tetra-positively charged and hence water soluble [15].

A previous study from our laboratory showed the binding of [Cu(2,3-tmtpa)]⁴⁺ and [Cu(3,4-tmtpa)]⁴⁺ as two cationic tetrapyridinoporphyrazines complexes to telomere G-quadruplex DNA. Both complexes induced some conformational changes in the G-quadruplex [17]. In this study, interaction of N,N',N'',N'''-tetramethyltetra-3,4-pyridinoporphyrazinatozinc(II) {[Zn(3,4-tmtpa)]⁴⁺} (Scheme 1) with G quadruplex DNA of the 27-nt G rich strand of the c-MYC NHE (G rich DNA), the i-motif of its complementary C rich strand (C rich DNA) and the GC rich double strand of the gene in which the G rich oligonucleotide hybridized with its complementary sequence (GC rich DNA) was investigated by spectroscopic techniques. Previously, Asadi et al. investigated the interaction of [Zn(3,4-tmtpa)]⁴⁺ with calf thymus DNA [16] but there is no report on the interaction of this complex with other forms of DNA.

2 Material and methods

2.1 Material

[Zn(3,4-tmtpa)]⁴⁺ was prepared and purified by the method described previously [16]. The G-rich and C-rich oligonucleotides purified by HPLC were purchased from Bioneer.

Scheme 1 Structure of $[Zn(3,4-tmtppa)]^{4+}$, $X = CH_3SO_4$

3 Methods

3.1 Preparation of G-quadruplex

The 27-nt G rich oligonucleotide (TGGGGAGGGTGGGGAGGGTGGGGAAGG) (sample 1) and its 27-nt C rich complementary strand (ACCCCTCCCACCCCTCCCACCCCTTCC) (sample 2) were dissolved in phosphate buffer 1 mM containing 150 mM KCl and EDTA 0.1 mM, pH 7. Samples 1 and 2 were mixed in equal proportions for preparation of sample 3. Then, all samples were heated to 90 °C for 5 min, gently cooled to room temperature and incubated at 4 °C overnight.

3.2 UV-Vis absorption spectroscopy

All UV-visible spectra were obtained on a Pharmacia Biotech Ultrospec 4000 UV/Vis Diode Array Spectrophotometer with 1200 lines/nm concave holographic grating monochromator and Tungsten Halogen and deuterium Arc lamps. All experiments were carried out at room temperature.

The path length of the quartz cell and the scan rate were 1 cm and 100 nm/min. The concentration of $Zn(3,4-tmtppa)^{4+}$ was determined using a molar extinction coefficient value of $9.9 \times 10^{-4} \text{ M}^{-1} \text{ cm}^{-1}$. UV-Vis absorption titrations were done by adding a G-quadruplex DNA stock solution of 60 μM to the cuvette containing 600 μL of the complex solution of 7.5 μM . The complex and the oligonucleotide were prepared in the same buffer. First, the spectrum of the porphyrazine was recorded in the range of 200–800 nm. Then, titration was carried out and the spectra were recorded after stepwise addition of DNA.

The difference spectra were used to generate Job plots for determination of the stoichiometry interactions between the complexes and the quadruplex DNA. The percentage of

hypochromicity was determined using the following equation:

$$\text{Hypochromicity (\%)} = \frac{|\varepsilon_f - \varepsilon_b|}{\varepsilon_f} \times 100$$

Where ε_f , ε_b correspond to the extinction coefficients of the free and bound forms of the complex.

3.3 Spectrofluorimetry

Fluorescence spectra were measured at 25 °C using a Perkin-Elmer (Varian, Australia) spectrofluorometer equipped with a pulsed Xenon lamp. The porphyrazine was excited at 655 nm and the emission spectra were recorded in the range of 665 to 750 nm. The measurements were done using a 1 cm quartz cell and a bandwidth of 5 nm for both excitation and emission monochromators. In this study, 1.5 μ M concentration of the complex was titrated with 2.5 μ M concentration of DNA. Fluorescence spectra were recorded after each addition of DNA to the fluorescent cuvette. Intervals between addition of DNA and recoding the spectra were 3 min.

3.4 Circular dichroism measurement

CD measurements were performed on an Aviv Model 215 Circular dichroism Spectrometer (Lakewood, NJ) at 25 °C in a quartz cell with a path length of 1.0 mm. The oligonucleotide concentration was 10 μ M and the concentrations of the complex were 1, 5, 10 and 20 μ M. The bandwidth was 1 nm. The molar ellipticity was determined as $[\theta] = (\theta 100 MRW)/(cl)$, where c is the oligonucleotide concentration in mg/mL, l is the light path length in centimeters, MRW is the mean residue weight of DNA and θ is the measured ellipticity in degrees at wavelength λ [17].

4 Results and discussion

All CD spectra were recorded in 150 mM KCl, pH 7 in absence and presence of the complex. The molar ratios of the complex to DNA were 0.1: 1, 0.5:1, 1:1 and 2:1. CD spectra of the G rich and GC rich in absence and presence of different concentrations of the complex are shown in Fig. 1. As shown in Fig. 1a, the CD spectrum of G rich DNA exhibits typical CD signals consistent with G-quadruplex DNA, in agreement with previous reports for the same sequence [18]. Obviously, unlike CD spectrum (spectrum 1 in Fig. 1a) has a positive peak near 260 nm and a negative peak near 240 nm indicating a parallel stranded G-quadruplex [18–20]. As shown in Fig. 1a, the CD spectrum of G rich DNA changes upon binding to the complex. Obviously, unlike position of the negative and positive bands, the intensity of the spectra changes after addition of zinc porphyrazine to the G rich DNA. As shown, the intensity of the spectra decreases proportional to the concentration of the complex. This result indicates that although the parallel stranded G-quadruplex structure is still present, the complex affects the compactness of its structure [13].

The CD spectra of GC rich DNA in presence and absence of the complex are shown in Fig. 1b. Obviously, the CD spectrum of GC rich DNA shows a positive wide peak near 270 nm and a negative peak near 240 nm. As previously reported [18], although the position and amplitudes of the CD bands of B-forms of synthetic polydeoxynucleotides differ markedly with sequence, all CD spectra are characterized by a positive long wavelength

Fig. 1 Circular dichroism spectra of the G rich (a) and GC rich (b) DNA in phosphate buffer 1 mM containing 150 mM KCl and EDTA 0.1 mM, pH 7 in absence and presence of tetrapyridinoporphyrinatozinc(II). The molar ratio of the complex to DNA equals 0.1: 1 (2), 0.5:1(3), 1:1(4), 2:1(5) and sample 1 is DNA without the complex

band at about 260–280 nm and a negative band around 245 nm. The difference among B-forms of synthetic polydeoxynucleotides is not only because of different conformational properties but also because chromophores differ. Accordingly, the spectrum of the GC rich DNA (Fig. 1b) indicates the formation of a double helix structure during folding of the GC rich oligonucleotide. In addition, based on the CD spectrum (Fig. 1b), quadruplex is probably another conformation of the GC rich oligonucleotide. We suggest that duplex and quadruplex DNA coexist in the GC rich oligonucleotide solution, because the negative peak at 240 nm and the positive peak near 210 are simultaneously shown in the CD spectrum of the G rich quadruplex (Fig. 1a) and the GC rich DNA (Fig. 1b). In other words, the CD spectrum of the GC rich oligonucleotide shows characteristics of both duplex and quadruplex structures. In Fig. 1b, the effect of complex on the structure of the GC rich DNA is observed. As shown, the intensity of spectra decreases proportional to the concentration of the complex. As noted, a similar change was observed in the CD spectra of the G rich DNA (Fig. 1a). Also, the intensity of the negative and positive bands of the CD spectra of C rich DNA decreases (data not shown) in presence of the complex. These results indicate that binding of the complex results in a decrease in compactness of the G, C and GC rich DNA.

Figures 2 and 3 show the change in the absorption spectra of the complex after titration with the oligonucleotides. Figure 2a shows the change in the absorption spectrum of the complex upon titration with the G rich oligonucleotide. Obviously, red-shift and hyperchromicity are shown in the spectra. The percentage of hyperchromicity at different concentrations of the complex is plotted and presented in Fig. 4. It can be clearly seen that the changes in the spectra of zn(3,4-tmtppa) upon addition of G rich DNA occur in two stages; in the first stage, a large hypochromicity (27%) and a very small red shift (1 nm) and in the second stage, a small hyperchromicity and a large red shift (35 nm) are shown. As shown in Fig. 2b, similar changes are also observed in the GC rich DNA; the changes of the first stage are almost the same and in the second stage, the increase in the intensity of the GC rich spectra is more than that for G rich ones. Obviously, the difference between the values of the red-shift of G and GC rich forms is very small in the two stages. In Fig. 3, the absorption spectra and in Fig. 4, the hyperchromicity of C rich are presented. As shown, unlike the G rich and GC rich, the changes occur in one step. The percentage of hypochromicity and red shift are 24% and 2 nm. As is well known, the mode of porphyrins binding to DNA is

Fig. 2 Absorption spectra of $[Zn(3,4-tmtppa)]^{4+}$ titrated with the G rich DNA (a) and GC rich DNA (b) in phosphate buffer 1 mM containing 150 mM KCl and EDTA 0.1 mM, pH 7

determined using an amount of hyperchromicity or hypochromicity and a shift in the wavelength of maximum absorbance. In the case of duplex DNA, intercalation of the porphyrin between the base pairs results in about a 15 nm red shift and a near 35% hypochromicity of the porphyrin solet band. Outside binding without stacking is demonstrated by a small red shift (about 6–8 nm) and hypochromicity (10%) or even hyperchromicity of the solet band. Outside binding with stacking is indicated by variable effects on the solet band [21–23]. Accordingly, for both forms of DNA, the changes of the first and the second stages are intermediate between those expected for intercalative and outside binding modes. It is important to remember that the values of the red shift and hypochromicity given above for different binding modes were determined for long pieces of duplex DNA where end stacking is insignificant. Here, it is not possible to discriminate end-stacking and intercalation but it seems that one of the binding modes for G rich and GC rich is end-stacking. Outside binding is also possible.

Fig. 3 Absorption spectra of $[Zn(3,4-tmtppa)]^{4+}$ titrated with the C rich DNA in phosphate buffer 1 mM containing 150 mM KCl and EDTA 0.1 mM, pH 7

Fig. 4 Increase in the absorbance of tetrapyridinoporphyrinatozinc(II) (hypochromicity) after addition of the G rich —*— the C rich —■— and the GC rich —□— DNA

Fluorescence is a very sensitive technique for studying the mode of ligand binding to DNA. Zn porphyrazine is a fluorophore molecule and here its emission spectra were recorded in presence of DNA to obtain information about binding of the complex to three forms of DNA. The result indicated that the intensity of Zn porphyrazine emission decreases upon addition of the three forms of DNA (data not shown). A Stern-Volmer plot (Fig. 5) were constructed for comparing the effect of the three forms of DNA on emission of the complex. The positive slope of the Stern-Volmer plots imply that emission of the complex was becoming quenched upon addition of excess of DNA. Furthermore, the plots related to the quenching effect of G rich and GC rich DNA exhibit a positive deviation from a straight line, suggesting both static and dynamic quenching are involved. The Stern-Volmer plots also indicate that the GC rich DNA quenches emission of the complex more than the G rich DNA. Obviously, unlike the G rich and the GC rich DNA, the Stern-Volmer plot of the C

Fig. 5 Stern-Volmer plots of G rich —*— the C rich —□— and the GC rich —◇— DNA

rich DNA is linear. In addition, the slope of the C rich Stern-Volmer plot is less than that for the two other forms of DNA.

As reported earlier, the changes in the local environment of chromospheres that reflect the interaction of the fluorophore with its environment can be detected by fluorescence spectroscopy. When a fluorophore ligand intercalates between bases of DNA, the fluorescence intensity increases, because energy transfer takes place between them. On the other hand, external binding leads to a decrease in the fluorescence intensity because of the quenching of ligand emission by the surrounding water molecules [24–26]. Accordingly, fluorescence hypochromicity observed upon binding of all forms of DNA to the complex supports external binding mode.

5 Conclusion

On the whole, the results indicate that the dominant binding mode of tetrapyridinoporphyrazinatozinc(II) to DNA is probably outside binding and end stacking. In addition, the binding results in a decrease in compactness of the three forms of DNA structure.

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