Biochemical and Biophysical Research Communications 404 (2011) 139–142

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Human telomere d[(TTAGGG)$_4$] undergoes a conformational transition to the Na^+-form upon binding with sanguinarine in presence of K^+

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ARTICLE INFO

Article history:
Received 11 November 2010
Available online 23 November 2010

Keywords:
Human telomere
Sanguinarine
G-quadruplex
Circular dichroism
Conformational transition

ABSTRACT

Guanine-rich telomeric sequences fold into G-quadruplex conformation and are known to bind a variety of ligands including potential drug candidates. By means of CD spectroscopy and fluorescence lifetime measurements we demonstrate that putative anticancer therapeutic sanguinarine (SGR) exhibits two distinct interactions with human telomere d[(TTAGGG)$_4$] (H24) in presence of K^+. Up to about 1:2 M ratio of H24:SGR (10 μM H24), two molecules of SGR bind H24. Above this molar ratio, SGR induces a conformational transition in H24 from the K^+-form to the Na^+-form. The demonstration of SGR-induced conformational transition in a G-quadruplex formed by a human telomeric sequence could provide new insights into interaction of drugs with quadruplex DNA structure.

1. Introduction

Telomeric sequences are short stretches of guanine (G)-rich DNA that occur at the termini of chromosomes and play an important role in chromosome duplication and are attractive targets for anticancer drugs [1–4]. They fold into G-quadruplexes and show conformational variability that depends on the nature of the counterion (Na^+/K^+; Fig. 1a), the sequence of the loops connecting the G-repeats and the terminal residues [5–9]. In order to fully exploit the potential of drugs that bind to telomeric sequences it is important that the mode of interaction between the two be elucidated at the molecular level. Typically, one G-quadruplex unit is known to bind two drug molecules, possibly in end stacked geometry, without inducing any conformational change in the G-quadruplex backbone [10–11]. Plant alkaloid, Sanguinarine (SGR), with putative anticancer activity [12], is one such important compound. Here, we show that in addition to the canonical binding of two SGR molecules, the human telomeric sequence d[(TTAGGG)$_4$] (H24) undergoes a conformational transition (in presence of K^+) from a mixed Type-I conformation, to the Na^+-form (see Fig. 1a), in presence of high concentration of SGR. The SGR-induced conformational change in H24, from a biologically relevant K^+-form to the Na^+-form has important implications.

2. Materials and methods

2.1. Materials

Human telomeric sequence (TTAGGG)$_4$, sanguinarine chloride, potassium di-hydrogen phosphate and di-potassium hydrogen phosphate were purchased from Sigma chemical company, USA. Buffers were prepared in autoclaved water that was purified through a Millipore (Bedford, MA) Milli-Q system. Human telomeric G-quadruplex (H24) and sanguinarine were prepared according to the standard method described earlier [13–14].

2.2. Circular dichroism (CD) spectroscopy and convex constraint analysis (CCA)

CD measurements were carried out in a JASCO J-815 spectropolarimeter (Jasco Corporation, Tokyo, Japan) in phosphate buffer (pH 6.9, 150 mM KCl) at 20 °C equipped with a temperature controller. The CD scans were recorded within the wavelength range of 200–400 nm at sensitivity set to 10 mdeg and scan speed of 50 nm per minute with step size of 0.5 nm. The time constant was 1 s and bandwidth was 0.2 nm. A solution containing 10 μM H24 was titrated with SGR up to 110 μM. All spectra are average of five runs. CCA [15] was performed on the spectral set in order to extract the basis spectra and the associated coefficients.

2.3. Fluorescence lifetime measurement

Fluorescence lifetimes were estimated from time-resolved fluorescence intensity decays using HORIBA JOBIN YVON (Scotland)

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doi:10.1016/j.bbrc.2010.11.081

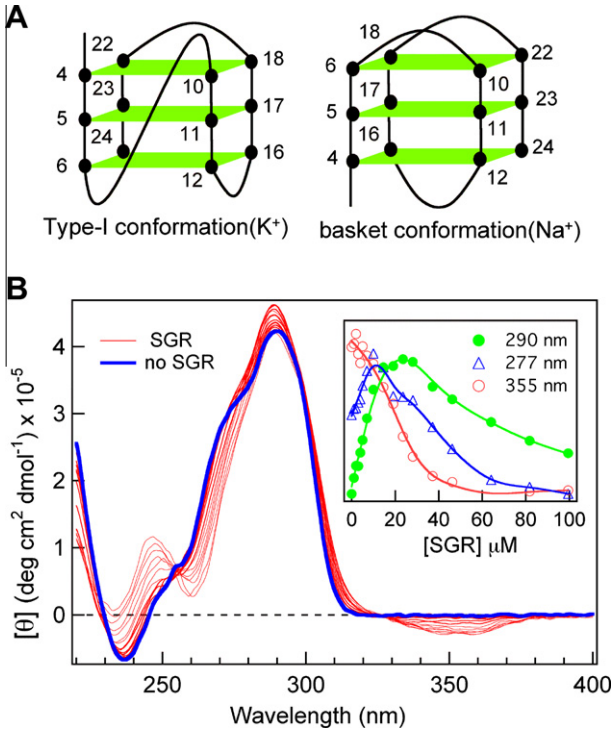

Fig. 1. (A) Cartoon representations of the K^+-form and the Na^+-form of H24 (guanine residues are numbered). (B) CD spectra of H24 (10 μM, 150 mM KCl, 10 mM phosphate buffer, pH 6.9) in absence (thick line) and in presence (thin line) of increasing amounts of SGR. Normalized variations in ellipticity at three selected wavelengths are shown in the inset (filled circle: 290 nm; open triangle: 277 nm; open circle: 355 nm).

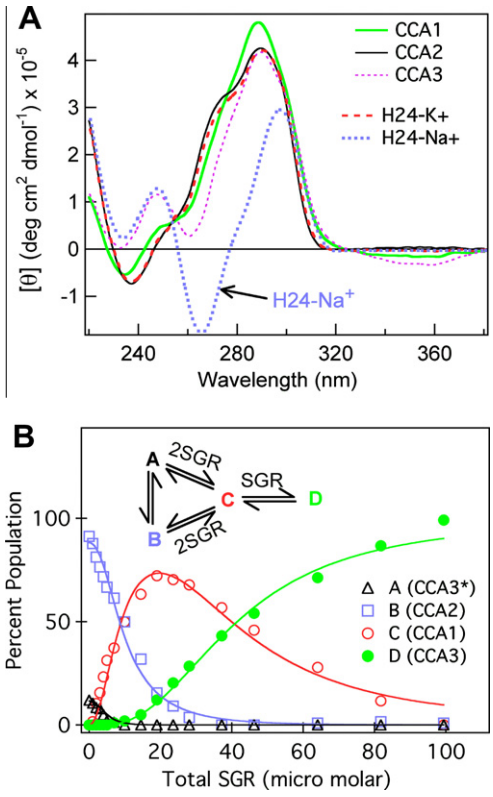

Fig. 2. (A) Basis spectra (CCA1, CCA2, CCA3) from CCA analysis of CD spectra in Fig. 1b along with CD spectra of H24 in Na^+ (Ref. [16]) and K^+ (this work). (B) Contributions of CCA components as a function of [SGR]. Solid lines indicate the best global fit ($K_{AC} = 0.015\ \mu M^{-2}$; $K_{BC} = 0.013\ \mu M^{-2}$; $K_{CD} = 9.22 \times 10^{-5}\ \mu M^{-1}$; $n = 2.5$; $\theta_{A0} = 0.12$) to Eq. (1). The CCA basis spectra are assigned to four inter-converting H24 species (A and B: major and minor H24 conformers in absence of SGR; C: SGR-bound H24 in K^+-conformation; D: SGR-bound H24 in Na^+-conformation) show schematically in the inset to panel b (see text for details).

luminescence spectrophotometer in the time-correlated single photon counting mode at 20 °C with a 340 nm NanoLED pulsed diode as the excitation source. To optimize the signal-to-noise ratio, 10,000 photon counts were collected in the peak channel. All experiments were performed using excitation and emission slits with a band-pass of 8 nm. Fluorescence Intensity decay curves so obtained were fitted using in-built software DAS6.

2.4. ^1H NMR spectroscopy

NMR spectra were recorded at 25 °C in a Bruker DRX-500 spectrometer in presence of 10% D_2O (for signal locking) and trimethylsilyl propionate (internal standard). 1D NMR spectra for H24 (600 μM) in absence and in presence of increasing concentrations of SGR (40, 120 and 225 μM) were recorded. For the H24-SGR complex, H24 was pre-incubated with appropriate concentrations of SGR for 30 min before the spectra were recorded. All samples were prepared in phosphate buffer (pH 6.9, 150 mM KCl). Data were analyzed using Bruker X-WIN NMR 3.5 software.

3. Results and discussion

3.1. SGR binding to H24 as monitored by CD and NMR

The CD spectrum of H24, in presence of K^+, is shown in Fig. 1B. The spectrum, characterized by a maximum at 290 nm with a hump at 274 nm and a minimum at 237 nm, is consistent with a previously reported CD spectrum of H24 [16], and characteristic of a mixed Type-I conformation. Twelve sharp imino signals in the ^1H-NMR spectrum of H24 (Fig. S1 of Supplementary Information) also indicated the presence of a dominantly folded conformation that was identified as the K^+-form I by comparison with a previously published NMR spectrum [6]. Upon addition of SGR (Fig. 1B), systematic changes were observed in H24 CD spectrum including the appearance of a negative band at ~355 nm. Achiral SGR exhibits two absorption maxima at 275 and 330 nm. Therefore, the negative signal at 350 nm with increasing SGR indicated binding of SGR to H24. SGR-binding was also evident from NMR data (Fig. S1) – upon titration with SGR (up to 240 μM SGR: 600 μM H24; NMR experiments could not be performed beyond 1:0.4 H24:SGR due to poor SGR solubility), the imino signals did not show significant shift indicating that at least in this concentration range the original H24 structure remained mostly intact. However, the observed line broadening was consistent with a model where there is a dynamic exchange between SGR-bound and free H24 forms.

3.2. CCA analysis of CD spectra indicates conformational transition at high SGR concentration

CD spectra of H24 changed non-linearly with increasing [SGR] (non-linear variation of $[\theta]_{290}$, $[\theta]_{277}$ and $[\theta]_{355}$; inset to Fig. 1B), indicating the presence of more than two underlying species. To resolve this complexity, the CD spectra were de-convoluted using the CCA method [15]. This yielded three basis spectra – CCA1, CCA2 and CCA3 (Fig. 2A). The first two (CCA1 and CCA2) matched with the CD spectra of SGR-bound and SGR-free (with minor contributions from SGR) H24 forms, respectively. Surprisingly, the third basis spectrum (CCA3) showed characteristic features of the Na^+-form of H24 [16] even though there was no Na^+ in the sample. Changes in the relative populations of the three species are shown

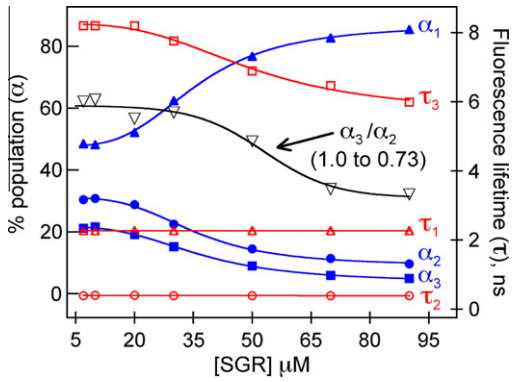

Fig. 3. Concentration dependence of fluorescence lifetimes (τ) and corresponding fraction populations (α) of SGR, in presence of 10 μM H24 (potassium phosphate buffer, pH 6.9, 150 mM KCl; raw data in Table S2). Fitting performed with $I(t) = \sum \alpha_i \exp(-t/\tau_i)$ where τ_1 was fixed at 2.27 ns (fluorescence lifetime of free SGR).

in Fig. 2B (C, B and D, corresponding to CCA1, CCA2 and CCA3, respectively). The initial decrease in CCA3 was considered to be arising from a fourth component (species A in Fig. 2B) not resolved in the CCA analysis, due to its minor presence. Fig 2B shows decreasing populations of two inter-converting SGR-free H24 species (A and B) and a concomitant increase in SGR-bound H24 species (C) till about 20 μM SGR (1:2 H24:SGR molar ratio). Beyond 20 μM SGR, species C decreases with a concomitant increase of species D (the Na^+-form of SGR-bound H24).

The presence of a major (B) and a minor (A) K^+-stabilized H24 conformation is consistent with published NMR data [7]. The saturation of SGR-bound H24 (C), roughly at 1:2 H24:SGR molar ratio, is also consistent with the canonical case of double ligand binding to G-quaruplexes [16]. However, the appearance of species D (SGR-bound Na^+-form of H24) with further increase of SGR was surprising. That this step involves a conformational transition is also strongly indicated by the sigmoidal nature of the increase of component D. To have a more quantitative understanding, the CCA coefficient data of Fig. 2B were fitted (global fitting) with appropriate equations (Eq. (1); see Supporting Materials), compatible with the scheme shown in the inset to Fig. 2B.

$$X_A = f_{A0}\left(1 - \frac{K_{AC}[SGR]^2}{1 + K_{AC}[SGR]^2}\right) / f_{B0}\left(1 - \frac{K_{BC}[SGR]^2}{1 + K_{BC}[SGR]^2}\right)$$
$$X_B = 1 \qquad (1)$$
$$X_C = K_{BC}[SGR]^2$$
$$X_D = K_{CD} \times K_{BC}[SGR]^{2+n}$$

The percent populations of the four CCA components are given by $X_i/\sum X_i$ (X_i defined in Eq. (1)) where K_{ij} are the appropriate association constants (species i to species j), n is the Hill coefficient, and, f_{A0} and f_{B0} are fraction populations of A and B in absence of SGR, respectively. The last step (rise of component D) could not be fitted without the use of a Hill parameter $n = 2.5$ (see Supplementary information), indicating a highly cooperative change ($n = 1$ corresponds to absence of cooperativity), typically characterized by conformational transitions.

3.3. Evidence of conformational transition at high SGR concentration from time-resolved fluorescence data

Fluorescence lifetimes of SGR were measured in presence and absence of H24 to further probe the SGR-H24 interaction. Free SGR exhibited a single exponential decay ($\tau = 2.27$ ns). In presence of H24, this changed to a triple exponential decay profile (Fig. 3). Compared to free SGR, a shorter and a longer lifetime component, with almost equal pre-exponential factors, arose as a result of the association that were ascribed to the two SGR molecules bound per H24, each in a different microenvironment. A rough estimate of K_{AC} (Fig. 2b inset) from the pre-exponential factors is consistent with CD data. At 1:1 SGR:H24 M ratio, $[SGR]_{bound} = 5.24$ μM, $[SGR]_{free} = 4.76$ μM, $[H24]_{bound} = 5.24/2 = 2.37$ μM, $[H24]_{free} = 10.0–2.37 = 7.63$ μM (see Table S1). Using these values, K_{AC} (or K_{BC}) can be estimated as: $2.37/(7.63 \times 4.762) = 0.014\ \mu M^{-2}$. These match very well with K_{AC} and K_{BC} values estimated from CD data (see legend to Fig. 2b).

In the regime where H24 undergoes a CD-detected conformational transition ([SGR] >20 μM), τ_2 remained constant while τ_3 and α_3/α_2 exhibited a sigmoidal change (Fig. 3), indicating that at higher SGR concentrations, two SGR molecules still bind H24, but the relative population of bound SGR at the two sites (α_3/α_2) and the nature of the second site (τ_3) is different. The sigmoidal change indicates a conformational transition, consistent with the CD data. A decrease in α_3/α_2 ratio indicates accumulation of extra H24-bound SGR population corresponding to the τ_2 site, probably originating from a third H24-bound SGR molecule. While two SGR molecules are known to stack on the G-quadruplex structure, three SGR molecules have been observed to bind two tandem G-quadruplex repeats, the third SGR probably binding in between the two G-quadruplex units [10]. It is tempting to suggest that at high SGR concentrations, H24 forms similar inter-molecular G-quadruplex structures, stabilized by a sandwiched SGR molecule.

4. Summary and perspective

The central finding of this work is the SGR-induced conformational transition in H24, at SGR concentrations greater than 20 μM, after the canonical SGR binding sites in H24 get saturated. SGR alters the physiologically relevant K^+-conformation to the non-physiological Na^+-conformation, even in the absence of Na^+ ions. It could be a plausible molecular mechanism of action of G-quadruplex-binding drugs that interfere with normal biological activities. For example, this could be a mechanism for the observed dose-dependent inhibition of Taq polymerase DNA synthesis by SGR [10]. Similar ligand-induced conformational transition in quadruplex structures has also been reported for the ligand, tetra-(*N*-methyl-4-pyridyl) porphyrin [17–18].

Acknowledgments

We acknowledge Barun Mazumder and Himal Ganguly for help with NMR experiments and CCA analysis. The work was supported by intramural funding from DAE, Govt. of India.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.11.081.

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