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Exploration of a multi-target ligand, dehydroevodiamine, for the recognition of three G-quadruplexes in c-Myb proto-oncogene by ESI–MS

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ABSTRACT

In this research, ESI mass spectrometry was used to probe the formation of G-quadruplexes from three G-rich sequences (S1–S3) in the upstream region of the transcription start site in human c-Myb proto-oncogene. A ligand, dehydroevodiamine, was found for its high binding affinities towards the c-Myb G-quadruplexes. In addition, dehydroevodiamine bound towards the Q1–Q3 with high selectivity over long-chain duplex DNA. High-resolution ESI–MS was utilized to investigate the binding competition of dehydroevodiamine in the mixture solution of Q1–Q3 G-quadruplexes, and it appeared to have the binding affinity in the following order: Q3 ≈ Q1 > Q2, which is consistent with the results in the single G-quadruplex solutions. The properties of dehydroevodiamine gave its potential in the future studies of c-Myb expression regulation.

1. Introduction

It is now well-established that G-tracts rich sequences in DNA (or RNA) are capable of folding into G-quadruplexes, a special secondary-structure related on Hoogsteen base paring [1,2], and those G-rich sequences are widely prevalent and observed in the human genomes [3–6]. The locations of the three million putative G-quadruplex-forming sequences are non-random, and they distribute in functional regions to affect important biological processes, such as telomere, promoters, 5′-UTR, and the non-coding regions [3,7]. Due to the existence and important effects of G-quadruplexes in genomic process, many putative G-quadruplex-forming sequences were proved to be targeting sites for gene expression modulation [3], for instance c-myc [8], bcl2 [9,10] and c-kit [11]. So G-quadruplex targeting with small molecules is emerging as a novel approach to gene expression modulation and cancer therapeutics [12].

c-Myb proto-oncogene is aberrantly expressed in leukemia and some solid tumors, therefore it is an important target for cancer therapy. G-rich sequences in the downstream region of c-Myb transcription start site have been proved to fold into G-quadruplex structures [13–15]. Recently, our bioinformatics analysis shows that there are three G-rich sequences [−80 to −49 (S1), −112 to −94 (S2) and −352 to −318 (S3)] in the upstream region of the transcription start site in c-Myb gene (Fig. 1a). It has been acknowledged that Electrospray ionization mass spectrometry (ESI–MS) is a useful tool for investigating G-quadruplexes and their interactions with ligands. Here, ESI–MS was used to probe the formation of G-quadruplexes from the S1–S3 sequences and their recognition by organic small-molecules. The results from ESI–MS combined with CD spectroscopy revealed that the G-rich sequences (S1–S3) formed stable G-quadruplex structures (Q1–Q3), respectively. In addition, dehydroevodiamine (Fig. 1b), a natural bioactive alkaloid separated from Chinese herbal medicine Evodia rutaecarpa with hypotensive [16], negative chronotropic [16], antiamnesic [17] and antiarrhythmic [18] effects, was screened by ESI–MS to selectively bind with Q1–Q3 G-quadruplexes other than long-chain duplex DNA, which provided the possibility to modulate the transcription of c-Myb gene by this ligand.

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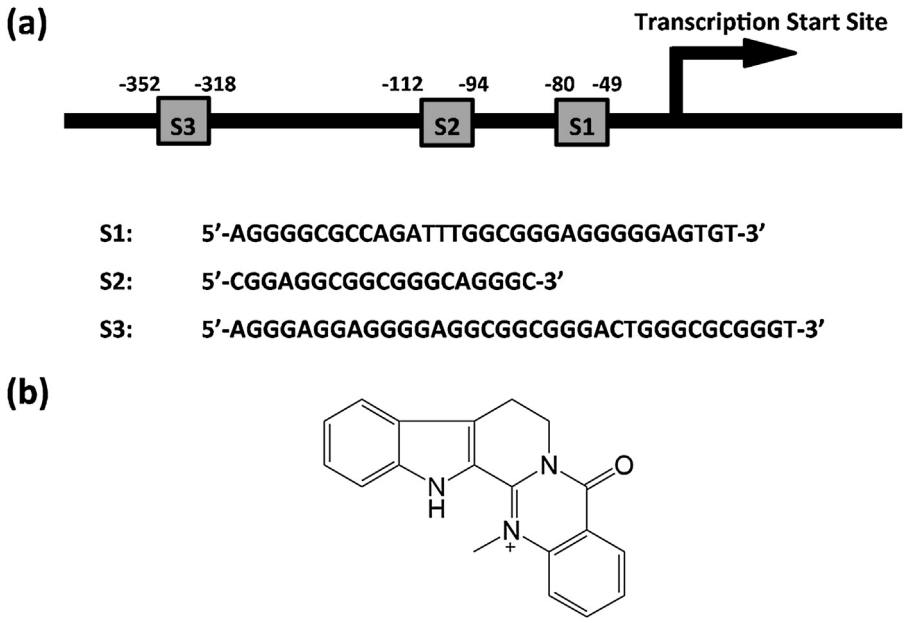

Fig. 1. (a) The G-rich sequences (S1–S3) in the upstream region of c-Myb transcription start site. (b) The chemical structure of dehydroevodiamine (P).

2. Experimental or methods

2.1. Materials

The guanine-rich sequences d[5′-$AG_4CGCCAGATTTG_2CG_3AG_5AGTGT$-3′] (S1, Mw = 10107.6), d[5′-$CG_2AG_2CG_2CG_3CAG_3C$-3′] (S2, Mw = 5960.9), d[$AG_3AG_2AG_4AG_2CG_2CG_3ACTG_3CGCG_3T$] (S3, Mw = 11130.2) were synthesized by Sangon Biotech Co., Ltd. (Beijing, China) with HPLC purification. The ligand dehydroevodiamine (98.0% purity, Mw = 302) was purchased from BioBioPha (Yunnan Province, China). The Calf Thymus DNA (CT-DNA) was subjected to sonication to obtain an average length of 500 base pairs (according to gel electrophoresis analysis with DNA ladder).

2.2. ESI mass spectrometry

ESI–MS spectra were obtained by a Finnigan LCQ Deca XP Plus ion-trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA). Negative ion mode was used with a capillary temperature of 140 °C (120 °C for S2) and a spray voltage of 3.0 kV (2.7 kV for S2). The sample infusing rate was 2 μL/min, and the sheath gas flow rate was 25 arb. 100 μM stock S1–S3 solutions were diluted in 25% CH_3OH (volume ratio, to improve electrospray efficiency) with or without 50 mM NH_4OAc to a final concentration of 10 μM. The mixture of the G-quadruplex and dehydroevodiamine were generated by adding 1, 2 and 4 equivalents dehydroevodiamine to the DNA solutions. Samples for the competition experiments with CT-DNA were prepared by mixing 100 μM annealed stock S1–S3 solution, ammonium acetate buffer and methanol, and then adding the CT-DNA solution. Four equivalents dehydroevodiamine was added to the mixture of the G-rich sequences and CT-DNA with a molar ratio of 1:1.

2.3. High-resolution ESI mass spectrometry

The binding competition experiments were carried out by a Bruker SolariX-XR Fourier transform ion cyclotron resonance mass spectrometer (Bruker, Billerica, MA, USA) with an ESI source. The samples contained S1–S3 with or without four equivalents dehydroevodiamine, and were tested in negative ion mode with a syringe flow rate of 3.0 μL/min, a capillary voltage of 3000 V and drying gas temperature of 140 °C.

2.4. Circular dichroism spectroscopy

Circular dichroism (CD) experiments were carried out using a J-815 CD spectrometer (JASCO, Japan). 2.5 μM S1–S3 were added to the solution of 150 mM KCl and 30 mM Tris-HCl (pH = 7.4). Before the DNA samples were tested, they were annealed at 95 °C for 5 min and then programmed cooling to 4 °C (by PCR). The samples of 2.5 μM S1–S3 with 50 mM NH_4OAc and 25% CH_3OH were also tested. The scans were carried out in a 0.1 cm path-length cuvette two times to obtain an average spectrum from 220 to 350 nm.

3. Results and discussion

3.1. G-quadruplex formation

ESI–MS was used to investigate the G-quadruplex formation of three G-rich sequences (S1–S3) in the upstream region of c-Myb gene transcription start site (Fig. 1a). The S1 mass spectrum in 25% CH_3OH (absence of NH_4OAc) shown a series of deprotonated ion peaks with charges distribution from 8- to 13- (Fig. 2a), which revealed an unfolded structure [14,15]. In 25% CH_3OH with 50 mM NH_4OAc, high charge ions from 8- to 13- disappeared and a base peak at m/z 1692.5 was observed, which belonged to the adduct ions $[S1+3NH_4^+-9H^+]^{6-}$, indicating the formation of G-quadruplex (Q1) [19], and the charge numbers was confirmed by the high-resolution ESI-FTMS (Fig. 2b). The similar characteristics were obtained also in the mass spectra of the S2 and S3 (Fig. 2c–f), indicating the formation of Q2 and Q3 G-quadruplexes.

The G-quadruplex formation of S1–S3 in 50 mM NH_4OAc and 25% CH_3OH have been confirmed by CD spectroscopy (Fig. S1). In addition, CD experiments were also performed in 150 mM KCl (simulation of physiological conditions) to confirm the formation of G-quadruplexes (Fig. 2g). The spectrum of 2.5 uM S1 in 30 mM Tris-HCl (pH = 7.4) showed random peaks indicating an unfolded structure. After KCl was added, the CD spectrum of S1 showed the positive peak at 262 nm and a negative peak at 240 nm, indicating