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Hsa-miR-1587 G-quadruplex formation and dimerization induced by NH_4^+, molecular crowding environment and jatrorrhizine derivatives

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ABSTRACT

A guanine-rich human mature microRNA, miR-1587, was discovered to form stable intramolecular G-quadruplexes in the presence of K^+, Na^+ and low concentration of NH_4^+ (25 mM) by electrospray ionization mass spectrometry (ESI-MS) combined with circular dichroism (CD) spectroscopy. Furthermore, under high concentration of NH_4^+ (100 mM) or molecular crowding environments, miR-1587 formed a dimeric G-quadruplex through 3′-to-3′ stacking of two monomeric G-quadruplex subunits with one ammonium ion sandwiched between the interfaces. Specifically, two synthesized jatrorrhizine derivatives with terminal amine groups could also induce the dimerization of miR-1587 G-quadruplex and formed 1:1 and 2:1 complexes with the dimeric G-quadruplex. In contrast, jatrorrhizine could bind with the dimeric miR-1587 G-quadruplex, but could not induce dimerization of miR-1587 G-quadruplex. These results provide a new strategy to regulate the functions of miR-1587 through induction of G-quadruplex formation and dimerization.

1. Introduction

G-quadruplexes widely exist in genome and transcriptome, such as telomeres, oncogenic promoters, immunoglobulin switches, mutational hot spot sequences, introns and the untranslated regions of mRNA [1–3]. With the increasing evidence of the existence and regulatory role of G-quadruplexes in biological processes [4], G-quadruplex structures have been considered as promising therapeutic targets and different kinds of G-quadruplex ligands have been developed [5–8].

DNA G-quadruplexes are highly polymorphic regarding to the relative strand orientations (parallel and antiparallel), glycosidic conformations of guanine bases (*anti* and *syn*), groove widths (wide, medium and narrow) and intervening loops (double-chain-reversal, diagonal and lateral loops) [9,10]. Based on these conformational features, a discrete DNA G-quadruplex structure generally has three kinds of typical folding topologies: parallel, hybrid and anti-parallel. Irregular folding topologies are also reported, such as snapback configurations [11–13], bulges in interrupted G-column [14,15] and V-shaped loops connecting two or three G-quartets [16–18]. Guanine-rich sequences with four, two or one G-tracts could form intramolecular, bimolecular or tetramolecular G-quadruplexes. Interestingly, sequences with four G-tracts capable of forming intramolecular G-quadruplexes are also reported to form bimolecular G-quadruplexes, such as the bimolecular c-kit2 [19] and N-myc [20] G-quadruplexes with six stacking G-quartets formed in K^+ solution. Moreover, DNA G-quadruplexes can also form high-order structures, interlocked G-quadruplexes [21–24] and end-to-end stacking G-quadruplexes [25–29]. Several factors including flanking sequences, the type and concentration of cations and molecular crowding environment have been reported to influence the stacking of DNA G-quadruplexes [25–30]. Besides, a perylene derivative, PIPER, has been reported to accelerate the association of bimolecular and tetramolecular G-quadruplexes [31].

In contrast to the polymorphic DNA G-quadruplex, RNA G-quadruplex is normally monomorphic. The 2′ hydroxyl groups in RNA limited the glycosidic conformations of guanine bases to adopt *anti* conformation, so that RNA G-quadruplex can only exist in parallel conformation with parallel strand orientations and double-chain reversal loops [32]. Moreover, reports about high-order RNA G-quadruplexes are relatively fewer [33–38] and factors that influence high-order RNA G-quadruplex formation are still under to be investigated.

Electrospray ionization mass spectrometry (ESI-MS) has been widely used as a robust and convenient technique in the determination of molecularity of biological macromolecules in solution [39,40]. Furthermore, several academic groups have shown that the high-order nucleic acids and their non-covalent complexes could be preserved and detected in mass spectrometer. Both intramolecular and intermolecular G-quadruplexes could preserve their structures and stably exist in ESI-MS conditions due to the formation of eight

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hydrogen bonds in G-quartets and electrostatic interactions between central cations and G-quartets [41,42]. In this study, ESI-MS, tandem MS and high resolution MS were utilized to study the formation and properties of a RNA G-quadruplex formed by miR-1587, r(5′-UUGGGCUGGGCUGGGUUGGG-3′), a guanine-rich human mature microRNA [43]. Utilizing these methods, herein we further demonstrated that the miR-1587 G-quadruplex formed dimeric G-quadruplex in high concentration of NH_4^+ or molecular crowding environment with high thermal stability. Specifically, we discovered that two jatrorrhizine derivatives could also induce the formation of the dimeric G-quadruplex. Our study demonstrated the formation of a human mature microRNA G-quadruplex and investigated several factors that induced the miR-1587 G-quadruplex dimerization, enhancing our understanding of dimeric RNA G-quadruplex formation.

2. Experimental

2.1. Materials

The oligonucleotide of miR-1587, r(5′-UUGGGCUGGGCUGG GUUGGG-3′), was purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). The sample was dissolved in ultrapure water

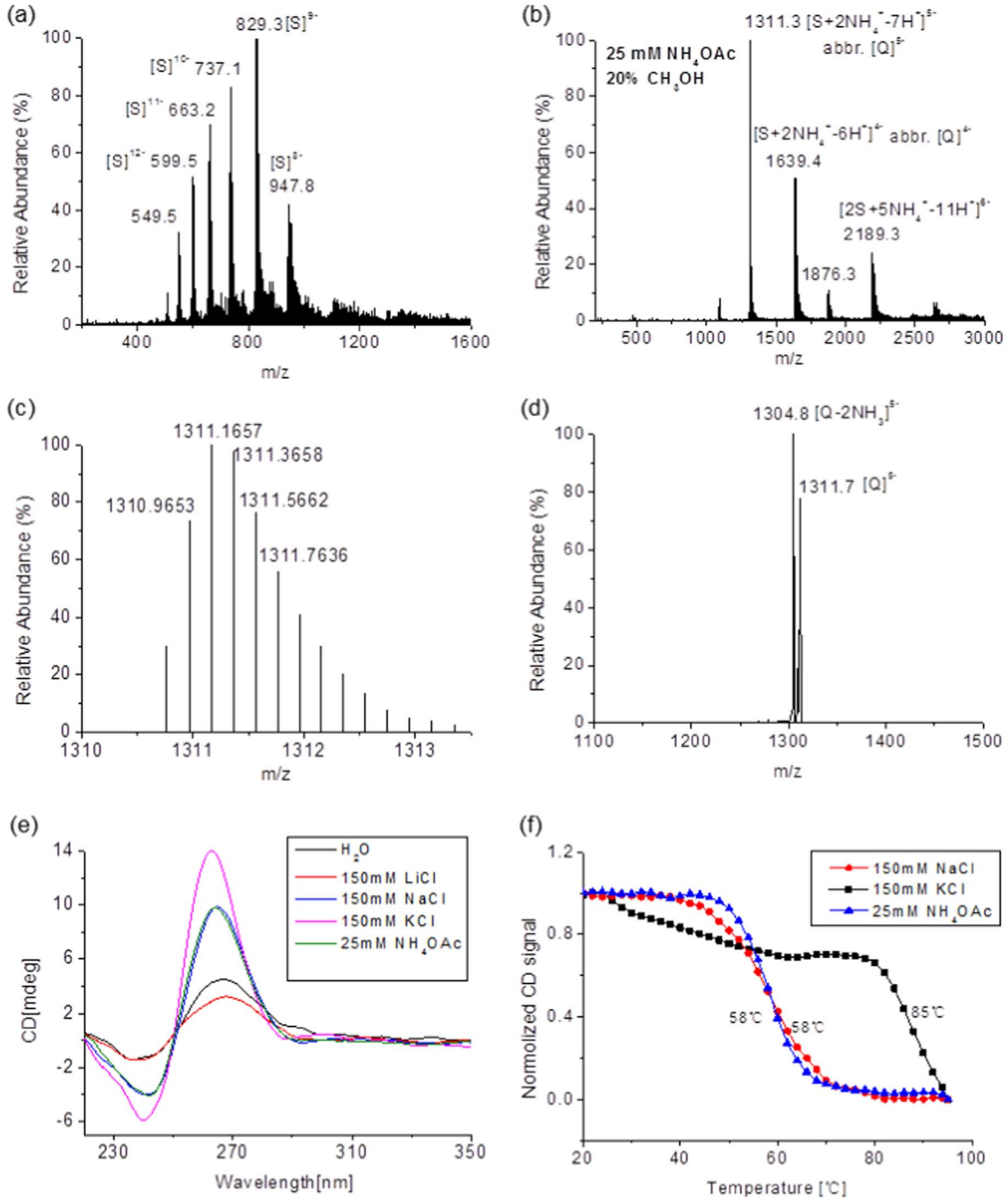

Fig. 1. ESI-MS spectra of 5 μM miR-1587 in 0 mM (a) and 25 mM NH_4OAc (b) with 20% CH_3OH. (c) Isotopic distribution of m/z = 1311.3 ion peak in high resolution mass spectrum. (d) MS/MS spectrum of the m/z = 1311.3 ion peak. (e) CD spectra of miR-1587 in H_2O, 150 mM LiCl, 150 mM NaCl, 150 mM KCl and 25 mM NH_4OAc with 20% CH_3OH. (f) CD melting curves of miR-1587 in 150 mM KCl, 150 mM NaCl and 25 mM NH_4OAc with 20% CH_3OH.