Analytica Chimica Acta 964 (2017) 161–169

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Human telomeric hybrid-2-over-hybrid-1 G-quadruplex targeting and a selective hypersaline-tolerant sensor using abasic site-engineered monomorphism

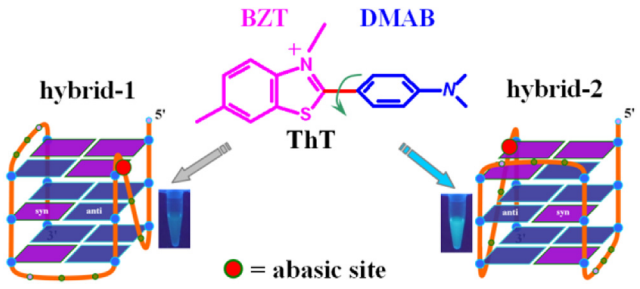
Tao Wu [a, 1], Meiyun Ye [b, 1], Tianyi Mao [b, 1], Fan Lin [a], Yuehua Hu [a], Ning Gan [c], Yong Shao [a, *]

[a] Institute of Physical Chemistry, Zhejiang Normal University, Jinhua, 321004, Zhejiang, China
[b] College of Chemistry and Life Sciences, Zhejiang Normal University, Jinhua, 321004, Zhejiang, China
[c] Faculty of Material Science and Chemical Engineering, Ningbo University, Ningbo, 315211, Zhejiang, China

HIGHLIGHTS

- Fluorescence discrimination of hybrid-2 G-quadruplex from hybrid-1 G-quadruplex in human telomere is achieved.
- Abasic site-induced monomorphism can be used as the best sensor platform.
- A selective K^+ sensor with a remarkable hypersaline-tolerant capability is developed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 October 2016
Received in revised form
13 January 2017
Accepted 21 January 2017
Available online 31 January 2017

Keywords:
Human telomeric G-quadruplex
Hybrid-1/Hybrid-2
Abasic site
Hypersaline
Fluorescent sensor
Selectivity

ABSTRACT

Coexistence of the polymorphic hybrid-1 and hybrid-2 conformers for a given human telomeric G-quadruplex-forming sequence (htG4) complicates its fine structure identification and limits its application as a sensor element. With help from abasic site (AP site)-engineered htG4s serving as the monomorphic representatives of the two typical hybrid conformers, we found that thioflavin T (ThT) can selectively target the hybrid-2 conformer over the hybrid-1 counterpart in monomer and tandem htG4 molecules. The htG4 that solely adopts the monomorphic hybrid-2 conformer engineered by the AP site is most efficient in lighting up ThT fluorescence in K^+ and a selective K^+ sensor is realized with a remarkable hypersaline-tolerant capability that can work even in 30000-fold excess of Na^+. At 600 mM Na^+, the dynamic range for K^+ detection can be extended to 30 mM with the limit of detection of 20 μM. This is the first report on the fluorescence discrimination of these two hybrid conformers of htG4 although they have long been categorized with their characteristically structural topologies. Our work will attract much interest in the development of sensors based on the monomorphic htG4 conformer since such high performance in sensor development has not been previously achieved.

1. Introduction

Along with gradual elucidation of unique biological functions of G-quadruplex (G4) structure in living cell [1,2], G4 has also been recognized as an essential element in development of innovative

* Corresponding author.
E-mail addresses: yshao@zjnu.cn, yshao@zjnu.edu.cn (Y. Shao).
[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.aca.2017.01.041
0003-2670/

sensors and devices due to the reliable tunability of its folding and stability [3–11]. Illustratively, some well-developed aptamers for, for example, ATP and thrombin, involve the G4 motif as the binding configuration. The representative human telomeric DNA G4 (htG4) has received much attention due to its importance in chromosome end protection [1] and many efforts have been made in finding ligands to stabilize and sense the variant htG4 structures [12–23]. The htG4 stabilizers are promising in developing into potential therapeutic drugs [24,25]. Concurrently, htG4 has also served successfully as a practical detection platform for telomerase activity [26–28], telomerase inhibitor [29], DNA oxidation [30], anticancer drugs [31], metal ions [16,32–39], and as a novel element in logic gates [40].

During developing htG4-based practices, the polymorphic coexistence for a given htG4 sequence [41] should be seriously considered when evolving towards a specific binding ligand. The strand orientation in htG4 has been resolved with variant conformer categories at least including the basket-type antiparallel topology in Na^+ solution, the hybrid topology (hybrid-1 and hybrid-2) in K^+ solution [16,42,43], and the parallel topology in K^+-containing crystal and molecular crowding condition [44–49]. Even for a given htG4 sequence, these conformers coexist through a sequence-dependent manner [50–56]. For example, the presence of an individual 5′-TA-3′ dinucleotide beyond the 5′ end of the htG4 tetrad core (TAQ, 5′-TAQ-3′, Q = 5′-$G_3(T_2AG_3)_3$-3′, Table S1) gives a G4 blend in K^+ with about 30% hybrid-2 (two successive lateral loops followed by one propeller loop from 5′ to 3′) and 70% hybrid-1 (one propeller loop followed by two successive lateral loops from 5′ to 3′). However, further extending another 5′-TT-3′ dinucleotide at the 3′ end of TAQ (to give TAQTT, 5′-TAQTT-3′) just reverses this hybrid-2-to-hybrid-1 ratio [50,53]. More interestingly, the lengthened htG4 thus with ability to form tandem G4s brings occurrence of hybrid-1 and hybrid-2 components in one strand [57–59], although other structure combination recipe has also been reported [48]. Due to the close structure similarity with the hybrid-2 and hybrid-1 conformers, their discrimination with a ligand is a great challenge. In practice, such conformer heterogeneity in a solution should also compromise the performance of htG4 as a sensor element.

Sklenář and Vorlíčková [60] recently found that introduction of an abasic site (AP site) into 5′-AQ-3′ (AQ) in replace of the adenine base can extremely narrow the structure distribution to a specifically monomorphic G4 conformer dependent on the position of the AP site. The replacement occurring at the adenine of the first loop (A7, counting from 5′ to 3′ end) favors the monomorphic hybrid-1 conformer in K^+ (thus named AP-H1, Fig. 1), while the replacement occurring at the adenine of the third loop (A19) supports the monomorphic hybrid-2 conformer (AP-H2, Fig. 1). Furthermore, the parallel G4 (AP-P, Fig. 1) is the dominant conformer when the three loop adenines (A7, A13, and A19, Fig. 1) are all replaced with AP sites. The parent AQ without any AP site, otherwise, forms polymorphic G4s in solution [60,61]. These findings inspire us to identify a ligand that can favorably target any of these hybrid conformers in order to improve the htG4-based sensor performance. In this work, we found that thioflavin T (ThT) can selectively bind with the hybrid-2 htG4 with an affinity-induced fluorescence brighter than binding with the hybrid-1 htG4. As a proof-of-principle application, we first develop a monomorphic hybrid htG4-based K^+ sensor that can even work at an extreme condition of highly concentrated salt. This sensor can endure 30000-fold excess of Na^+ for 20 μM K^+ detection. Our work demonstrates competence of htG4 in sensor development by engineering it towards a monomorphic conformer.

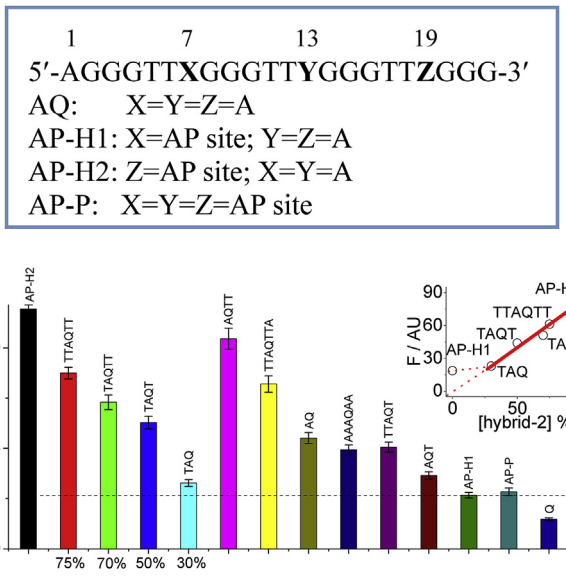


Fig. 1. Dependence of ThT fluorescence (0.5 μM) at 488 nm on the htG4 sequences (1 μM) in 0.1 M K^+ (pH 7.5). The sequences given above are the typical htG4s showing the position of adenine replacement with the AP site. The percentage labeled at abscissa is the population of hybrid-2 conformer acquired by previous NMR experiments. The dotted line reflects the fluorescence level for AP-H1 with solely the hybrid-1 conformer. Inset: dependence of ThT fluorescence on the reported population of the hybrid-2 conformer.

2. Experimental section

2.1. Materials and reagents

DNA oligonucleitides (Table S1) were synthesized by TaKaRa Biotechnology Co., Ltd (Dalian, China) and purified by HPLC. The nucleic acid concentrations (in strand unit) were measured by first dissolving DNA in pure water and detecting the UV absorbance at 260 nm using extinction coefficients calculated by nearest neighbor analysis. Buffers, metal ions (nitrate salt), NH_4Ac, and EDTA were of analytical grade (Sigma Chemical Co., St. Louis, USA) and used without any purification. $NaNO_3$ at the highest commercially available purity was used as the hypersaline condition. Thioflavin T (ThT, ultrapure grade) was obtained from AAT Bioquest, Inc. (California, USA) and used as received. For the abasic site-containing AP-H1 and AP-H2, tetrahydrofuran residue was used as the chemically stable abasic site analogue for replacement of the naturally tautomeric deoxyribose structure, as used in Ref. [60]. Milli-Q water (18.2 mΩ; Millipore Co, Billerica, USA) was used throughout the experiments.

2.2. Fluorescence measurements

Fluorescence spectra were acquired with a FLSP920 spectrofluorometer (Edinburgh Instruments Ltd., Livingston, UK) at 20 ± 1 °C, which was equipped with a temperature-controlled circulator (Julabo Labortechnik GmbH, Seelbach, Germany). Fluorescence was measured in a quartz cell with path length of 1 cm. If not specified, in order to prepare the htG4 solution with stable conformation, the nucleic acid strand was annealed in a thermocycler (first at 92 °C, then slowly cooled to room temperature) and stored at 4 °C overnight. ThT at the specified concentration was added into the nucleic acid solution, and the resulting solutions allowed incubation for 30 min before fluorescence measurements. Tris buffer (pH 7.5) containing the corresponding metal salt was used in the htG4 targeting investigation.