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Development of a novel antioxidant assay technique based on G-quadruplex DNAzyme

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ABSTRACT

Study on antioxidants' radical scavenging processes and antioxidant capabilities is important for understanding the protective role of antioxidants against oxidative damages associated with some chronic diseases and food degradation. Traditional methods to monitor the radical scavenging by antioxidant require expensive instrument and sophisticated synthesis process. Herein, we report a novel, simple, colorimetric DNAzyme-based method to detect radical-scavenging capacity of antioxidant. In this new strategy, horseradish peroxidase (HRP) mimicking DNAzyme catalyzes the oxidation of $ABTS^{2-}$ (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) by H_2O_2 to generate blue/green $ABTS^{\bullet-}$ radical, which can be scavenged by antioxidants resulting in color change. The typical kinetic curve of antioxidant-inhibited generation of $ABTS^{\bullet-}$ shows distinct biphasic pattern, involving a lag phase (stage I) and a linear increase phase (stage II). *kt* value, the product of lag time (*t*) and the slope of the curve in stage II (*k*), was used as the parameter for antioxidant capacity determination. This DNAzyme-based antioxidant assay has been effectively used to quantitatively detect the concentrations of antioxidants and evaluate the antioxidant capabilities of a variety of antioxidants and some real samples. Compared with traditional antioxidant assays, this method is thermostable, pH stable, and time-saving, which presents a promising platform for antioxidant assay.

1. Introduction

In recent years free radical reactions have attracted much attention because of their involvement in the degradation of polymer or food and the oxidative damage of DNA, protein and lipid (Coenjarts et al., 2003; Nau, 1998; Shigenaga and Ames, 1993; Shigenaga et al., 1994). Free radical induced oxidative damage has been implicated in many chronic diseases, such as cancer, atherosclerosis, cardiovascular diseases and neurodegenerative disorders (Ignatov et al., 2002). Antioxidants have been proven as effective free radical scavengers, which play a key role in preventing the damage of radical reactions (Niki and Noguchi, 2004; Ricciarelli et al., 2002). It is therefore important to study antioxidants' radical scavenging processes and antioxidant capabilities. Electron spin resonance (ESR) measurement is the traditional method for monitoring the radical scavenging by antioxidant (Guo et al., 2002), which is specific for radical detection but requires expensive ESR instrument and lacks sensitivity. Some fluorescent methods based on fluorophore-nitroxide probes have been applied in the detection of radical-scavenging ability of antioxidants (Aliaga et al., 2003). However, the specific fluorescent probes require sophisticated synthesis. Therefore, it still remains a requirement to develop new simple, economical, and reliable methods to probe the radical scavenging by antioxidants.

DNAzymes, which are selected by the systematic evolution of the ligand by the exponential enrichment (SELEX) process, are a kind of artificial enzyme that attracts substantial research efforts directed to the development of novel biocatalysts (Breaker, 1997, 1999; Chen et al., 2009; Emilsson and Breaker, 2002). In recent years, they have been employed as catalysts for numerous biochemical reactions such as cleavage of RNA or DNA phosphoesters (Breaker and Joyce, 1995; Carmi et al., 1996), porphyrin metalation (Li and Sen, 1996), DNA self-modification and DNA ligation (Cuenoud and Szostak, 1995; Levy and Ellington, 2001; Sugimoto and Wakizaka, 1998). Horseradish peroxidase (HRP) mimicking DNAzyme, PS2.M (Travascio et al., 1998, 2001), contains a special G-quadruplex structure with a intercalated hemin, which can catalyze the oxidation of $ABTS^{2-}$ by H_2O_2 to produce the colored radical ion ($ABTS^{\bullet-}$) (Deng et al., 2008; Li et al., 2007a, 2009a, 2009b; Nakayama and Sintim, 2009; Shlyahovsky et al., 2007; Teller et al., 2009), thereby causing a detectable color change. With this property, this DNA peroxidase mimic has been employed to develop many colorimetric biosensors for the specific and sensitive detection of various targets. For example, Pb^{2+}-specific DNAzyme

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coupled with the HRP-mimic DNAzyme was utilized to develop the biosensor for aqueous Pb^{2+} ion (Elbaz et al., 2008). Similarly, blocked aptamer–DNAzyme conjugates were used to act as aptasensor for the amplified analysis of adenosine or lysozyme (Li et al., 2007a). Recently, our group employed the DNAzyme and DNAzyme-based DNA machine in DNA methyltransferase (MTase) and restriction endonuclease assays (Li et al., 2010). All the existing HRP-mimic DNAzyme biosensing platforms employed the colored radical ion ($ABTS^{\bullet-}$), generated by DNAzyme's catalysis, as colorimetric signal read-out. However, the intrinsic property of $ABTS^{\bullet-}$ as a free radical is largely neglected in the design of DNAzyme-based methods. And, to the best of our knowledge, no such methods are currently available for radical-scavenging assay.

Herein, we develop a novel, simple, colorimetric DNAzyme-based method to detect radical-scavenging capacity of antioxidant, which extends the DNAzyme-based technology to sense antioxidant. Taking advantage of DNAzyme as a novel biocatalyst, $ABTS^{2-}$ is oxidized by H_2O_2 to generate blue/green $ABTS^{\bullet-}$ radical, which can be scavenged by antioxidants resulting in color change. This method can be used to quantitatively detect the concentrations of antioxidants and evaluate the antioxidant capabilities of a variety of antioxidants. Although a few of $ABTS^{\bullet-}$ derived methods have been employed in antioxidant assay, the advantages of this DNAzyme-based method over traditional methods using $ABTS^{\bullet-}$ have also been shown.

2. Materials and methods

2.1. Chemicals and instruments

HAP (High Affinity Purification) purified oligonucleotide (5′-GGGTAGGGCGGGTTGGGT-3′), ABTS obtained as the diammonium salt, hemin (bovine) and 2-[4-(2-hydroxyethyl)-1-piperazine] ethanesulfonic acid (Hepes) were obtained from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and used as received. Myoglobin (from equine skeletal muscle, Mb) and Triton X-100 were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used without further purification. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from J&K CHEMICA (New Jersey, USA) and used as an antioxidant standard. Baicalin, kaempferol, ferulic acid and 3′,4′-dihydroxyflavone were from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Quercetin, caffeic acid and protocatechuic acid were kindly provided by Prof. Chen and Dr. Liu at Hunan Normal University. The purity of these antioxidant compounds was ≥98%. Human serum was obtained from Hospital of Hunan University and stored in the blood storage refrigerator (Haier, China). Hydrogen peroxide (30%, w/v), in stabilized form, was purchased from Shanghai Taopu Chemical Plant (Shanghai, China). All other chemicals were of analytical grade.

DNA oligomers were dissolved in water and stored at 4 °C. Hemin solution (4 mM) was prepared by dissolving accurately weighted amount of hemin in 1 mL of dimethylsulfoxide (DMSO) and ultrasonicating until fully dissolved. Diluted hemin stock solution (40 μM) was made up in DMSO and frozen in the dark at −20 °C. Stock solutions (1 mM) of baicalin and kaempferol were prepared in DMSO. Solutions of 2 mM ferulic acid and 0.2 mM Trolox were prepared freshly in water. Stock solutions (1 mM) of quercetin, caffeic acid, 3′,4′-dihydroxyflavone and protocatechuic acid were prepared in ethanol. The UV–Vis spectra of all the above-mentioned solvents were recorded, no interference was found to the antioxidant measurement. Nanopure water (18 MΩ) was used in the experiments.

Experiments were performed on the Beckman spectrophotometer model DU-800 fitted with peltier temperature control. The detection was done in the kinetic mode at 734 nm, except otherwise described.

2.2. Colorimetric measurements for the DNAzyme-based assay

A solution of DNA (1×10^{-6} M) in a buffer consisting of NaCl (200 mM), KCl (20 mM), Hepes (25 mM), Triton X-100 (0.05%, w/v) was prepared. Hemin (8×10^{-7} M, final concentration) was added to the oligonucleotide solution (at oligomers/hemin ratio of 1.25:1 mol/mol), and the mixture was gently mixed for 30 min at 20 °C to yield the DNA–hemin complex. And then, $ABTS^{2-}$ (1.8×10^{-4} M, final concentration) was added into the solution. The reaction was initiated with the addition of H_2O_2 (4.4×10^{-4} M, final concentration).

2.3. Colorimetric measurements at different DNAzyme concentrations

This assay followed the similar protocol as the aforementioned DNAzyme-based assay, except that DNA–hemin complex was at different concentrations.

2.4. Colorimetric assay for antioxidants, beverage and serum samples

The DNA–hemin complex solution was prepared as aforementioned. The antioxidants (at varying concentrations), beverage (2 μL) or serum (1 μL) samples and $ABTS^{2-}$ (1.8×10^{-4} M, final concentration) were added into the DNA–hemin complex solution, then H_2O_2 (4.4×10^{-4} M, final concentration) was added to initiate the reaction.

2.5. Spectroscopic study of DNA–hemin interactions

As the binding of DNAs to hemin can be reflected by the hyperchromicity of the hemin Soret band (Travascio et al., 1998, 1999), the Soret band of hemin (centered at 396–405 nm) was recorded using the UV–Vis spectrophotometer. In order to investigate the influence of antioxidant, the antioxidant with a final concentration of 10 μM was added into the DNA–hemin complex solution. The UV–Vis spectrum was recorded from 350 nm to 540 nm.

2.6. Study on the influence of temperature and pH to catalytic activity of DNAzyme and myoglobin

For the study on the influence of temperature or pH, the experiment followed the same protocol as described in DNAzyme-based assay, but with some modifications. The DNA–hemin complex and Mb (3.6×10^{-6} M) solutions were prepared in the test tubes before the measurements. In the study on the influence of temperature, the test tubes were heated at 20 °C, 40 °C, 60 °C and 80 °C for 30 min, respectively. While for the study of pH, the DNA–hemin complex or Mb was incubated in buffer at different pH (3, 5, 7, and 9) for 30 min. The buffers of different pH were prepared according to the reference (Travascio et al., 1998). After 30 min incubation, $ABTS^{2-}$ and H_2O_2 were added to initiate the reaction. All determinations were carried out at least three times.

3. Results and discussion

3.1. DNAzyme-based assay for measuring antioxidant capacity

Fig. 1A depicts the design principle of this DNAzyme-based antioxidant assay. In the presence of coordination cation (K^+), the single-stranded DNAzyme sequence is able to fold into a G-quadruplex conformation, which can bind hemin to form the