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Review

Analytical potential of the quadruplex DNA-based FRET probes

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Abstract

DNA exhibits structural flexibility and may adopt also tetraplex structures known as guanine-quadruplexes or G-quadruplexes. These G-quadruplexes have recently received great attention because G-rich sequences are often found in genome and because of their potential links to mechanisms that relate to cancer, HIV, and other diseases. The unique structure of quadruplexes has also stimulated development of new analytical and bioanalytical assays based on fluorescence resonance energy transfer (FRET). Intramolecular folding of a flexible single-stranded DNA molecule into a compact G-quadruplex is a structural transition leading to closer proximity of its 5′- and 3′-ends. Thus, labeling both ends of a DNA strand with donor and acceptor fluorophores enables monitoring the quadruplex formation process by means of the FRET signal.

This review shows how FRET technique contributes to G-quadruplex research and focuses mainly on analytical applications of FRET-labeled quadruplexes. Applications include studies of structural transitions of quadruplexes, FRET-based selection of ligands that bind to quadruplexes, design of molecular probes for protein recognition and development of sensors for detection of potassium ions in aqueous solution.

Keywords: Fluorescence; FRET; G-quadruplex; Potassium ion; Proteins; Quadruplex-binding ligands; Telomeric DNA; Tetraplex DNA; Thrombin aptamer

Contents

1. Introduction

Fluorescence spectroscopy is used widely in nucleic acids research to study the structure of nucleic acids as well as their interactions with small organic molecules and proteins. In addition, a large number of fluorescence-based assays have been reported for the detection and quantitation of DNA and RNA. Almost all of these investigations have targeted the classical DNA duplex. However, there are evidences that DNA exhibits structural flexibility and may adopt also tetraplex structures, which is essential for some DNA functions. Such tetraplex structures known as G-quadruplexes or G4 DNA can be formed by single-stranded DNA with guanine-rich sequences [1,2]. These G-quadruplexes have recently received great attention because G-rich sequences are often found in genome and because of their potential links to mechanisms that relate to cancer, HIV, and other diseases [3–5]. For example, telomeric DNA sequences consist of several G-rich motifs; the T_2AG_3, T_4G_4, and T_2G_4 are the repeats of the telomeric DNA in vertebrates, *Oxytricha*, and *Tetrahymena*, respectively. These sequences can associate to form G-quadruplexes with highly polymorphic structures that depend on the metal cation present in solution. The fact that

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appropriate DNA sequences can interconvert to G-quadruplex structures in the presence of alkali metal cations, suggest the existence of ion-driven regulatory mechanisms in vivo.

Beside metal cations, a number of promising small organic molecules have been devised to selectively promote the formation and/or stabilization of guanine-quadruplex structures, ranging from derivatives of anthraquinones to porphyrins, acridines, and others planar compounds [6–10]. These ligands have the common feature of extended planar aromatic electron-deficient chromophore with cationic substituents.

Another interest in G-quadruplexes is connected with their biological significance. Beside involvement in the regulation of telomerase activity, G-quadruplexes were linked to proteins that either specifically bind to or promote the folding of quadruplex structures [11,12]. Thrombin-binding aptamer (5′-d(GGTTGGTGTGGTTGG)-3′) is an example of oligonucleotide that exploits quadruplex motif for thrombin-binding event [13].

Several techniques are employed to explore formation and properties of G-quadruplexes including X-ray diffraction, NMR spectroscopy, circular dichroizm (CD), mass spectrometry, and UV–vis spectroscopy. Fluorescence spectroscopy, and particularly resonance energy transfer technique (FRET), has been recognized as powerful tool to study G-quadruplexes due to its high sensitivity and multidimensionality. Using such fluorescence parameters as intensity, anisotropy, lifetimes, spectra, and fluorescence resonance energy transfer (FRET), different aspects of the molecular structure can be revealed as well as information on the concentration, binding events, and interstrand motion can be obtained.

This review will show briefly how FRET technique contributes to the G-quadruplex research and focuses mainly on the FRET application. Review will summarize the current developments in the field of the molecular aptamer probes for protein recognition and for visualization of potassium ions in aqueous solution with the use of quadruplex-based sensors (PSO—potassium sensing oligonucleotide).

2. Fluorescence resonance energy transfer

Fluorescence resonance energy transfer (FRET) is a spectroscopic method that provides distance information on macromolecules in solution and is particularly suited to the analysis of the structural changes in proteins and nucleic acids [14,15]. In a typical FRET experiment, a biopolymer is labeled with two different fluorophores, a donor and an acceptor, covalently attached at different locations. Interactions between the electronic excited states of these dye molecules lead to the transfer of excitation energy in a non-radiative process from a donor molecule to an acceptor molecule. The acceptor must be within the distance of 10–80 Å from the donor to provide a reasonable energy transfer signal [14]. FRET efficiency (E) can be measured by looking at the decrease in the fluorescence (or lifetime) of the donor or at the increase in the fluorescence of the acceptor. Förster showed that FRET efficiency depended on the inverse sixth power of the distance (R) between the two fluorophores (Eq. (1)) and this is the basis of the use of the technique to provide structural information [16]:

$$E = (1 + R^6/R_0^6)^{-1} \quad (1)$$

R_0 is the characteristic Förster radius for a given donor–acceptor pair, which is given by:

$$R_0^6 = 8.8 \times 10^{-28} \Phi_D \kappa^2 n^{-4} J(\nu) \quad (2)$$

where Φ_D is the fluorescent quantum yield of the donor in the absence of the acceptor, κ^2 is parameter that depends on the relative orientation of the donor and acceptor transition moments, n is the refractive index of the medium, and $J(\nu)$ is the spectral overlap between donor emission and acceptor absorption. From the Eq. (1) it is clear that for $R = R_0$, the efficiency of FRET is 50%.

FRET techniques have been used in a number of studies focused on nucleic acids. Successful applications involve study of conformational changes of nucleic acids [17,18], investigation of DNA hybridization and melting profiles [19], DNA triple helix formation [20], elucidation of the overall geometry of four-way DNA junctions [21], or development of molecular beacons and quantitative PCR analysis [22,23]. The last two examples constitute bioanalytical applications that exploit hybridization process (Watson-Crick pairing) for the analytical signal generation.

3. Guanine quadruplexes

The DNA tetraplexes or G-quadruplexes exhibit four-stranded structures containing one or more nucleic acid strands, in parallel or antiparallel orientations [24]. Four guanines on

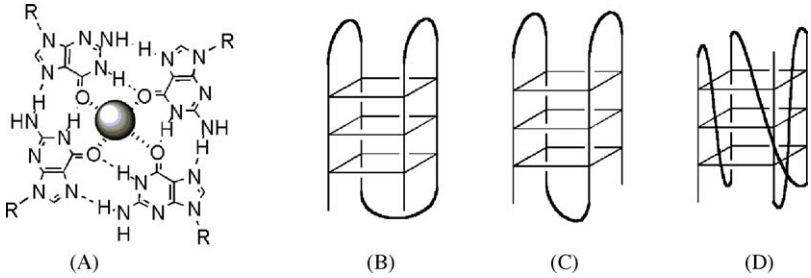

Fig. 1. (A) Structure of G-tetrad showing hydrogen bonds between four guanines and the interaction with a cation (filled circle). Schematic representation of G-quadruplex structures: an intramolecular antiparallel “chair-type” G-quadruplex with all lateral loops (B), an intramolecular antiparallel “basket-type” G-quadruplex with one diagonal and two lateral loops (C), and an intramolecular parallel quadruplex with all loops positioned alongside the grooves (D).