 

# Synthesis and Characterization of a Fluorescein-Labeled Circular G-Quadruplex

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A fluorescein-labeled circular G-quadruplex was readily synthesized chemically using a linear G-rich precursor. Hydrolysis tests and fluorescence spectroscopic examination confirmed that the product in our investigation has a circular backbone and possesses a fluorescein moiety.

A G-quartet is a cyclic array of four guanine bases assembled through intermolecular hydrogen bonds.[1] Composed of two or more stacks of such G-quartets, a G-quadruplex can be formed via either unimolecular assembling of DNA or through intermolecular association of two or more strands of G-rich sequences.[2–10] Certain guanine-rich sequences that exhibit potential for forming G-quadruplex were found in the past in a number of important DNA regions, such as those present at the ends of telomeres, in the promoter region of c-myc and in the upstream of the insulin gene.[11] In addition, a variety of proteins have been discovered, thus far, that are capable of binding to certain structural forms of G-quadruplexes.[12] With the aim of probing the interaction between G-quadruplex and its binding proteins, we previously synthesized certain circular features of G-quadruplex through chemical ligation reactions.[13] One of the advantages of utilizing circular oligonucleotides as molecular probes is that they have higher melting points than their linear counterparts and resist the hydrolysis by exodeoxyribonucleases.[14] In this study, we report the synthesis and characterization of circular G-quadruplexes labeled with fluorescein moieties. Fluorescein-tagged circular G-quadruplexes could be useful molecular probes for identifying new types of G-quadruplex-binding proteins.

Our strategy for the synthesis of circular G-quadruplex tagged with a fluorescein moiety is depicted in Fig. 1. Sequence 1 (Fig. 1) is a linear 32-mer oligodeoxyribonucleotide containing a fluorescein moiety covalently linked to carbon-5 of a thymine within the sequence. It was thought that the fluorescent moiety would have little effect on the formation of the G-quadruplex because the covalently modified thymine would be located in the loop region of G-quadruplex. Accordingly, a stock solution of sequence 1 (2 μM) in a buffer solution containing 100 mM MES (pH 6.0) and 100 mM KCl was heated to 100 °C and kept at the same temperature for three minutes followed by cooling the mixture to 25 °C over one hour. A reaction mixture containing 1 μM of annealed sequence 1, 100 mM MES (pH 6.0), 100 mM KCl, 10 mM $MnCl_2$, and 10 mM *N*-cyanoimidazole in a total volume of 20 μL were then prepared and incubated at 25 °C for 24 h. The reaction was then terminated by the addition of a loading buffer, and the resultant ligation product was analyzed by polyacrylamide gel electrophoresis.

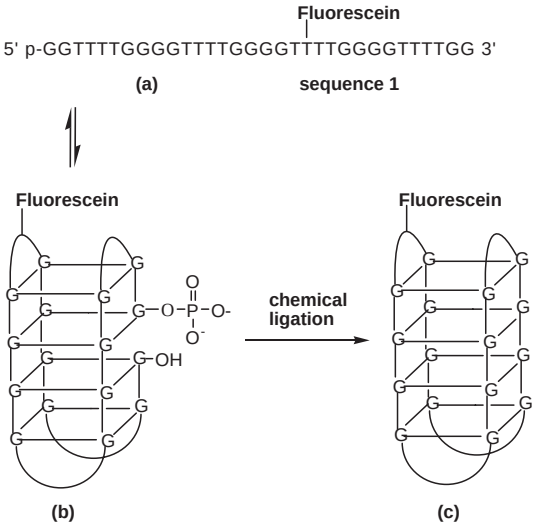


Fig. 1. Schematic representation of our synthetic route toward fluorescein-labeled circular G-quadruplex.

As shown in the autoradiogram in Fig. 2, a new product was formed during the ligation reaction, which has a slower mobility shift than that of sequence 1 (upper band of lane 5) and was thought to be the desired fluorescein-labeled circular G-quadruplex (Fig. 1c). As controls, circularization reactions of a non-fluorescein-labeled oligonucleotide **5′pGGTTTT-GGGGTTTTGGGGTTTTGGGGTTTTGG3′**, sequence 2, were also carried out (Lane 2) under the same conditions as the one for the sample loaded into Lane 5, Fig. 2. The circularization product for non-fluorescein-tagged oligonucleotide (Lane 2) possessed a faster rate of mobility shift than that of fluorescein-tagged oligonucleotide (Lane 5). The mobility shift difference between these two circular products can be attributed to the possession of an extra fluorescein moiety within the fluorescein-tagged circular product (Fig. 1c).

To verify that circularization occurred, product (upper band

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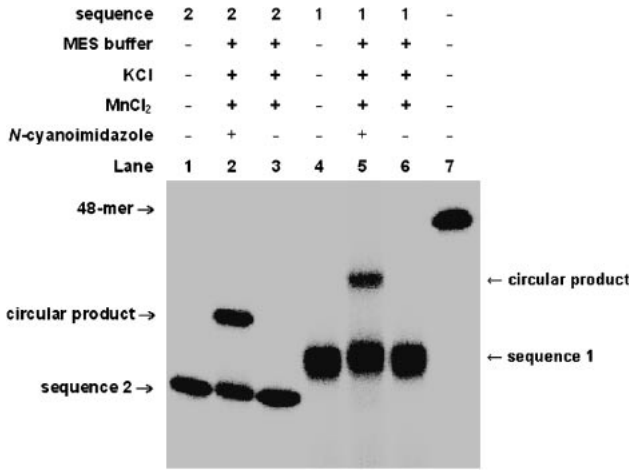


Fig. 2. Electrophoretic analysis of fluorescein-labeled circular G-quadruplex. Lane 1: sequence 2 alone. Lane 2: same reaction as the one loaded in Lane 5 except for replacing sequence 1 with sequence 2. Lane 3: same reaction as the loaded in Lane 2 except for the absence of *N*-cyanoimidazole. Lane 4: sequence 1 alone. Lane 5: a reaction mixture containing 1 μM annealed sequence 1, 100 mM MES (pH 6.0), 100 mM KCl, 10 mM $MnCl_2$, and 10 mM *N*-cyanoimidazole in a total volume of 20 μL was incubated at 25 °C for 24 h. Lane 6: same reaction as the loaded in Lane 5 except for the absence of *N*-cyanoimidazole. Lane 7: 48-mer oligonucleotide as a molecular weight marker.

in Lane 5 of Fig. 2) was purified and digested with exonuclease VII (an exodeoxyribonuclease that hydrolyzes linear DNA from both 3′ and 5′ termini). Because it has no open ends in its structure, circular oligodeoxyribonucleotide should, in theory, resist the degradation by this exonuclease.[14] In Fig. 3, the product from the circularization reaction completely resisted the hydrolysis by exonuclease VII (Lane 4) as expected. In contrast, a linear sequence (sequence 2) was hydrolyzed to completion by exonuclease VII (Lane 2) under the same reaction conditions. In addition to degradation by exodeoxyribonuclease, hydrolysis of the fluorescein-labeled circular G-quadruplex was carried out by using DNAse I (an endonuclease hydrolyzing both single and duplex forms of DNA in a randomized pattern). A major band was observed upon partial hydrolysis of our fluorescein-labeled circular product by the endonuclease (Fig. 4). This degradation product displayed the same rate of mobility shift as that of its linear precursor (Lane 3), which is characteristic of randomized partial hydrolysis of circular oligonucleotides by enzyme or chemicals.[15] The results of both exonuclease and endonuclease hydrolysis in these studies are consistent with a circular backbone structure.

It is well known that when fluorescein-labeled oligonucleotides were examined with fluorescence spectroscopy, emission is readily detectable.[16] In order to verify that the molecular moiety of fluorescein was indeed present in our circular G-quadruplex, fluorescence spectroscopic measurements were carried out on the corresponding samples of oligonucleotides. As shown in Fig. 5, the circular G-quadruplex had an emission maximum at 520 nm when the wavelength of excitation was set at 495 nm while a non-fluorescence-tagged oligonucleotide (sequence 2) displayed no emission in the corresponding range

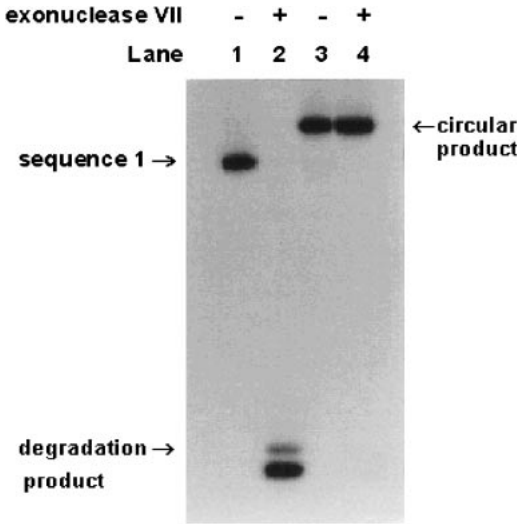


Fig. 3. Hydrolysis of fluorescein-labeled circular products by exonuclease VII. Lane 1: sequence 1 alone. Lane 2: sequence 1 after treatment with 20 units of exonuclease VII at 37 °C for 2 h. Lane 3: fluorescein-labeled circular G-quadruplex alone. Lane 4: fluorescein-labeled circular G-quadruplex after treatment with 20 units of exonuclease VII at 37 °C for 2 h.

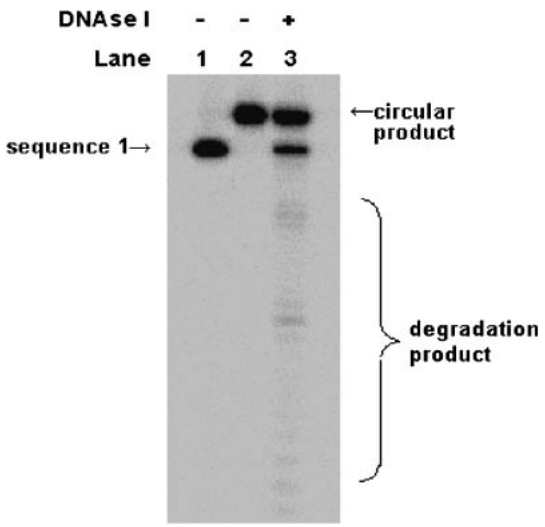


Fig. 4. Partial hydrolysis of the fluorescein-labeled circular products by DNAse I. Lane 1: sequence 1 alone. Lane 2: fluorescein-labeled circular G-quadruplex alone. Lane 3: fluorescein-labeled circular G-quadruplex after treatment with 10 units of DNAse I at 25 °C for 30 min.

of wavelengths. In other words, the fluorescein moiety is included in the circular G-quadruplex.

In summary, a fluorescein-labeled G-quadruplex was readily synthesized through chemical ligation reactions. This fluorescein-tagged circular oligonucleotide resists hydrolysis by an exodeoxyribonuclease, as anticipated. Fluorescence spectroscopic analysis revealed that fluorescein moiety is attached to the circular G-quadruplex. It is anticipated that our newly designed fluorescein-labeled circular G-quadruplex will be useful probes for identifying new types of enzymes and other functional proteins correlated with G-quadruplex in vivo and

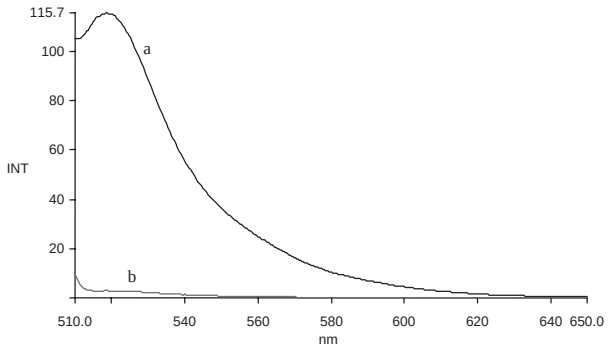


Fig. 5. Fluorescence emission spectra of fluorescein-labeled circular G-quadruplex (a) and non-fluorescein-labeled linear oligonucleotide, sequence 2 (b). Samples in the measurements contained 1 μM oligonucleotides and 10 mM Tris buffer (pH 7.0) and were examined at 20 °C with excitation set at 495 nm.

for tracing the location and distribution of G-quadruplex-binding proteins in living cells by detection of the emission of the fluorescein covalently linked to circular G-quadruplex.

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