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Assessing DNA structures with ^{125}I radioprobng

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Summary

Iodine-125 radioprobng is based on incorporation of radioiodine into a defined position in a nucleic acid molecule. Decay of ^{125}I results in emission of multiple low energy Auger electrons that, along with positively charged residual daughter nuclide, produce DNA strand breaks. The probability of such strand breaks at a given nucleotide is in reverse proportion to the distance from the ^{125}I atom to the sugar of that nucleotide. Therefore, conclusions can be drawn about the conformation or folding of a DNA or RNA molecule base on the distribution of ^{125}I decay-induced strand breaks. Here we describe in detail an application ^{125}I radioprobng to study the conformation of quadruplex structures, and discuss the advantages and limitations of the method.

Keywords

Iodine-125; radioprobng; DNA structure; telomere; quadruplex

1. Introduction

Probing the chemical reactivity of DNA or RNA nucleotides with various agents is widely used in studies of the conformation of nucleic acids. Accessibility of the nucleotides to the agents is usually revealed by the analysis of the consequent strand breaks. Methods based on such probing provide valuable information on the conformation of nucleic acids and often complement and verify structural data obtained by X-ray crystallography and NMR. For example, the discovery of the quadruplex structure in G-rich oligonucleotides was based on the observation that the N7 position of guanines was protected from dimethylsulfate modification (1). Iodine-125 radioprobng is a similar methodology but different in approach (2). It is based on the analysis of nucleic acid strand breaks produced close to the site of decay of an ^{125}I atom. The majority of local breaks produced by ^{125}I decay result from the direct action of radiation. The probability of these breaks occurring depends mostly on the distance from the radionuclide decay site to the corresponding nucleotides (3), and to a lesser extent on their accessibility to solution like in other probing methods that rely on a diffusible chemical reagent.

Iodine-125 belongs to a class of radioisotopes called Auger-electron-emitters that decay by electron capture or internal conversion. Both of these processes create a vacancy in an inner atomic shell that results in a cascade of electron transitions accompanied by the emission of a number of orbital electrons, so-called Auger electrons (4). Decay of ^{125}I produces on average 21 such electrons. The energy of the majority of these electrons is below 1 keV which is considerably less than the energy of nuclear decay-generated beta particles. Auger

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²We use 20x40 cm glass plates with 0.4 mm spacers.

electrons produce breaks in DNA or RNA strands either directly or by generation of OH radicals in the “bound” water. In addition, the nucleotides located next to that site are affected by the positively charged (+21 on average for ^{125}I) daughter nucleus which, by stripping electrons from the neighboring bonds, may also contribute to the strand breakage (5).

Iodine-125 can be easily incorporated into DNA as [^{125}I]-iododeoxycytosine (^{125}I -dC) using a DNA polymerase and commercially available ^{125}I -dCTP as a precursor (6). Most of the breaks (90%) produced by decay of ^{125}I are located within approximately one helical turn from the ^{125}I -dC incorporation site (7). Analysis of strand breaks in DNA and DNA-protein complexes with known 3-D structure confirmed experimentally that the probability of breaks depends primarily on the distance from ^{125}I to the sugar of a target nucleotide (7–9). The strand breaks are due to the energy deposition or radical attack in the sugar-phosphate moiety since damage to the DNA bases generally does not lead to strand scission. Therefore, radioprobng, like “low resolution” NMR, allows one to obtain information on the interatomic distances and, in principle, to reconstruct the 3D-structure of nucleic acids in complexes with proteins.

Unfortunately, due to the complex nature of DNA breaks and multiple mechanisms involved, currently there is no comprehensive theoretical model that would establish a relationship between the probability of breaks at a given nucleotide and the distance to its atoms. Therefore, the analysis of the strand breaks is limited to the establishment of the order, in term of distance, in which the nucleotides are located relative to the ^{125}I decay site. However, even this simple analysis proved to be useful in providing unique information on the conformation of DNA and DNA-protein complexes studied so far (10–12). Here we describe application of radioprobng to the analysis of quadruplex DNA structures in oligonucleotides.

2. Materials

2.1. Oligodeoxyribonucleotides

Oligodeoxyribonucleotides (ODN) (primer and template, Fig. 1) were synthesized on an ABI394 DNA synthesizer (PE Applied Biosystems, Foster City, CA), and purified by denaturing polyacrilamide gel electrophoresis (PAGE) as described in (11). The concentration of single stranded ODN was measured at 260 nm on a HP 8452A Diode Array Spectrophotometer, and was calculated with an online oligonucleotide properties calculator (19).

2.2. Oligonucleotides labeling

2.2.1 Telomeric ODN labeling with [γ - ^{32}P]-ATP and T4 PNK

1. T4 Polynucleotide kinase (T4 PNK) 10 U/ μL , supplied with 10x reaction buffer: 500mM Tris-HCl (pH 7.6 at 25°C), 100mM MgCl_2 , 50mM DTT, 1mM spermidine and 1mM EDTA (Fermentas, Hanover, MD; cat. no EK0031).
2. EasyTides® [γ - ^{32}P]-adenosine 5'-triphosphate ([γ - ^{32}P]-ATP), Specific Activity: 6000 Ci/mmol, 10 mCi/mL, 50mM Tricine pH 7.6 (Perkin Elmer, Waltham, MA; cat. no NEG502Z500UC).
3. 0.5 M EDTA pH 8.0: dissolve 18.61 g EDTA in 80 ml of water. Adjust pH to 8.0 with NaOH (approx 2 g NaOH). Bring the volume to 100 mL with water.
4. MicroSpin™ G-25 Columns (GE Healthcare, Piscataway, NJ; cat. no 27-5325-01 or equivalent).

2.2.2. Incorporation of ^{125}I -dCTP by primer extension reaction with Klenow fragment of DNA polymerase I

1. 5- ^{125}I Iodo-2'-deoxycytidine-5'-triphosphate (^{125}I -dCTP), Specific Activity: 2200 Ci/mmol, in a solution containing methanol:water 3:1 (Perkin Elmer, Waltham, MA; cat. no NEX074S).
2. Klenow fragment of DNA polymerase I (Klenow fragment) 10 U/mL, supplied with 10x reaction buffer: 500mM Tris-HCl (pH 8.0 at 25°C), 50mM MgCl_2 , 10mM DTT (Fermentas, Hanover, MD; cat. no EP0051).
3. dNTP set - 100mM aqueous solutions at pH 7.0 of each of dATP, dCTP, dGTP and dTTP (Fermentas, Hanover, MD; cat. no R0181).

2.2.3. PAGE purification

1. Formamide stop solution (USB Corporation, Cleveland, OH; cat. no 70724).
2. SequaGel® Sequencing System consists of SequaGel Concentrate (237.5 g of acrylamide, 12.5 g of methylene bisacrylamide, and 450.5 g urea in 1 L of deionized aqueous solution), SequaGel Diluent (7.5M urea in deionized water), and SequaGel Buffer: 200 ml bottles containing 0.89M Tris-Borate, 20mM EDTA pH 8.3 (10X TBE) and 7.5M urea (National Diagnostics, Atlanta, GA; cat. no EC-833).
3. Tris-buffered saline (TBS): Prepare 10X stock with 1.37M NaCl, 27 mM KCl, 250 mM Tris-HCl, pH 7.4. Dilute 100 mL with 900 mL water for use.
4. Spin-X Centrifuge Tube Filter 0.22 mm Cellulose Acetate (Corning Incorporated, Corning, NY; cat. no 8160).

2.3. Required equipment

1. Vacuum centrifuge with a cold trap (Jouan RC 1010 or equivalent) – needed to dry ^{125}I -dCTP from methanol/water mixture.
2. Power supply for sequencing PAGE, capable of providing up to 3000V, 60W.
3. Gel dryer.
4. Freezers (-20°C and -80°C).
5. Water bath or incubator at 37°C .

3. Methods

A repeated non-coding DNA sequence $\text{d}(\text{TTAGGG})_n$ is present in the telomeric ends of all human chromosomes. These repeats can adopt multiple inter- and intra-molecular non-B-DNA conformations that may play an important role in biological processes. NMR spectroscopy and X-ray crystallography have solved several intra-molecular structures of the telomeric oligonucleotides - antiparallel, parallel and “mixed-type” hybrid type I and II structures containing both parallel and anti-parallel strands (Fig. 2) (13–18).

We applied ^{125}I -radioprobe to determine the conformation in solution of the human telomeric quadruplex in $\text{dAGGG}(\text{TTAGGG})_3$ (Fig. 1), the same sequence that was used in the NMR and crystallographic analyses. An oligonucleotide containing four human telomeric repeats was labeled with ^{125}I at a single internal position and was allowed to fold into quadruplexes under different ionic conditions and then frozen for decay accumulation. Probabilities of break at individual nucleotides were determined by sequencing gel-electrophoresis. Analysis of the quadruplex fold was based on a comparison of break

probability with the distance from ^{125}I to the corresponding nucleotides in different quadruplex models.

3.1. Oligonucleotide labeling

3.1.1 Telomeric ODN labeling with $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$ with T4 PNK

1. Take 20 pmol of G4-forming oligonucleotide (primer) and mix it with 2 μL of 10x T4 PNK buffer, 40–80 pmol of $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$
2. Add 1 μL (10 units) of T4 PNK. Adjust volume of the reaction mixture to 20 μL with water.
3. Incubate the sample at 37°C for 40 minutes.
4. Stop reaction by addition of 30 μL of 20 mM EDTA pH 8.0.
5. Separate labeled DNA from unincorporated $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$ by gel filtration through a MicroSpin column.
6. Dry the sample in a vacuum centrifuge.

3.1.2. Incorporation of $^{125}\text{I}\text{-dCTP}$ by primer extension reaction with Klenow fragment of DNA polymerase I

1. Vacuum dry approximately 80–100 pmol of $^{125}\text{I}\text{-dCTP}$ (see Note 1)
2. Reconstitute dried oligonucleotide in the mixture of 1 μL of Klenow fragment reaction buffer and 6 μL of water.
3. Add 24 pmol of the complementary template oligonucleotide in 1 μL .
4. Incubate for 15 min at 37°C to form a duplex.
5. Mix lyophilized $^{125}\text{I}\text{-dCTP}$ with the annealed duplex.
6. Dilute 1 μL (10 U) of Klenow fragment with 9 μL of 1x Klenow fragment reaction buffer
7. Add to reaction mixture 1 μL of 10 mM dATP and 1 μL (1 U) of diluted Klenow fragment.
8. Incubate for 10 min at room temperature then add 1 μL of 10 mM of dNTP mixture and incubate for another 5 min at room temperature.
9. Stop reaction by addition of 30 μL of 20 mM EDTA pH 8.0.
10. Separate labeled DNA from unincorporated nucleotides by gel filtration through a G25 MicroSpin column.
11. Dry the sample in a vacuum centrifuge.

3.1.3. PAGE purification

1. Reconstitute sample in 3–5 μL of formamide stop solution.
2. Boil oligonucleotide sample briefly (1 min) in water bath, transfer to ice and load onto 12% polyacrylamide sequencing gel with urea.

¹Consult your Radiation Safety Department on the local regulations regarding work with radioiodinated compounds. $^{125}\text{I}\text{-dCTP}$ is supplied in a methanol/water mixture. It is not volatile and does not require special iodination hood for handling. Once dried it degrades rapidly. To dry such an amount it usually takes 30–40 min. Plan ahead to minimize the time that dried $^{125}\text{I}\text{-dCTP}$ will spend on the bench.

3. Run electrophoresis at 55W for approximately 1 hour.
4. Excise band from the gel, crush it in an Eppendorf tube and elute with ~200 μ L of $1\times$ TBS for 1 hour on shaking platform at room temperature.
5. Purify sample from acrylamide debris on 0.22 μ m cellulose acetate Spin-X filter, precipitate oligonucleotide with ethanol.
6. Wash the pellet with 70% ethanol stored at -20°C , and dry the pellet.
7. Re-suspend sample in 50 μ L of water and desalt by gel filtration through a MicroSpin column (GE Healthcare or equivalent packed with G-25 resin equilibrated in water).
8. Dry the sample in a vacuum centrifuge.

3.2. Analysis of break distribution

3.2.1. Sample preparation

1. Take ~0.1–0.3 μ Ci to prepare mixtures with different counter-ions and/or quadruplex binding compounds and (see Note #3).
2. Incubate for at least 1 hour at 37°C to allow quadruplex structure formation.
3. Quickly freeze the samples in liquid nitrogen and store for two weeks at -80°C to accumulate breaks.

3.2.2. Analysis of break distribution

1. After two weeks thaw the samples and analyze by 12% denaturing PAGE (Fig. 3).
2. Quantify DNA strand breaks analyzing digitized autoradiograph using image analyzing software (Note 4). The probabilities of breaks for the samples in lanes 1 and 2 in Figure 3 are plotted in Figure 4.

Higher probabilities of breaks at the nucleotides from A(–3) to T12 in presence of 150 mM KCl reflect folding of telomeric ODN into a compact structure. The maximum of breaks is observed in the loop T5-A7 that is adjacent to ^{125}I -dC18 in the basket and chair anti-parallel structures (Figure 2). Therefore, folding of the telomeric DNA is detected by radioprobings and distribution of probability of breaks is consistent with the presence of the antiparallel conformations in solution.

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³To decrease non-specific degradation of DNA, 10% DMSO may be used.

⁴To measure the intensity of the individual bands we are using Image Gauge software (FUJI Medical Systems USA) to generate the intensity profile of each lane from the digitized gel image. The profile should be deconvoluted to a series of the Lorentz-type peaks corresponding to individual bands as described in detail in (20). The probability of breaks are calculated from the areas of the individual peaks using a recursive formula and assuming that probability of breaks at [^{125}I]-dC equals 1 (Fig. 4) as described in detail in (7). Another way to analyze digitized gel images is by using SAFA software (21).

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Figure 1.

Sequences of oligodesoxyribonucleotides used. Nucleotides incorporated by the primer extension reaction are underlined.

Figure 2. The schematic diagram of four intramolecular conformations of human telomeric quadruplexes. Position of ^{125}I -C is shown by the mark. Arrows illustrate the distances between ^{125}I and T5-A7 loop; shorter in the antiparallel basket and chair conformations and longer in the mixed and parallel propeller conformations of G-4 quadruplex.

Figure 3.

Analysis of DNA strand breaks in 12 % denaturing PAGE. Lane 1: telomeric ODN + 150 mM KCl; lane 2: duplex of telomeric ODN and complementary template + 150 mM KCl.

Figure 4. Distribution of ^{125}I induced strand breakage probability in the telomeric ODN calculated from the data on Figure 3): in 150 mM KCl (●) and in duplex form (○).