Photoimmunodetection: A Nonradioactive Labeling and Detection Method for DNA


ABSTRACT

We describe a simple detection system for DNA based on antibody detection of UV-induced photoproducts. It includes a convenient and inexpensive labeling procedure, which is completed in 5–10 min. The only equipment required is a UV source such as an ordinary transilluminator or a DNA crosslinker. Using a monoclonal antibody specific for thymine dimers, coupled to horseradish peroxidase, we are able to detect subpicogram amounts of UV-irradiated DNA directly, and approximately 10 pg homologous DNA indirectly by hybridization with an irradiated probe.

INTRODUCTION

Nonradioactive labeling of DNA is typically based on the enzymatic incorporation of modified nucleotides, carrying a small chemical moiety such as biotin, digoxigenin or fluorescein. These tags are subsequently detected by specific reagents such as streptavidin or a specific antibody coupled to a signal-producing enzyme. Although very efficient and reliable, labeling by in vitro polymerization is time-consuming, expensive and may require various post-label purification steps to remove an excess of unincorporated precursors. Here we present an alternative strategy for DNA labeling, based on the UV-induced formation of thymine dimers and the detection of these photoproducts by specific antibodies.

The use of anti-thymine dimer antibodies for the detection of DNA probes has been reported previously (3,4). However, the low affinity and specificity associated with polyclonal antibodies limited their practical use. Here, we have extended these studies using monoclonal antibodies (MAbs) specific for the (5′-6′) cyclobutane type of thymine dimers, which can be produced specifically using moderate doses of 312-nm light in the presence of acetone or acetophenone (10). The antibodies have no detectable affinity for nonirradiated DNA, and an irradiated probe will easily allow detection of a single copy gene in small to medium-sized genomes.

MATERIALS AND METHODS

Anti-Thymine Dimer MAb

The isolation of an anti-thymine dimer specific MAb (H3) has been described previously (7). The antibodies were prepared and affinity-purified according to Roza et al. (7) and conjugated to horseradish peroxidase (HRP) by a method developed at Amdex A/S (Copenhagen, Denmark) (9).

DNA Material

Double-stranded (ds) DNA from phage φX174 and genomic RNA from Brome mosaic virus were obtained from Promega (Madison, WI, USA), while single-stranded (ss) φX174 DNA was from Life Technologies (Gaithersburg, MD, USA). Chromosomal DNA of the bacterial strains C-1a (C wild-

Figure 1. Detection of UV-irradiated DNA and RNA with anti-thymine dimer antibodies. Single-stranded φX174 DNA (A) and Brome mosaic virus RNA (B), respectively, were irradiated with 40 kJ 312-nm light per m² in the presence of 2 mM acetophenone, and a series of dilutions were immobilized on a Quantum Yield membrane. Following incubation with HRP-conjugated H3 antibodies (1:10000), signals were detected with ECL. IR = irradiated; NI = nonirradiated control.
type; Reference 5) and C-1592 (C-1a P2cox; Reference 5) was prepared by standard techniques (8). The pEN21 plasmid probe used for hybridizations, was derived from pET21 (Novagen, Madison, WI, USA) by insertion of a fragment from pEE709 (2) comprising the A gene of phage P2.

UV Irradiation

DNA or RNA samples at a concentration of 10 ng/µL in 10 mM Tris-HCl, pH 7.5, were exposed to UV light as a drop of 100 µL resting in an open siliconized petri dish. In the case of 312-nm radiation, the Tris buffer was in all cases supplemented with 2 mM acetophenone. Various UV sources were tested, but defined doses were given with a TFL Crosslinker (Vilber Lourmat, Marne la Vallée, France) equipped with 6×15 W tubes showing emission peaks at 254 nm (T-15.C) or 312 nm (T-15.M), respectively. When an ordinary transilluminator was used, the effect (110 W/m²) was measured by a CDR-1 Monitor (Ultra-Lum, Carson, CA, USA), giving an approximate dose of 40 kJ/m² after 6 min.

Slot Blot

Irradiated samples were diluted in 6× standard saline citrate (SSC), and 50-µL aliquots were immobilized on a membrane using a Minifold® II apparatus (Schleicher & Schuell, Dassel, Germany).

Figure 2. Antibody detection of irradiated DNA as a function of dose and wavelength. Single-stranded φX174 DNA was irradiated in the presence of acetophenone with either 254- (A) or 312-nm (B) light for various time periods. Dilutions of irradiated DNA were immobilized and detected as in Figure 1.
Southern Blotting

Enzyme-digested chromosomal DNA was separated in a 0.7% agarose gel. Following depurination (0.25 M HCl for 10 min), denaturation (0.5 M NaOH, 1.5 M NaCl; 2×20 min) and neutralization (0.5 M Tris-HCl, pH 7.0, 1.5 M NaCl; 2×20 min), the DNA was transferred to a nylon membrane (Quantum Yield) by capillary blotting in 10×SSC as described by Sambrook et al. (8). Plaque lifts were performed according to Sambrook et al. (8). Plaques of P2 vir1 (5) or λ were formed on a lawn of C-1a indicator cells on LB plates overnight at 37°C.

Hybridization

The plasmid probe was heat-denatured for 10 min, chilled rapidly on ice and added to the prehybridization solution (6×SSC, 5×Denhardt, 0.5% sodium dodecyl sulfate [SDS], 50 μg/mL sonicated and denatured herring sperm DNA). Following overnight incubation (16 h) at 65°C in a hybridization oven (Hybaid, Teddington, England, UK), membranes were washed at 65°C in three successive steps with decreasing salt concentration: 15 min in 6×SSC, 0.5% SDS; 2 times 15 min in 2×SSC, 0.5% SDS; 15 min in 1×SSC, 0.5% SDS. Membranes were rinsed briefly in TBS-T (20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 0.1% Tween® 20) before detection with antibodies.

Immunochromelal Detection

Detection of the labeled DNA was performed identically for total DNA detection (slot blots) and hybridizations. Before incubation with antibodies, the membrane surface was saturated with 5% nonfat milk (Difco Laboratories, Detroit, MI, USA) in TBS-T for 1 h at room temperature. The thymine dimer specific antibodies were added directly to the blocking solution (1:10000) and incubation continued for 1 h. Following washing for 4×5 min in relatively large volumes of TBS-T (2–300 mL), the signals were developed by chemiluminescence using the ECL™ substrate from Amersham International plc (Little Chalfont, Bucks, England, UK) as recommended by the manufacturer.

RESULTS

Specificity of Antibodies

To explore the potential of photoimmunodetection (3,4), we have used a monoclonal antibody (H3) raised against thymine dimers (7). Its affinity is specific for the cyclobutane type of homothymine or thymine-cytosine heterodimers (L. Roza, unpublished observations). As can be seen in Figure 1A, at least 0.5 pg of irradiated ssDNA can be detected using these antibodies. Figure 1B shows their reaction with a comparable RNA sample. The relative difference in sensitivity is approximately 1:500, which may reflect a difference in affinity and a lower efficiency of pyrimidine dimer induction in RNA under the conditions used. As a practical consequence, the presence of RNA will show negligible interference with the detection of a DNA sample. We have also tested another unrelated antithymine dimer antibody, commercially available from Kamiya Biochemical (Tukwila, WA, USA) with comparable results (not shown).

Optimization of Labeling

Figure 2 shows the detection limit for irradiated DNA, with respect to wavelength and dose. A 100-μL drop of ssDNA from phage φX174 was irradiated for various time periods with either of two UV sources, having emission maxima at 254 or 312 nm, respectively. As can be seen in Figure 2A, 254-nm light leads to rapid formation of detectable photo products, up to a maximum after 10–15 kJ/m². However, at higher doses, a substantial decline in the signal strength is seen. This is consistent with previous observations (3), and probably reflects a combination of photo reversal and fragmentation of the target DNA. In contrast, 312-nm radiation (in the presence of acetophenone) leads to a slower, but steady increase in the signal strength up to a saturation level at 50–80 kJ/m² (Figure 2B). Compared to 254-nm radiation, the improvement in maximum sensitivity is about 5–10 fold.

Signal Detection

The HRP-conjugated antibodies were added directly to the blocking solution at an empirically determined dilution factor of 1:10000. Due to the polymeric nature of this conjugate, where up to several hundred antibody and enzyme molecules are interconnected on a dextran matrix (9), it is not possible to state the exact concentration of antibodies. However, compared to a 1:1 type of conjugate, previous studies suggest a 10-fold improvement in sensitivity and increased stability of the reagent (9). In a parallel experiment, we tested unconjugated antibodies, followed by detection with HRP-conjugated anti-mouse antibodies (Dako, Glostrup, Denmark). In addition to the inconvenience of introducing an extra incubation step, the sensitivity was in this case reduced by 80% (not shown).

Examples of Photoimmunodetection

We have applied the photoimmunodetection method to standard DNA hybridizations, such as plaque-lift and Southern blotting. Figure 3A shows de-
tection of P2 plaques using an irradiated plasmid probe (pEN21; 7.8 kbp), which carries a copy of the P2 A gene (2.4 kbp). Since supercoiling will be relaxed by UV-induced nicks, there is no need to linearize the plasmid before heat denaturation. As negative control, a similar membrane with a comparable number of λ plaques is shown in Figure 3B.

In Figure 4A, the same UV-labeled plasmid is used to probe a Southern blot. DNA prepared from an E. coli strain (C-1592) carrying a P2 prophage, was digested with one of three different restriction enzymes and separated in an agarose gel. A particular advantage of this labeling method is the convenience by which virtually any standard DNA sample, such as a fragment size marker, can be visualized (Figure 4A, lane mw). In all cases, the sizes of the hybridizing fragments are as expected from the restriction map of P2 (Figure 4B; adapted from Reference 2). In the linear genome prepared from phage particles (Figure 4A, lane d), the EcoRI fragment comprising gene A is flanked by cos on the right-hand side. Due to the Campbell type of integration, this fragment becomes continuous with the left-hand side of the genome in the prophage state, which explains the larger fragment in this case (Figure 4A, EcoRI, compare lanes b and d).

In conclusion, the gene A fragment of the prophage is clearly detectable from a 50-ng sample of E. coli DNA. Assuming that the haploid human genome is approximately 1000-fold larger than E. coli, the sensitivity of this method should permit the detection of a single copy gene from 50–100 µg of human DNA. However, this possibility remains to be tested.

DISCUSSION

This study demonstrates the practical use of UV-labeled probes for the detection of picogram amounts of target DNA in filter hybridization. A major advantage of the method lies in simplicity of probe labeling, which can be performed in only 5 min using any of several commonly found UV sources. Since no enzymes or any post-label purification is required, UV-labeling is...
inexpensive and very reproducible.

The cyclobutane ring is chemically stable in neutral and alkaline conditions (1), and we have not observed any reduced sensitivity of labeled DNA probes stored over a period of 6 months (results not shown). For some applications, such as Southern blotting, a minor disadvantage is that an electrophoresis gel containing target DNA cannot be inspected under UV light. For the purpose of measuring fragment sizes, however, any DNA size marker can easily be visualized on the final blot by having it UV-labeled separately, before electrophoresis.

The H3 antibodies have >10^6 higher affinity for irradiated vs. nonirradiated DNA (Figure 1A). However, they require the induced dimers to be presented in single-stranded form (7), suggesting that the hybridization signals arise from non-hybridized parts of the probe. While there will always be regions where an homologous probe is not completely annealed to the target, an improvement in sensitivity can be expected when the probe sequence is part of a nonhomologous carrier molecule like a plasmid or an M13 vector.

A cyclobutane lesion will only exert a minor effect on the helix structure and thus the melting temperature (T_m) of an annealed probe. According to Rahn and Patrick (6), the introduction of one (5'-6') dimer per 100 bp DNA reduces T_m by as little as 0.8°C. In principle, oligonucleotides could also be labeled by UV, but the T_m of a short probe will be relatively more affected by internal pyrimidine dimers. Moreover, as short probes will hybridize more or less completely with its target, a nonhomologous tail is essential in this case to present the dimers in single-stranded form. Our results (not shown) suggest that a tail of minimum 10–20 thymine residues is required for efficient labeling of oligonucleotide probes (see also Reference 7).

We believe that photoimmunodetection is a rapid, reliable and low-cost supplement to existing methods for nonradioactive DNA labeling. Its strength lies in tasks requiring speed and simplicity rather than extreme sensitivity, e.g., library screening or analysis of small to medium-sized genomes. It may also be a convenient alternative in the case of routine diagnostics, exemplified by the PCR-ELISA technique (Boehringer Mannheim). Finally, it could be a valuable tool for the detection and quantification of limited amounts of DNA in cases where in vitro labeling is not possible, such as the analysis of DNA fragmentation observed during cell apoptosis.

REFERENCES


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