Use of Modified Agarose Gel Electrophoresis to Resolve Protein-DNA Complexes for Electrophoretic Mobility Shift Assay

Electrophoretic mobility shift assay (EMSA) is the mainstay for quantitative determination of DNA-protein interactions. In this analysis, small DNA fragments or, more commonly, double-stranded oligomers 25–100 bp long are labeled and then combined with nuclear extracts or purified DNA-binding factors (1,2). Many approaches have been used for resolving the unbound DNA probe and the protein-DNA complexes that result from in vitro binding interactions. Generally, 3.5%–5% polyacrylamide gel electrophoresis (PAGE) has been used for this purpose. The use of agarose gel electrophoresis for gel shift analysis has been reported, but its use is restricted to mobility shifts of relatively large DNA fragments (>200 bp) and shifts caused by the binding of extremely large protein complexes to small DNA fragments (2,8). Mixed polyacrylamide-agarose gel electrophoresis has also been used to resolve various polymeric protein-DNA complexes (5).

In addition, a commercially available polysaccharide matrix containing AgarCryl™ (TreviGen™ 500; Trevigen, Gaithersburg, MD, USA) can be used as an alternative to polyacrylamide for EMSA (7). We report that agarose gels containing a commercially available, nontoxic synergistic gelling and sieving agent (Synergel™; Diversified Biotech, Boston, MA, USA) can be used to effectively analyze protein-DNA complexes. Synergel is a chemically modified galactomannan that acts as a liquid-phase sieve when immobilized in an agarose matrix, thus greatly improving the resolution of small DNA fragments (3,4). The method described here combines the convenience of agarose gel electrophoresis with the resolution of PAGE and can be used for DNA fragment lengths as small as 30 bp.

The modified agarose EMSA method was used to assay the binding of purified glucocorticoid receptor (GR) to a 30-bp double-stranded DNA fragment from the human tyrosine amino transferase (hTAT) gene, which included the glucocorticoid receptor element (GRE). GR is a transactivating factor that, when complexed with glucocorticoid hormone, elicits a response by binding to a GRE. The hTAT DNA oligomer probe had the following sequence: CTAGGC TGT ACA GCC TGT TCT GCC TAG, in which the 15 bp underlined constitute the hTAT GRE (6). For EMSA, 50 ng of the hTAT DNA fragment (Affinity Bioreagents, Golden, CO, USA) were end-labeled with 10 μCi of [γ-32P]ATP (New England Nuclear, Boston, MA, USA) in the presence of 1× kinase buffer (Promega, Madison, WI, USA) and 10 U of T4 polynucleotide kinase (Promega) in a 20-μl volume. The reaction was incubated for 30 min at 37°C, the labeled DNA fragment was separated from unincorporated ATP using a NucTrap® Probe Purification Column (Stratagene, La Jolla, CA, USA) according to the manufacturer’s directions, and 1 μL of the purified probe was counted in a TopCount® Scintillation Spectrophotometer (Packard Instrument, Meriden, CT, USA) in the presence of Micro-Scint™-20 scintillation fluid (Packard Instrument).

The in vitro binding reactions were performed essentially as described in the protocol provided with the purified recombinant human GR and hTAT GRE probe (both from Affinity Bioreagents), except that binding was done at 4°C as well as at room temperature. The reaction mixture contained 4 μL of 5× EMSA buffer (100 mM HEPES, 300 mM KCl, 25 mM MgCl₂, 10 mM dithiothreitol [DTT], 50% glycerol), 200 ng poly(dI-dC), 100 μg bovine serum albumin [BSA], 1 μL of purified recombinant human GR and 1 μL labeled hTAT DNA probe (40 000 counts per minute [cpm]) in a total volume of 20 μL. The control reaction contained all the components except for GR. The binding reaction was allowed to proceed either on ice or at room tempera-

Figure 1. Electrophoresis of hTAT DNA probe and GR protein-DNA complex on a modified agarose and polyacrylamide gel. (A) UV transillumination of ethidium bromide staining demonstrating 50-bp DNA ladder migration in a horizontal modified agarose gel after resolution of EMSA binding reactions. The gel was composed of 1.2% agarose and 1.2% Synergel in 0.5× TBE buffer. (B) Autoradiograph demonstrating resolution of the DNA-protein complex from unbound probe using a modified agarose gel. The image was obtained by vacuum drying the modified agarose gel shown in Panel A and exposing to X-ray film for approximately 5 h. (C) Autoradiograph demonstrating resolution of the same DNA-protein complex and unbound probe using conventional PAGE. The vertical gel contained 3.5% polyacrylamide (acrylamide/bisacrylamide 19:1) and 0.5× TBE buffer. The image was obtained by vacuum drying and exposing the gel to X-ray film for approximately 5 h. Lane 1: DNA molecular mass ladder; lane 2: control in vitro binding reaction with hTAT DNA probe alone; lane 3: in vitro binding reaction with hTAT DNA probe and purified GR.
ture (room temperature data not shown) for 45 min.

Following the binding reaction, DNA-protein complexes were resolved on a modified agarose gel composed of 1.2% agarose/1.2% Synergel in a 0.5x TBE buffer prepared as follows: 3 mL of absolute ethanol were added to 1.2 g of Synergel in a beaker, taking care to disperse the gel thoroughly. To this mixture, 1.2 g of agarose (SUN BIOSciences, Branford, CT, USA) were added and dispersed thoroughly while avoiding clumps. Then, 100 mL of 0.5x TBE (45 mM Tris base, 45 mM sodium borate, 1 mM EDTA) were added to the mixture, which was then boiled in a microwave until all the solids were dissolved. After the mixture had cooled slightly, it was poured into an 11- x 14-cm gel tray and allowed to solidify at 4°C. The gel was submerged in 0.5x TBE in a Horizon® 11-14 Horizontal Gel Electrophoresis Apparatus (Life Technologies, Gaithersburg, MD, USA), the wells were loaded with 10 µL of 1x EMSA buffer containing 0.5% bromophenol blue dye, and the gel was pre-run at 4°C for 45 min at 70 V. The binding reaction mixtures were loaded into wells without any tracking dye or additional loading buffer. A suspension containing 1 µg of a 50-bp DNA ladder (Life Technologies) with 1 µg ethidium bromide was loaded into a separate well. Ten microliters of 1x EMSA buffer containing 0.5% bromophenol blue dye were added to the last empty well to monitor migration. The gel was run at 4°C for about 3 h at 70 V, at which time the tracking dye had migrated to the midpoint of the gel. The gel was viewed on a UV transilluminator to determine migration of the DNA ladder. Using a Pasteur pipet, a small agarose plug was removed at positions corresponding to migration of molecular mass markers as shown in Figure 1A, and the gel was photographed. The gel was then transferred onto filter paper, vacuum-dried at 60°C on top of an additional sheet of filter paper and exposed to Fuji RX X-ray film (Fuji Medical Systems, Stamford, CT, USA) for about 5 h (Figure 1B). Agarose plug markings on the dry gel can be radioactively or fluorescingly labeled prior to exposure to film. All manipulations were performed using appropriate shielding of radioactivity. A dryer dedicated to gels containing radioactive samples was used and checked for contamination after use.

As demonstrated in Figure 1B, when the modified agarose gel was used, the mobility shift of the probe due to binding to GR was comparable to that observed when a like reaction was resolved on a 3.5% nondenatured vertical polyacrylamide gel (Figure 1C). However, the modified agarose method is easier to set up, avoids the use of toxic acrylamide and allows DNA marker progress to be periodically monitored by UV transillumination. This method shares many of the benefits ascribed to the use of TreviGel 500 polysaccharide matrix for analysis of protein-DNA interactions (7). The horizontal agarose gel does not require disassembly and is much easier to transfer onto filter paper for drying, thus minimizing exposure to radioactivity. In addition, the Plexiglas® cover on the horizontal gel electrophoresis apparatus and the fact that the gel is submerged in aqueous buffer actually afford more protection from sample radioactivity than do the exposed glass plates of some vertical PAGE apparatuses. Another advantage of the modified agarose format is that capillary action can be used to transfer protein-DNA complexes to membranes for further analysis such as Western blotting and N-terminal peptide sequencing. Modified agarose gels with improved sieving action may therefore serve as a safe and convenient alternative to PAGE for evaluation of protein-DNA complex formation.

REFERENCES


Supported by National Institutes of Health Grant No. R01-HL44986 (to W.W.S.) and No. P30-DK40561, Clinical Nutrition Research Center at Harvard (to S.F.A.). Address correspondence to Steve F. Abcouwer, Massachusetts General Hospital, Division of Surgical Oncology, Cox 626, 100 Blossom St., Boston, MA 02114, USA. Internet: abcouwer.steven@mgh.harvard.edu

Received 10 June 1997; accepted 27 August 1997.

Soundararajalu Chandra-sekhar, Wiley W. Souba and Steve F. Abcouwer
Massachusetts General Hospital and Harvard Medical School
Boston, MA, USA