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Alternative to Polyacrylamide Gels Improves the Electrophoretic Mobility Shift Assay

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ABSTRACT

In this paper we outline a simplified protocol for the electrophoretic mobility shift assay utilizing TreviGel™ 500, a nontoxic alternative to polyacrylamide. The TreviGel 500 matrix combines the strength and resolution of polyacrylamide with the simplicity and flexibility of agarose in the casting of gels. Therefore, this method provides a simple, rapid and nontoxic alternative to current protocols for the investigation of protein:DNA interactions.

INTRODUCTION

The electrophoretic mobility shift assay (EMSA) provides a rapid method for investigating the nature of protein:DNA interactions (3). Current methods involve the use of polyacrylamide gels to separate bound from unbound forms of a protein:DNA binding complex. We present an alternative protocol utilizing TreviGel™ 500 (Trevigen, Gaithersburg, MD, USA), a separation matrix with polyacrylamide properties, which simplifies the gel-shift procedure and provides significant benefits to current methods. In our test experiment, we utilized AP-1 consensus binding sequences (1) to investigate the utility of the TreviGel 500 matrix and compared the results to those obtained with polyacrylamide gels. The resolution, clarity and sensitivity of the assay appear to be identical to that found with polyacrylamide.

MATERIALS AND METHODS

Electrophoretic Mobility Shift Assay

HeLa nuclear extract and AP-1 consensus oligonucleotide were purchased from Trevigen. Nuclear extracts were provided at a concentration of 10 µg/µL. Double-stranded AP-1 consensus sequence (shown below) was 5′ end-labeled using T4 polynucleotide kinase to a specific activity of 2.8×10^9 counts per minute (cpm)/µg DNA (3.6×10^7 cpm/pmol) and gel-purified.

AP-1 (c-jun) binding consensus sequence (AP-1 oligonucleotide)	5′-CGCTTGATGAGTCAGCCGGAA-3′ 3′-GCGAACTACTCAGTCGGCCTT-5′

Labeled oligonucleotide (100000 cpm) was incubated in 0.5× Dignam Buffer D [1× Dignam Buffer D = 20 mM HEPES pH 7.9, 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol (DTT) and 0.5 mM phenylmethylsulfonyl fluoride (PMSF) (2)], in the presence of 1 µg poly(dI-dC):poly(dI-dC) and increasing amounts of HeLa nuclear extract, both in the presence and absence of 100-fold excess AP-1 double-stranded oligonucleotide as specific competitor. The final reaction volume was 20 µL. Incubation was performed at room temperature for 30 min, after which one-half of the reaction (10 µL) was loaded directly onto each of a 2% TreviGel 500 horizontal gel or a 5% polyacrylamide vertical gel.

Preparation of Gels

The 2% TreviGel 500 was prepared by boiling 1 g of the powder in 50 mL of 0.5× TBE buffer (10× = 1.0 M Tris Base, 0.83 M boric acid, 10 mM EDTA). The gel was microwaved for 6 min, which is slightly longer than typical for agarose. Distilled water was added back to maintain the 50-mL volume, and the gel was poured within 1 min after microwaving, as the gel becomes viscous upon cooling. The gel was cast in a 10- × 14-cm casting tray (total gel thickness was 3 mm). For comparison, a 14- × 18-cm, 1.5-mm-thick, vertical 5% polyacrylamide gel (20:1 acrylamide:bisacrylamide) was cast using 0.5× TBE.

Electrophoresis

Samples (10 µL) were loaded onto either the TreviGel 500, or polyacrylamide gels and separated by electrophoresis at 150 V, until the unbound DNA was within one centimeter of the bottom of the gel, as determined by relative migration of Orange G tracking dye loaded into an empty well. Total electrophoresis time was about 1 h for TreviGel 500 and 1.5 h for the polyacrylamide gel. Following electrophoresis, the gels were dried with a vacuum gel dryer for 1 h at 70°C and exposed to XAR-5 film (Eastman Kodak, Rochester, NY, USA) for 4 h.

RESULTS

Figure 1 shows a side-by-side comparison of the electrophoretic mobility shift assay using either a 2% TreviGel 500 or 5% polyacrylamide gel. Lane 1 shows free AP-1 probe in the absence of nuclear extract. Increasing amounts of HeLa nuclear extract (0.2, 0.5, 1.0 and 5 µg, lanes 2–5, respectively) produce the single band specific to the AP-1:DNA complex

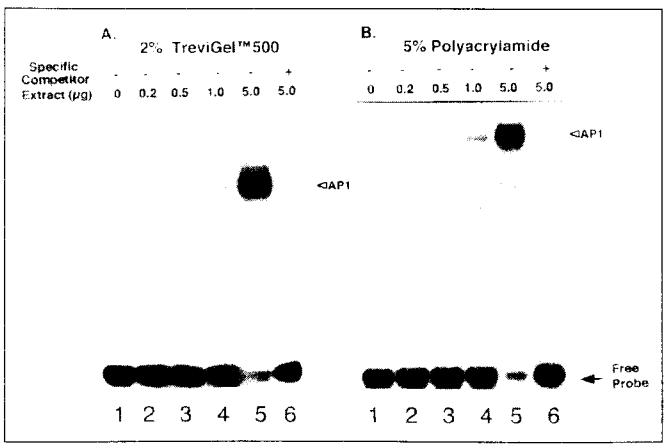

Figure 1. Comparison of electrophoretic mobility shift assays resolved in TreviGel 500 or polyacrylamide gels. AP-1 consensus double-stranded oligonucleotide was labeled to high specific activity using T4 polynucleotide kinase and [γ-^{32}P]ATP. Labeled AP-1 probe (equivalent to 100000 cpm) was incubated in the absence (lane 1) or presence of increasing amounts of HeLa nuclear extract (0.2, 0.5, 1 and 5 µg, lanes 2–5, respectively) or in the presence of both nuclear extract (5 µg) and 100-fold excess unlabeled AP-1 oligonucleotide specific competitor (lane 6). All lanes contain 1 µg poly(dI-dC):poly(dI-dC) as a nonspecific competitor. Total reaction volume was 20 µL. Free AP-1 probe is indicated by the lower arrow. Ten-µL samples were resolved on either a 2% TreviGel 500 (A) or a 5% polyacrylamide (B), 0.5× TBE gel run at 150 V. Total time for electrophoresis was 1 h for the TreviGel 500 or 1.5 h for the polyacrylamide gel. Gels were dried at 70°C for 1 h with a vacuum gel dryer and exposed to autoradiographic film for 4 h.

(visible only in lanes 3–5). Addition of 100-fold excess unlabeled AP-1 oligonucleotide as a specific competitor (lane 6) totally eliminated the shifted band, which demonstrates the specificity of the binding event. The AP-1:DNA complex in both gel matrices formed a single major band. A minor nonspecific band was detected in the polyacrylamide gel and was also seen in longer exposures of the TreviGel 500 gel. By increasing the concentration of nonspecific competitor, this minor band was completely eliminated.

DISCUSSION

EMSA is often used as a first approximation in defining the components of a protein:DNA complex. The protocol outlined above enhances the assay by providing a simpler and safer method for the separation of bound and unbound forms of the complex. The method is fast, uses standard laboratory equipment and offers the following improvements as compared to current protocols: First, the gel matrix is nontoxic, inexpensive and can be easily cast in a horizontal format. Second, resolving properties of TreviGel 500 compare with those of polyacrylamide at the concentrations used for gel shift assays. Third, the strength of the gel provides the ability to cast very thin (<4 mm) gels, which can be further manipulated and quickly dried at high (up to 80°C) temperatures. Fourth, the horizontal format allows one to cast a single gel with multiple combs for the simultaneous processing of a great number of samples. Finally, the matrix allows for capillary transfer of the shifted complex to immobilizing membranes such as nitrocellulose or nylon, which allows further analysis of the shifted complex (i.e., Western blot or N-terminal sequencing), without the need for electrotransfer methodologies as required by polyacrylamide.

The superior strength of the TreviGel 500 matrix allows for easy handling of very thin gels, making it a versatile, nontoxic alternative to polyacrylamide. The ability to cast and run the gel in a horizontal format expands and simplifies the EMSA protocol without sacrificing resolution of protein:DNA complexes separated by electrophoresis.

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