SDS Agarose Gels for Analysis of Proteins

Minjie Wu and Noriko Kusukawa
FMC BioProducts, Rockland, ME, USA

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ABSTRACT

A new agarose-based protein electrophoresis gel system is described. The system consists of a highly resolving agarose, MetaPhor® XR (FMC BioProducts, Rockland, ME, USA) dissolved in urea and TBE buffer and a stacking gel composed of a high gel-strength agarose, SeaKem® Gold (FMC BioProducts). TBE containing sodium dodecyl sulfate (SDS) is used as electrophoresis buffer. The disadvantages of traditional agarose gels have been overcome, and several advantages over polyacrylamide gels have been demonstrated. The system is capable of high-resolution separation of small proteins and has a dynamic separation range equivalent to a 4%–20% gradient polyacrylamide gel. Furthermore, the staining of protein bands by Coomassie® Brilliant Blue is very uniform in this gel, and depending on the protein, higher detection sensitivity can be obtained compared to SDS polyacrylamide gels. In Western blotting, proteins are more efficiently transferred to the membrane from the agarose gel than from polyacrylamide gels. Finally, the exceptional stability of agarose allows for gels to be precast and stored for a year.

INTRODUCTION

Agarose gels have been used for protein separation for many decades. Although they are particularly useful for analytical and preparative separation of large proteins and protein complexes, their main drawback has been the lack of sufficient resolution to analyze small (<30 kDa) proteins and peptides. We have found a way to overcome this disadvantage using a new system of sodium dodecyl sulfate (SDS) agarose gel electrophoresis. Not only does this system resolve small proteins well, but it also has several advantages over SDS polyacrylamide gels. First, no toxic components are used in the preparation of the gel. Also, this gel has a dynamic separation range equivalent to a 4%–20% gradient polyacrylamide gel so that proteins ranging in size from 6 to over 200 kDa can be analyzed using a single concentration gel. Furthermore, the staining of protein bands by Coomassie® Brilliant Blue is uniform and provides up to 50-fold higher detection sensitivity compared to SDS polyacrylamide gels stained under equivalent conditions. Some proteins are also better resolved by agarose than by polyacrylamide. In Western blotting, proteins are efficiently transferred to the membrane, as monitored by colloidal gold staining. In particular, high-molecular-weight proteins appear to transfer better from this gel than from polyacrylamide gels. Finally, the stability of agarose allows for gels to be precast and stored for a long period of time.

MATERIALS AND METHODS

MetaPhor® XR agarose (FMC BioProducts, Rockland, ME, USA) (1) was dispersed in ice-cold resolving buffer (0.5 M Tris, 0.2 M boric acid, 1 mM EDTA, adjusted to pH 8.6 with HCl) to make a 7% (wt/wt) solution. The mixture was allowed to hydrate at room temperature for 30 min then microwaved at high power for 45 s and swirled to mix. The agarose was dissolved by heating in a boiling water bath for 40 min, swirling to mix at 15 and 30 min. Water loss by evaporation was replaced by boiling water. Urea crystals were dissolved in the agarose solution to a final concentration of 1 M, microwaved for 10 s and incubated in a 70°C water bath to allow bubbles to clear before casting. For the stacking gel, SeaKem® Gold agarose (FMC BioProducts) was dispersed in stacking buffer (0.125 M Tris-HCl, pH 6.8) to make a 1% (wt/wt) solution, microwaved at high power for 75 s with swirling to dissolve and kept at 70°C until ready to cast.

Plastic mini-cassettes ($8 \times 8 \times 0.1$ cm; Novex, San Diego, CA, USA), a 60-cc syringe and an 18-gauge needle were warmed to 65°C. The resolving solution (6 mL) was injected into each cassette using the syringe attached to a caulking

gun. Then it was allowed to level at 65°C. The stacking solution (2 mL) was pipetted on top of the resolving solution, which was still fluid, and combs were inserted. The gels were set at room temperature for 15 min and then chilled to 4°C for 30 min to complete gelation.

Samples were prepared in a standard 1× sample buffer (10% glycerol, 0.125 M Tris-HCl, pH 6.8, 2% SDS, 1% 2-mercaptoethanol, 0.0005% bromophenol blue) (4), and were separated by electrophoresis in 1× TBE-SDS buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, 0.1% SDS) at 15 V/cm constant voltage. Protein bands were stained with 0.125% Coomassie Brilliant Blue R-250 in 40% methanol, 10% acetic acid for 1 h in a 50°C water bath with gentle agitation. Destaining was performed in 5% methanol, 7.5% acetic acid for 2 h at 50°C with gentle agitation.

For Western blotting, Immobilon® P membranes (Millipore, Bedford, MA, USA) and the Semi-Dry Electroblotter Transfer System (JKA-Biotech, Vaerlose, Denmark) were used. The electroblot transfer buffers were as previously reported (2). The membranes were incubated with S-protein-alkaline phosphatase conjugate (FMC BioProducts), then protein bands were detected using LUMI-PHOS™ 530 (PHOTO-

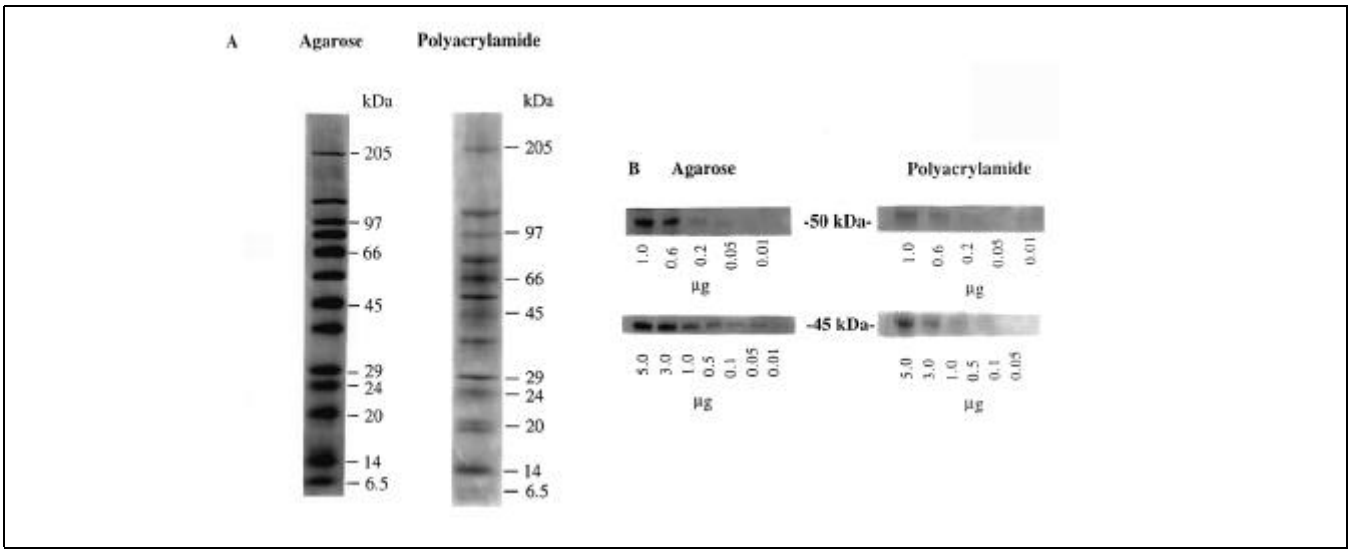

Figure 1. Comparison of the SDS agarose gel and a 4%–20% SDS polyacrylamide gradient gel (acrylamide and bisacrylamide, 37.5:1), freshly made according to the Laemmli formulation (3). The proteins analyzed were SigmaMarkers™, Wide Molecular Weight Range (Sigma Chemical, St. Louis, MO, USA). Gels were stained with Coomassie Brilliant Blue. In the polyacrylamide gel, note the poor resolution of ovalbumin (45 kDa) and the poor staining of myosin (205 kDa), phosphorylase B (97 kDa), trypsinogen (24 kDa) and aprotinin (6.5 kDa) compared with the agarose gel. One of the two bands at 20 kDa in the polyacrylamide gel is an artifact of the particular batch of markers used (A). Segments of gels shown in Panel B indicate that ovalbumin (45 kDa) was undetectable below 500 ng in a polyacrylamide gel, while in the agarose gel it was detectable at 10 ng. The detection limit of the 50-kDa engineered protein (Novagen, Madison, WI, USA) was 50 ng in agarose, while in this example it was around 200 ng in polyacrylamide.

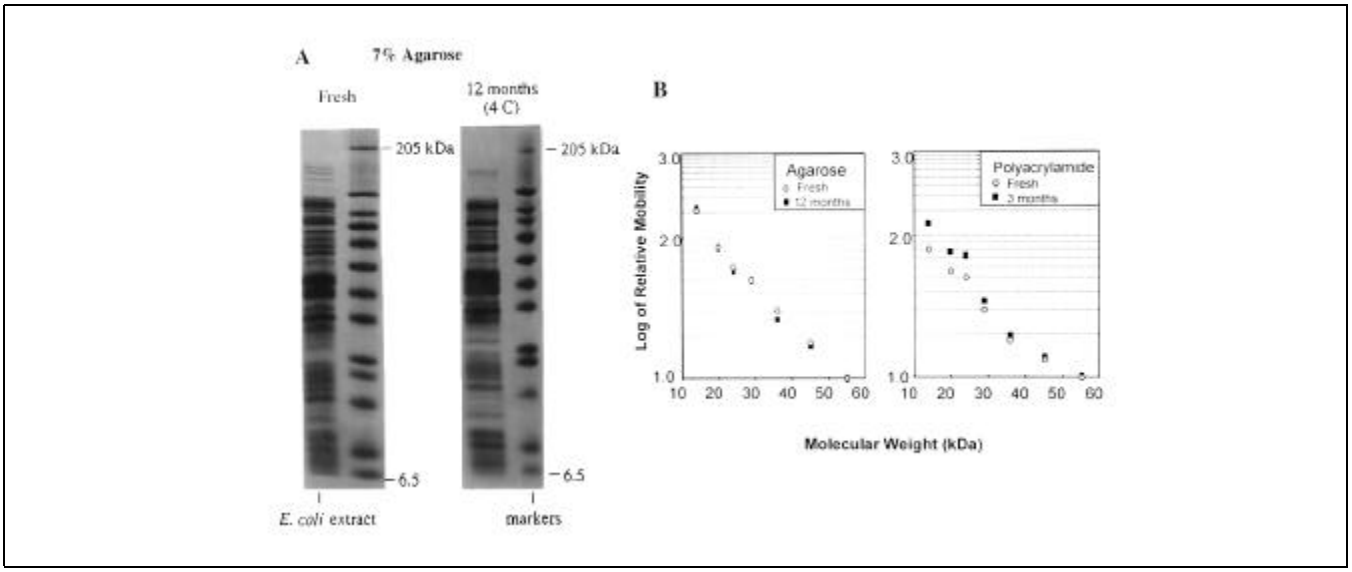

Figure 2. Stability of precast gels. The performance of freshly made gels was compared with gels stored at 4°C. Agarose gels (A) were stored for 12 months and polyacrylamide gels for three months. The SigmaMarker set was electrophoresed and stained with Coomassie Brilliant Blue. In Panel B, the relative mobilities of each protein for the fresh and stored gels are shown. Values were normalized against the 55-kDa protein. Only the low-molecular-size range is shown because it is more sensitive to aging of the gel. The relative mobilities of the higher-molecular-size proteins did not change in agarose or in polyacrylamide gels during storage. Gels were stored in heat-sealed, foil-laminated pouches.

BLOT™ Chemiluminescent System; Life Technologies, Gaithersburg, MD, USA). To check the transfer efficiency, membranes were stained with colloidal gold (AuroDye™ forte; Amersham Pharmacia Biotech, Piscataway, NJ, USA).

RESULTS AND DISCUSSION

A set of molecular size markers was separated on the agarose gel and compared against a 4%–20% gradient polyacrylamide gel. The dynamic ranges of the two gels were shown to be comparable (Figure 1A). In both gels, proteins between 6.5 and 205 kDa were separated. Some proteins, such as ovalbumin (45 kDa), resolved poorly in polyacrylamide gels, giving diffuse bands, whereas all proteins tested resolved well in agarose.

Protein staining with Coomassie Brilliant Blue was more uniform and intense in agarose than in polyacrylamide gels. This translated into differences in detection sensitivity. For instance, as shown in Figure 1B, the agarose gel detected ovalbumin (45 kDa) at 50-fold higher sensitivity, and an engineered 50-kDa protein at about fourfold higher sensitivity than polyacrylamide. This highlights the fact that the detection limit of proteins in polyacrylamide gels using Coomassie Brilliant Blue is highly variable. Although it is usually considered to be around 100–200 ng (as was the case with the 50-kDa protein), in reality it could be as high as 500 ng to 1 µg if the protein either does not resolve well (like ovalbumin) or stain well in polyacrylamide gels.

In a comparison of Western blotting, we found that proteins between 25 and 100 kDa transferred efficiently from both gel types to the membrane as detected by the chemiluminescent probe. However, the transfer efficiency of a larger 150-kDa protein was markedly higher from agarose than from polyacrylamide. The probe barely detected this protein on membranes lifted from polyacrylamide gels but easily detected it from the agarose gel (data not shown). Further, hybridization membranes were stained with colloidal gold to monitor the overall efficiency of protein transfer from gels. With agarose, the membrane stained with a clear background, and proteins were transferred nearly completely within 30 min of electroblotting. In polyacrylamide gels, the membrane stained with a very high background. Most of the proteins were still in the gel at 30 min, and completion of transfer required 1 h of electroblotting (data not shown). The agarose gels handled similarly to polyacrylamide gels and were not difficult to manipulate for staining or electroblotting.

We compared the performance of a fresh agarose gel and a gel that had been stored at 4°C for 12 months. The performance of the gel was not appreciably changed during storage (Figure 2). This is particularly demonstrated by the complex pattern of the *E. coli* protein extract (Figure 2A). Gels stored for 12 months showed only a slight decrease in the mobility of proteins relative to the position of the ion front, and the relative mobility among the molecular markers was remarkably consistent (Figure 2B). In contrast, polyacrylamide gels were not functional after 4 months at 4°C, and even at 3 months, the relative mobility of the smaller fragments was noticeably decreased (Figure 2B).

In summary, we have demonstrated an agarose-based protein gel system that has several advantages over the conventional SDS polyacrylamide gel, giving superior sensitivity, resolution, dynamic range and long-term stability. As long as a consistent filling technique and cooling regime is used, many gels can be prepared in a batch operation and will perform with high reproducibility over time.

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Address correspondence to Dr. Minjie Wu, FMC BioProducts, 191 Thomaston Street, Rockland ME 04841, USA. Internet: minjie_wu@fmc.com