Highly purified agarose as stacking gel in sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

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A modified sodium dodecyl sulphate/polyacrylamide-gel-electrophoretic method is described that utilizes highly purified agarose as stacking gel. The same electrophoretic resolution of different marker proteins is found as when polyacrylamide is used as stacking gel, but the background staining seen when polyacrylamide is used as stacking gel is decreased.

SDS/polyacrylamide-gel electrophoresis is a widely used technique for molecular-weight determination of macromolecules. Polymerization of polyacrylamide gels can either be chemically induced (e.g. with sodium persulphate) or photo-induced (e.g. with riboflavin). Some macromolecules have been shown to be sensitive to persulphate or products formed during photopolymerization [for a review, see Maurer (1971)]. In these cases, pre-electrophoresis (i.e. electrophoresis before sample application) can be applied, but such a procedure is time-consuming.

Highly purified agarose gels have been shown to be suitable matrices for isoelectric-focusing experiments (Rosén *et al.*, 1979). These agarose gels can also be used as stacking gels in SDS/polyacrylamide-gel electrophoresis. Since they do not contain any polymerization initiators, the samples to be electrophoresed will never be exposed to polymerization initiators, not even those used for the polymerization of the separation polyacrylamide gels. These initiators are separated from the sample macromolecules by the agarose stacking gel, and they will always migrate ahead of the macromolecules. Besides the above-mentioned advantage of agarose compared with polyacrylamide stacking gels, the agarose stacking gels are quicker to prepare (5 min or less) and they are non-toxic.

Experimental

Acrylamide was from Bio-Rad laboratories (Richmond, CA, U.S.A.) and agarose (HSIF grade) was from Litex (Glostrup, Denmark). Molecular-weight-marker proteins were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Coomassie Brilliant Blue R-250

was from Serva Feinbiochemica (Heidelberg, Germany). The other chemicals were from Merck (Darmstadt, Germany). The SDS/polyacrylamide-gel-electrophoresis buffer system described by Laemmli (1970) was used, with 0.375 M-Tris/HCl/SDS (1 g/litre), pH 8.8, in the separation gel, 0.125 M-Tris/HCl/SDS (1 g/litre), pH 6.8, in the stacking gel, and 0.025 M-Tris/HCl/0.192 M-glycine/SDS (1 g/litre), pH 8.3, as electrophoresis buffer. The sample buffer was 63 mM-Tris/HCl/0.7 M-mercaptoethanol/10% (v/v) glycerol/SDS (20 g/litre)/Bromophenol Blue (0.01 g/litre), pH 6.8.

Normally, 11% (w/v) separation polyacrylamide slab gels were used; they were polymerized with 2 mM-*NNN'*N'-tetramethylethylenediamine and 1 mM-sodium persulphate. The ratio of cross-linker to acrylamide was 1:37. Stacking gels contained various concentrations of agarose (see the Results and discussion section). Electrophoresis was performed with 20 mA/cm² of gel surface in a slab-gel-electrophoresis apparatus maintained at 10°C; the electrophoresis time was 4–5 h. After electrophoresis the gels (including the agarose stacking gels) were fixed for 1 h at 56°C in 75% (v/v) acetic acid/25% (v/v) methanol, stained 1 h at 56°C in Coomassie Blue (2.5 g/litre)/trichloroacetic acid (10 g/litre)/50% (v/v) methanol/7.5% (v/v) acetic acid and destained at 56°C in 35% (v/v) methanol/7.5% (v/v) acetic acid/5% (v/v) glycerol. The gels can easily be dried after destaining.

Because of the increased fragility of agarose as compared with polyacrylamide stacking gels, a modified (plastic) comb for preparing sample compartments in the stacking gel was designed by drilling a hole inside each tap of the comb. Stoppers were placed in each hole when the comb was placed in the warm agarose solution and removed when the agarose was solidified. A little air was pumped

Abbreviation used: SDS, sodium dodecyl sulphate.

Fig. 1. SDS/polyacrylamide-gel electrophoresis of five marker proteins

Densitometric scans are shown for 11%-polyacrylamide separation gels with: (a) 0.75% agarose in stacking gel; (b) 1.50% agarose in stacking gel; (c) 4% polyacrylamide in stacking gel; (d) 1.50% agarose in stacking gel. The separation gel in (d) contains, in addition, 0.5% agarose. The arrow indicates the top of the separation gel and indicates the baseline. From left to right the five marker proteins were: bovine serum albumin (M_r 66000), ovalbumin (M_r 45000), trypsinogen (M_r 24000) β -lactoglobulin (M_r 18400) and lysozyme (M_r 14300).

through each hole so that the agarose lamellae were liberated from the comb material. In this way the comb could be removed without breakage of the lamellae.

Results and discussion

Fig. 1 shows the densitometric scans of different SDS/11% polyacrylamide gels with two different concentrations of agarose in the stacking gel (Figs. 1a and 1b) compared with a 4% polyacrylamide stacking gel (Fig. 1c). As can be seen, there are no differences in the migration and the peak sharpness of the marker proteins. However, there was more background staining when the 4% polyacrylamide gel was used as stacking gel; indeed, when agarose stacking gels were used, no background staining was found at all (see Fig. 1). Of the two agarose-stacking-gel concentrations used, 1.5% is recommended, because agarose at this concentration has the best physical strength.

Fig. 1(d) shows a scan from an electrophoresis where 0.5% agarose had been incorporated into the 11% polyacrylamide gel; the 1.5% agarose stacking gel was used in this electrophoretic run. The marker-protein peaks in this scan are broader than those of the gels without agarose in the separation gel, but for experiments where sharp bands are not needed, the 0.5% agarose/11% polyacrylamide separation gels can be useful because they are very easy to prepare. It is not necessary to seal the glass plates for separation-gel preparation because the agarose in the separation-gel mixture will solidify instantly when poured between the glass plates (i.e. no problems of leakage).

When agarose stacking gels are used it is recommended that one prepares several ready-to-use separation gels. They can be stored for months in the separation-gel buffer at 4°C. In this way SDS/polyacrylamide-gel electrophoresis can be set up within a few minutes when desired.

References

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