[Technical Note]

Inverse-gradient polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate for better separation of protein samples

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SUMMARY

We found that the relationship between the molecular mass of a protein and its mobility in polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate on an inverse-gradient (15–5%) gel is linear on a double logarithmic plot. In contrast, this relationship is not linear for proteins resolved on a standard 10% gel, but is fitted to two straight lines. Both gel types gave a similar slope in resolving proteins smaller than 100 kDa but inverse-gradient (15–5%) gel separated proteins with wider range of molecular mass. On the other hand, the standard 10% gel provided better separation of proteins larger than 100 kDa. These results suggest that the 15–5% inverse-gradient gel is best suited for the separation of proteins smaller than 100 kDa. The advantage of inverse-gradient gel SDS-PAGE may stem from the fact that unstacking of SDS-protein complexes in inverse-gradient gels is faster and more complete than in standard 10% gels.

Key words: inverse-gradient gel, SDS-PAGE, protein migration

INTRODUCTION

SDS-PAGE is a widely used technique for separating protein mixtures based on molecular size. In conjunction with immunoblotting, the method provides a convenient and effective tool for protein analysis. At least two independent mechanisms determine the migration properties of proteins in SDS-PAGE under discontinuous buffer conditions¹). First, separation of proteins in SDS-PAGE relies on the sieving effect of the polyacrylamide gel. Thus, a suitable polyacrylamide gel concentration must be chosen to analyze target proteins. For the analysis of a wide range of protein sizes, such as are found in tissue homogenates or cell lysates, a concentration-gradient gel is the preferred method, because the gradient gel ensures a wide range of separation in terms of molecular size. Second, unstacking of SDS-protein complexes by trailing ions plays a crucial role in separating proteins smaller than 20 kDa. SDS-PAGE is often utilized to obtain samples for mass spectrometry or 2-D phospho-peptide mapping by cutting out a portion of the gel for subsequent analysis of the separated proteins. The technique is also used for in vitro analyses in which the size range of target proteins is already known. For example, an in vitro kinase assay or limited digestion of proteins involves substrates and products that may be of similar size and often share the same immunoreactivity. In these instances, much higher resolution of similarly sized proteins is required to be isolated or be analyzed for further analysis.

We examined the use of inverse-gradient gel SDS-PAGE for the separation of size markers of protein. This modified SDS-PAGE technique traps high molecular size proteins at the upper part of the gel, which contains a higher concentration of acrylamide. On the other hand, separation of smaller molecular size of proteins is accelerated with decreasing acrylamide concentration at the bottom of the gel. As a result, the method provides wider separation of proteins with smaller molecular sizes. Here, we report the migration characteristics of inverse-gradient gel SDS-PAGE and discuss its potential advantages.

MATERIALS AND METHODS

Casting inverse-gradient gels

A gel cassette was inversely placed on a gel-casting stand (NIHON EIDO CO., LTD., Tokyo, Japan). A specially designed comb was placed at the bottom of the gel cassette.
to establish a tight seal, and then the stacking gel solution was cast. After washing with distilled water to remove unpolymerized gel solution, the gel cassette was connected to a gradient-mixer (CHG042AA, ADVANTEC TOYO KAISHA, LTD., Tokyo, Japan). High-percentage gel solution was then placed in the mixing chamber of the gradient chamber and a linear-gradient gel was cast in the regular manner. In this way, the higher-percentage acrylamide solution remained undisturbed during the polymerization process. The acrylamide stock solutions used for gel preparation contained 30% acrylamide and 0.8% N,N'-methylenebisacrylamide. Once polymerized, the inverse-gradient gel was removed from the disassembled gel cassette. In some experiments, 10% precast gels or 15–5% inverse-gradient acrylamide precast gels were custom-ordered to Bio-Rad Laboratories Inc. (Tokyo, Japan).

**Electrophoresis**

A Tris-glycine buffer system 2) was used for the separation of ProteoLadder 150, 20- to 150-kDa protein standards (Norgen Biotek, Thorold, ON, Canada). The Tris-glycine separating buffer contained 25 mM Tris, 192 mM glycine, and 0.1% SDS. Electrophoresis was carried out with less than 60 mA of current using a constant current power supply.

To monitor the progress of electrophoresis, phenol red (1%) was added to the stacking gel 3, 4). The leading edge of the bright-red phenol dye was considered as the migration front and the migration distances of Coomassie-stained proteins were measured and relative mobility (Rf) values were calculated against the migration dye front. The molecular mass at half migration distance (MD$_{50}$) was determined to quantify the dependence of protein migration distance on gel composition. The gel density profile and the relationship between Rf value and molecular mass were plotted for each protein using Origin 7.5 (OriginLab, Northampton, MA).

**RESULTS AND DISCUSSION**

To resolve proteins with different molecular masses, a proper concentration of acrylamide should be chosen so that the proteins localize around the middle of the gel. Thus, the MD$_{50}$ is a convenient measure for selecting a suitable acrylamide concentration. In a preliminary experiment, we measured the MD$_{50}$ value for 7.5%, 10%, 12.5%, and 15% acrylamide gels and found 81-, 50-, 37-, and 30 kDa, respectively, while that for 15–5% inverse-gradient gel was 56 kDa. Thus the resolution and range of separation of 15–5% inverse-gradient gels were compared with that of conventional linear gels containing 10% acrylamide.

Typical separations of 20- to 150-kDa protein standards

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**Fig. 1.** Protein separation on (A) standard 10% gels and (B) inverse-gradient gels. Representative images of Coomassie-stained gels (left) and associated profiles of optical density (right) are shown. The relationship between mobility and molecular mass of marker proteins was plotted on a linear (C) or double-logarithmic (D) scale.
by each gel type are shown in Fig. 1. The standard 10% gel resolved proteins as small as 30 kDa (Fig. 1A). A sharp band of Coomassie-stained protein just under the 30-kDa band indicates that unstacking of SDS-protein complexes was incomplete. Thus, the lower part of the running gel was wasted because of no separation of proteins in it. It should be noted that proteins with a high molecular mass (e.g., 100–150 kDa) were reasonably separated.

Inverse-gradient gels separate proteins with different characteristics when compared with standard 10% gels (Fig. 1B). In contrast to the standard 10% gel, the proteins with a high molecular mass (150-, 125-, and 100-kDa) were packed tightly at the top of the inverse-gradient gel, although these marker proteins were clearly distinguishable. Notably marker proteins larger than 25 kDa were separated with greater resolution. A sharp protein band at the very bottom of the lane in the gel indicates the presence of stacked SDS-protein complexes (Fig. 1B).

Comparison of the inverse-gradient gel with the standard 10% gel revealed that unstacking of the SDS-protein complexes occurred on much lower position of the gel faster in the inverse-gradient gel and made possible to resolve 25 kDa protein as the smallest. The high acrylamide concentration (up to 15%) at the top of the inverse-gradient gels decelerates migration of SDS-protein complexes so that the trailing glycine ions can catch up to the SDS-protein complexes more quickly than that in standard 10% gels.

The migration characteristics of inverse-gradient and standard 10% gels were further analyzed by plotting Rf values against the molecular mass of the protein markers. The migration curves of the inverse-gradient gel and the standard 10% gel deviated in the range below 100 kDa (Fig. 1C). On the other hand, a double-logarithmic plot of mobility versus molecular mass for a 15–5% inverse-gradient gel offered a straight line for a wider range of molecular size of proteins than that in a standard gel SDS-PAGE.

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ABBREVIATIONS

SDS-PAGE, sodium dodecyl sulfate poly acrylamide gel electrophoresis; MD_{50}, molecular mass at half migration distance; Rf, relative mobility

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