High Speed SDS-PAGE of Proteins

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Abstract

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a widely used method for assessing in a reproducible manner the relative mass of denatured polypeptide chains and the purity of a protein preparation. For more than 40 years, the Laemmli SDS-discontinuous system based on Tris-glycine buffer has been used with slab gels for high-resolution fractionation of protein mixtures under dissociating conditions. However, the electrophoresis run time is long for a standard 10 x 10 cm gel (~60 min) since the gel cannot be run at higher voltage without generating excess heat and compromising the resolution of separated protein bands. Through the years, various gel chemistries and buffering systems have been developed to improve protein band resolution at higher speed, but always with increased cost. In this work, we show that a simple change to the running buffer can provide high separation speed with enhanced resolution of protein bands. Tris-glycine mini-gels (precast and homemade) cast with the traditional Laemmli recipe can be run 50-60% faster (run time <25 min) using our new proprietary Tris-SDS buffer. Gels are run at higher voltage without generating excessive heat and impacting the clarity of protein bands. Faster electrophoresis time improves the overall throughput of the protein lysate to Western blot work flow.

Introduction

For more than 40 years, the Laemmli SDS discontinuous buffer system has been used with Tris-glycine polyacrylamide gels for high-resolution fractionation of protein mixtures (1). The gel matrix acts as a molecular sieve, and the Tris-Glycine-SDS running buffer provides the ionic strength for the electric field. When current is applied, proteins migrate in the electric field based on their net charge, size and shape of the protein or its subunits. If the voltage is set too high, the running buffer will become hot prompting the gel matrix to heat up and then break down causing protein bands to diffuse and lose sharpness.

Standard Tris-glycine mini-gels prepared with the Laemmli recipe are generally run at 125 V using TGS buffer (2) resulting in long electrophoresis run-times (60-90 min). Since these gels cannot be run at significantly higher voltage without generating excess heat, this fact provided an opportunity to develop a new buffering system that would permit protein electrophoresis at higher voltages without compromising the gel matrix.

We report a new electrolytic buffer solution which can be run at increased voltages without generating high heat and impacting the clarity of protein bands. Preliminary data shows protein electrophoresis run times <25 minutes for traditional 10% Tris-glycine (TG) mini-gels (precast and home-made) using our new proprietary FAST*Run*™ Tris SDS PAGE running buffer.

Material and Methods

Gel Specifications, Running Buffers, and Apparatus

Gel type: precast Tris-glycine gel (3) or homemade TG mini-gel (4)
Acrylamide percentage: 10% and 16% gels or 4-20% gradient gel
Gel dimensions: 10 cm x 10 cm
Comb: 10 well
Thickness: 1.0 mm or 0.80 mm

Running Buffer

Tris-Glycine-SDS (TGS) buffer, 10X solution (4)
BP881 FAST*Run*™ Tris SDS PAGE Running Buffer, 10X solution (Fisher Scientific)

Apparatus

XCell SureLock™ Mini-Cell Electrophoresis System (Thermo Fisher Scientific)
Mini-PROTEAN® Tetra Cell or Mini-PROTEAN 3 Cell (Bio-Rad)
FisherBiotech™ Vertical Electrophoresis System (Fisher Scientific)

Protein MW Standards, Cell Lysate, and Stain

BP3602 EZ-Run™ *Rec* Protein Ladder (Fisher Scientific), 10 – 200 kDa (14 protein bands)
BP3603 EZ-Run Prestained *Rec* Protein Ladder (Fisher Scientific), 10 – 170 kDa (10 protein bands)
LC5800 Novex® Pre-Stained Protein Standard (Life Technologies), 3.5 – 260 kDa (12 protein bands)
LC5677 Mark12™ Unstained Standard (Life Technologies), 2.5 – 200 kDa (12 protein bands)
PI26628 Low Range Protein Ladder (Thermo Scientific), 1.7 – 40 kDa (6 protein bands)
E. coli cell lysate from BP4000 TransMax™ Competent Cells, FB5α (Fisher Scientific)
BP3620 EZ-Run Protein Gel Staining Solution (Fisher Scientific)

In these experiments, we utilized electrophoresis tanks from different suppliers which were compatible with precast and home-made mini-gels. Speed of protein separation under different applied voltages was analyzed using 10% or 16% TG polyacrylamide gels with either traditional TGS running buffer or the new FAST*Run* Tris SDS PAGE running buffer.

Results

Experiment 1: Speed of Separation

Protein gel electrophoresis was performed in 10% TG precast and homemade gels (Table 1) using 1X TG-SDS running buffer and 1X FAST*Run* buffer (Fig. 1). Partial separation of the protein ladder was observed after 25 min run time using the gel manufacturer's recommended 125 V for TG-SDS buffer (Fig. 1a). However, complete separation of 10 protein bands (10 – 170 kDa) after 25 min at 200 V occurred with the use of FAST*Run* buffer in precast and homemade gels (Fig. 1b,c). In contrast, about 90 min were required to separate the protein ladder in the 10% gel using TG-SDS running buffer (data not shown). A low MW protein ladder (1.7 – 40 kDa) was separated in 35 min on a 16% TG gel using FAST*Run* buffer (Fig. 1d).

Table 1. Gel Type and Electrophoresis Run Conditions in Experiment 1

Gel Type	Running Buffer	Voltage	Run Time	Temperature (Cathode Chamber)
Fig. 1a - 10% TG Precast	1X TG-SDS	125 V	25 min	24° C
Fig. 1b - 10% TG Precast	1X FAST*Run*	200 V	25 min	34° C
Fig. 1c - 10% TG Homemade	1X FAST*Run*	200 V	30 min	31° C
Fig. 1d - 16% TG Precast	1X FAST*Run*	200 V	35 min	33° C

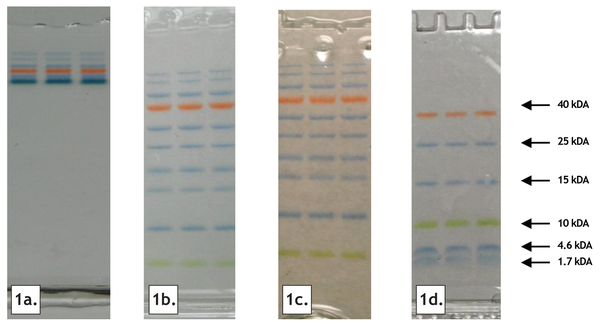

Fig. 1a-d. Electrophoretic separation of a protein ladder (BP3603) in a 10% TG precast gel after 25 min using TG-SDS running buffer (**1a**) and FAST*Run* buffer (**1b**). Same protein ladder separated in a 10% TG homemade gel after 30 min using FAST*Run* buffer (**1c**). Low MW range protein ladder (PI26628) fractionated in 35 min on a 16% TG precast gel using FAST*Run* buffer (**1d**).

Experiment 2: Band Clarity at Different Voltages

FAST*Run* buffer is an electrolytic solution which can run at higher voltages without generating high heat and impacting the clarity of protein bands. For example, protein electrophoresis in 10% precast TG mini-gels (10 cm x 10 cm) was evaluated with increasing voltages (Table 2) using a full (700 mL) buffer tank of FAST*Run* buffer. Electrophoresis run times decreased from 23 min at 200 V to 13 min at 300 V without a significant increase to the operating temperature (buffer temperature in cathode chamber was about 35° C at end of run). Protein markers remained sharp with little evidence of band diffusion (Fig. 2a-c).

Table 2. Electrophoresis Run Conditions in Experiment 2

Voltage	Run Time	Current (mA)		Temperature (Cathode Chamber)
		Start	End	
Fig. 2a - 200 V	23 min	98	54	34° C
Fig. 2b - 250 V	18 min	135	75	34° C
Fig. 2c - 300 V	13 min	169	109	36° C

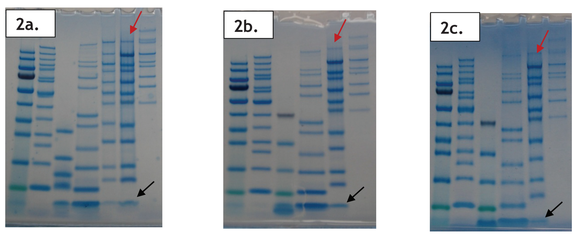

Fig. 2a-c. Decreasing electrophoresis run time with increased voltage in a 10% precast TG gel using FAST*Run* buffer: 23 min at 200 V (**2a**), 18 min at 250 V (**2b**), and 13 min at 300 V (**2c**). Protein bands are sharp and fully separated from 3.5 kDa (black arrow) to 260 kDa (red arrow).

Experiment 3: Cell Lysate and Protein Separation in <25 min

E. coli cell lysate was loaded on a 10% TG gel and electrophoresed using 1X FAST*Run* buffer (Fig. 3a). Lanes 3 and 4 had 10 µl and 5 µl, respectively, of *E. coli* lysate. The complex *E. coli* protein mixture fractionated well as did individual proteins (Fig. 3b) such as carbonic anhydrase (29 kDa in lane 3) and albumin from chicken egg white (45 kDa in lane 4).

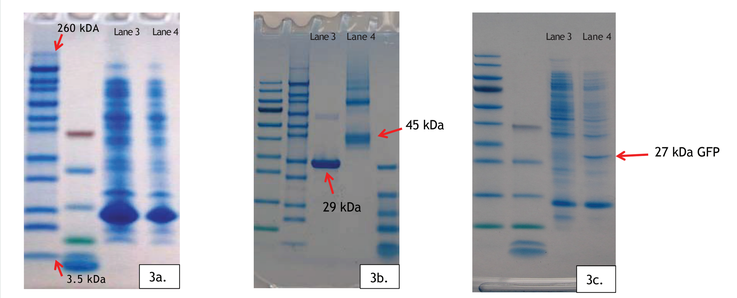

Fig. 3a-c. Separation of *E. coli* lysate in lanes 3 and 4 using 10% TG gel with 1X FAST*Run* buffer (**3a**). Individual proteins isolated with FAST*Run* buffer protocol (**3b**), carbonic anhydrase (29 kDa in lane 3) and albumin from chicken egg white (45 kDa in lane 4). *E. coli* lysates with and without green fluorescent protein (**3c**, lanes 3 & 4 respectively) on 10% TG gel with 1X FAST*Run* buffer.

Discussion

Since the mid-1970s Laemmli's method based on a Tris-glycine-SDS buffer system has been widely used for analyzing protein mixtures by PAGE. Laemmli's gel and buffer system provides clear and accurate molecular weight estimation of proteins in complex samples from a wide variety of sources, but at the cost of long electrophoresis time. Recent improvements of the SDS-PAGE method to reduce total run-time have been limited primarily to high-priced precast gels. Indeed, the general industry point of view is that modification of electrokinetic methods involving only the polyacrylamide gel matrix may lead to advances in separation speed and resolution of proteins.

In this work, we demonstrate an improved Laemmli method which provides significant cut in electrophoresis run-time by 50 to 60% through patent pending modifications made solely to the running buffer system. Traditional TG polyacrylamide gels (precast or homemade) can be operated at higher voltages using the new FAST*Run* Tris SDS PAGE running buffer without generating high heat (Fig. 1,2).

A major drawback of the Laemmli SDS-discontinuous buffer system is that proteins smaller than 15 kDa are very poorly separated, i.e. they co-migrate within the electrophoretic migration front. However, a 10% TG gel run with FAST*Run* buffer permitted complete separation of the lower molecular weight proteins (Fig. 2,3a) which is similar to the resolving power of a 4-20% gradient gel (2) or a 10% gel with Tricine SDS running Buffer (5).

Conclusions

In the protein characterization workflow, the speed to complete separation and identification of a protein is important. FAST*Run* Tris SDS PAGE Running buffer for use with standard Tris-glycine gels provides the speed and resolution required by researchers to complete in timely manner downstream applications like Western blotting for identifying particular protein species.

References

1. Laemmli, U.K. (1970). *Nature*. 227: 680.
2. Hames, B.D. (1990). One-dimensional Polyacrylamide Gel Electrophoresis. In *Gel Electrophoresis of Proteins, A Practical Approach*, 2nd Edition, (ed. B.D. Hames and D. Rickwood), Oxford University Press, New York.
3. Novex® Pre-Cast Gel , Electrophoresis Guide, Rev date: October 2010, Invitrogen.
4. Scopes, R.K. and Smith, J.A. (1998). Analysis of Proteins. In *Current Protocols* in *Molecular Biology*, Vol. 2, (ed. F.M. Ausubel et al.), John Wiley & Sons, Inc.
5. Schägger, H. and Von Jagow, G. (1987). *Anal Biochem*. 166: 368.

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