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Separation of Protein Oligomers by Blue Native Gel Electrophoresis

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Abstract

Native gel electrophoresis is used as a tool to assess structural differences in proteins. This note presents an application to separate oligomeric forms of proteins, such as HIV-1 reverse transcriptase monomers and homodimers. Technical difficulties encountered with various native gel techniques and ways to circumvent them are described.

Human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT) is a multifunctional enzyme that catalyzes the conversion of genomic RNA into double-stranded proviral DNA. The enzyme is a heterodimer of 66 kDa and 51 kDa subunits, which can also form homodimers. The p51 subunit is derived by proteolytic cleavage of the C-terminal RNase H domain of p66. Analytical ultracentrifugation experiments determined K_d values of 4.2 and 230 μ M for homodimerization of p66 and p51 [1]. To evaluate dimerization over a range of conditions, we tried a commercially available kit for blue native polyacrylamide gel electrophoresis (BN-PAGE). Purified p66 behaved normally with one or two bands depending on solution conditions, but purified p51 produced a ladder of bands under conditions in which only monomer is present in solution. Therefore, we explored a variety of native gel electrophoresis techniques [2-6], and developed a modified protocol for blue native agarose gel electrophoresis (BN-AGE) that gave superior results for p51 as well as other soluble proteins.

Novex Bis-Tris gel system and NativeMarkTM protein standards were purchased from Invitrogen Corporation (Carlsbad, CA). SeaKem Gold agarose was purchased from Lonza (Rockland, ME). Kaleidoscope precision plus protein standards were purchased from BioRad (Hercules, CA). EZ-Run Protein Gel Staining solution was purchased from Fisher Scientific (Fair Lawn, NJ). Bovine serum albumin (BSA) and T4 DNA ligase were purchased from Roche Diagnostics (Indianapolis, IN). Carbonic anhydrase, aprotinin, cytochrome c, and other biochemical reagents were purchased from Sigma Chemicals (St. Louis, MO). HIV-1 p66 and p51 were purified as previously described [1].

BN-PAGE was carried out using the Novex Bis-Tris system according to the manufacturer's specifications. Pre-cast NativePAGETM Novex 4–16% (v/v) Bis-Tris gels were run with near neutral pH at 90 V at 4 °C with stirring for 4.5 h. Protein samples (10 μ L) were mixed with the sample buffer provided (2.5 μ L) and 5% (w/v) Coomassie blue G-250 (0.3 μ L). Gels were stained in EZ-Run Protein Gel Staining solution for 1-2 h and destained in water.

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BN-AGE was carried out using a BioRad mini-sub cell DT unit. A 3% (w/v) horizontal SeaKem Gold agarose gel (10 cm × 6 cm × 5 mm) was prepared using native agarose gel buffer (NAGB; 25 mM Tris, 19.2 mM glycine, pH 7.0 or 8.5) [4]. The horizontal gel was submerged in the apparatus containing NAGB and electrophoresis was performed at room temperature at 40 V for 4.5 h. Protein samples (10 µL) were mixed with sample buffer (2.5 µL, NAGB containing 30% (w/v) glycerol) and 5% (w/v) Coomassie blue G-250 (0.3 µL, Novex gel system). Gels were stained and destained using the same protocol as BN-PAGE.

The patterns of p66 and p51 generated in BN-PAGE are shown in Fig. 1. Fig. 1A shows BN-PAGE of p66 solutions containing monomeric and dimeric forms. Fig. 1B shows BN-PAGE of a p51 solution containing only monomers. Severe laddering of monomeric p51 was witnessed. To confirm that the multiple p51 bands were an artifact of BN-PAGE, the bands from BN-PAGE were cut out, soaked, and subjected to SDS-PAGE (not shown). The protein bands that originally migrated to different positions by BN-PAGE now migrated to positions identical to the p51 standard by SDS-PAGE. This confirms that protein degradation did not cause the ladder pattern.

We tried several permutations of the native PAGE system. (1) BN-PAGE was run in the absence of Coomassie Blue G-250. p51 remained in the sample well and did not enter the gel (not shown). (2) Detergents were added to ensure p51 solubility during BN-PAGE. Protein samples containing either 1% (w/v) n-dodecyl-β-D-maltoside or 1% (w/v) digitonin were prepared as described above. (3) Voltage was reduced to eliminate the possibility of thermal denaturation during BN-PAGE. Gels were run at 60 V at 4 °C with stirring for 8 h. (4) BioRad Criterion™ Tris-HCl gels 8–16% (v/v) pH 8.5 were run at 100 V at 4 °C with stirring for 2.5 h. (5) Non-gradient 16 cm native polyacrylamide gels were poured using the Protean II xi cell with a 3% (v/v) stack and 7.5, 10, or 12.5% (v/v) resolving gel. Gels were run using the Novex Bis-Tris buffer system at 90 V at 4 °C with a circulating water system for 8–10 h. Neither omission of Coomassie blue G-250, addition of detergents, lowering the voltage, increasing the pH from 6.8 to 8.5, nor replacing gradient with standard gels resolved the laddering. It is possible that an interaction of p51 with the polyacrylamide matrix contributes to the peculiar behavior, despite identical amino acid sequences of p51 and the first 440 residues of p66. Therefore, agarose was evaluated as an alternative support medium [2,3].

In the Invitrogen BN-PAGE kit and a published BN-AGE protocol, electrophoresis is carried out at near neutral pH [2,4,5,6]. Figs. 2A and 2B show the migration of several proteins with different isoelectric points (pI) run on 3% (w/v) agarose gels in the presence and absence of Coomassie blue G-250 at pH 7.0. As expected in the presence of Coomassie blue G-250, all proteins migrated toward the cathode (Fig. 2A). Aprotinin, cytochrome c, BSA, and T4 DNA ligase migrate as single bands with approximately the same mobility, carbonic anhydrase migrates as three bands, and p51 migrates as a single band only a short distance from the well. In the absence of Coomassie blue G-250, aprotinin (pI = 10.0-10.5) migrates as a single band and cytochrome c (pI = 10.0-10.5) migrates as a diffuse band toward the anode, BSA (pI = 4.7) and T4 DNA ligase (pI = 6.0-6.2) migrate as single bands toward the cathode, and carbonic anhydrase (pI = 6.6-7.2) and p51 (pI = 8.7) remain near the well (Fig. 2B). Although p51 migrates as a single band on agarose gels at pH 7.0, the mobility is low in both the presence and absence of Coomassie blue G-250.

Native agarose gels have been run at pH 8.5 [4]. In the presence of Coomassie blue G-250, aprotinin, carbonic anhydrase, BSA, T4 DNA ligase, and p51, all migrate as single bands and cytochrome c migrates as a long diffuse band toward the cathode (Fig. 2C). In the absence of Coomassie blue G-250, the migration of the proteins was driven by their respective pIs: aprotinin and cytochrome c migrated toward the anode as single bands; carbonic anhydrase, BSA, T4 DNA ligase and p51 migrate toward the cathode as single bands (Fig. 2D).

Because p51 migrated cleanly by BN-AGE at pH 8.5, we tried to separate monomeric and dimeric p51 under these conditions. Fig. 1C shows the migration of wildtype (wt) and mutant p51 proteins in the presence of Coomassie blue G-250 as discrete bands representing monomer and homodimer. The mutant proteins W401A and L234A are dimerization deficient [7,8]. Efavirenz is an inhibitor that enhances dimerization of RT subunits [1,9]. The wt and W401A p51 samples incubated with efavirenz clearly show dimer formation as a result of drug binding, whereas L234A does not bind the drug [9].

The results presented here extend the use of blue native gel electrophoresis to the separation of oligomeric forms of proteins. Protein migration under native conditions is dependent on molecular mass, pI, buffer pH, and type and percentage of gel matrix. In the presence of Coomassie blue G-250, migration is also dependent on nonspecific binding of the dye by the protein to provide a net negative charge. Agarose acts as a superior solid phase for separation of monomeric and dimeric HIV p51 and may work well for other proteins. Availability of sieving agarose for high resolution separations has allowed us to develop a novel protocol to study small proteins and protein-protein interactions. In conclusion, our experience dictates the use of due diligence in the choice of system and conditions used. The appropriate conditions may prove to be counter intuitive to theoretical expectations.

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References

1. Venezia CF, Howard KJ, Ignatov ME, Holladay LA, Barkley MD. Biochemistry 2006;45:2779–2789. [PubMed: 16503633]
2. Niepmann M, Zheng J. Discontinuous native protein gel electrophoresis. Electrophoresis 2006;27:3949–3951. [PubMed: 16991206]
3. Kim R, Yokota H, Kim S-H. Electrophoresis of proteins and protein-protein complexes in a native agarose gel. Anal Biochem 2000;282:147–149. [PubMed: 10860512]
4. Henderson NS, Nijtmans LGJ, Lindsay JG, Lamantea E, Zeviani M, Holt IJ. Separation of intact pyruvate dehydrogenase complex using blue native agarose gel electrophoresis. Electrophoresis 2000;21:2925–2931. [PubMed: 11001305]
5. Schagger H, Cramer WA, von Jagow G. Analysis of molecular masses and oligomeric states of protein complexes by blue native electrophoresis and isolation of membrane complexes by two-dimensional native electrophoresis. Anal Biochem 1994;217:220–230. [PubMed: 8203750]
6. Swamy M, Siegers GM, Minguet SM, Wollschield B, Schamel WWA. Blue native polyacrylamide gel electrophoresis (BN-PAGE) for the identification and analysis of multiprotein complexes. Sci STKE 2006;345:1–14.
7. Powell MD, Ghosh M, Jacques PS, Howard KJ, Le Grice SFJ, Levin JG. Alanine-scanning mutations in the “primer grip” of p66 HIV-1 reverse transcriptase result in selective loss of RNA priming activity. J Biol Chem 1997;272:13262–13269. [PubMed: 9148945]
8. Tachedjian G, Aronson H-EG, de los Santos M, Seehra J, McCoy JM, Goff SP. Role of residues in the tryptophan repeat motif for HIV-1 reverse transcriptase dimerization. J Mol Biol 2003;325:381–396. [PubMed: 12559908]
9. Tachedjian G, Orlova M, Sarafianos SG, Arnold E, Goff SP. Nonnucleoside reverse transcriptase inhibitors are chemical enhancers of dimerization of the HIV type 1 reverse transcriptase. Proc Natl Acad Sci USA 2001;98:7188–7193. [PubMed: 11416202]

Fig. 1. Migration of HIV-1 p66 and p51 subunits by blue native electrophoresis. 5 μ g of protein loaded per well; (% monomer, % homodimer) calculated from K_d values. (A) BN-PAGE: Lane 1: Native Markers, Lane 2: 1 μ M p66 (75% p66, 25% p66/p66), Lane 3: 5 μ M p66 (60% p66, 40% p66/p66). (B) BN-PAGE: Lane 1: Native Markers, Lane 2: 5 μ M p51 (96% p51, 4% p51/p51), Lane 3: 10 μ M p51 (92% p51, 8% p51/p51). (C) BN-AGE: Lane 1: 5 μ M wt p51 (96% p51, 4% p51/p51), Lane 2: 5 μ M wt p51 + efavirenz (46% p51, 54% p51/p51), Lane 3: 5 μ M W401A p51 (100% p51), Lane 4: 5 μ M W401A p51 + efavirenz (~95% p51, ~5% p51/p51), Lane 5: 5 μ M (100% p51) L234A p51, Lane 6: 5 μ M (100% p51) L234A + efavirenz.

Fig. 2.

Migration of proteins by native agarose gel electrophoresis. 5 µg of protein loaded per well. Lane 1: aprotinin (pI = 10.0–10.5), Lane 2: cytochrome c (pI = 10.0–10.5), Lane 3: carbonic anhydrase (pI = 6.6–7.2), Lane 4: BSA (pI = 4.7), Lane 5: T4 DNA ligase (pI = 6.0–6.2), and Lane 6: p51 (pI = 8.7). (A) pH 7.0 with Coomassie blue G-250, (B) pH 7.0 without Coomassie blue G-250, (C) pH 8.5 with Coomassie blue G-250, (D) pH 8.5 without Coomassie blue G-250. Gels in (A–C) destained 36 h, gel in (D) destained 72 h.