Highly Sensitive and Fast Protein Detection with Coomassie Brilliant Blue in Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

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Detection of proteins in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is an important first step for protein analysis. For years, various experimental efforts have been directed to develop and improve the protein detection methods[1-4] with particular interests in their sensitivity. Among the available protein detection methods, staining with Coomasie brilliant blue (CBB) is most frequently used in laboratories. However, despite the popularity of the method, its sensitivity is still low, and needs to be improved significantly for detecting low-abundance proteins in analytical 2-D gels for proteomic applications. Previously, *Neuhoff et al.* successfully increased the sensitivity of CBB staining by using large amount of ammonium sulfate in acidic alcoholic media[5,6] where the dye molecules are aggregated into colloidal particles. Despite its great improvement in sensitivity, so called colloidal CBB staining method, still requires at least 10 ng of proteins in a band for a routine detection.[7] Furthermore, it was also observed that the colloid formation slowed down the staining process resulting in prolonged staining time.[6] When toxic methanol added in the staining solution was replaced by ethanol, even longer staining time, at least 24 hours incubation in staining solution, was recommended for optimal visualization.[4]

In dyeing protein-based fabrics, aluminum (III) ions have been used to improve the binding of the dyes to fabrics and also to accelerate the staining by enhancing penetration of the dyes into fiber.[8] As an attempt to improve protein detection with CBB-G250 in SDS-PAGE, in the present study, we modified the staining protocol by adding aluminum sulfate ($Al_2(SO_4)_3 \cdot (H_2O)_x$, x = 14-18) to the staining solution as well as replacing methanol with less toxic ethanol. We found that incorporating aluminum sulfate to the CBB-G250 staining solution considerably increased the binding of CBB molecules to protein bands and accelerated the staining process to a great extent.

The sensitivity improvement by incorporating aluminum sulfate and ethanol in the CBB-G250 staining process is demonstrated in Figure 1. Compared to the gel stained with the colloidal CBB-G250 (Fig. 1A), which is the most sensitive CBB-G250 based staining method reported previously,

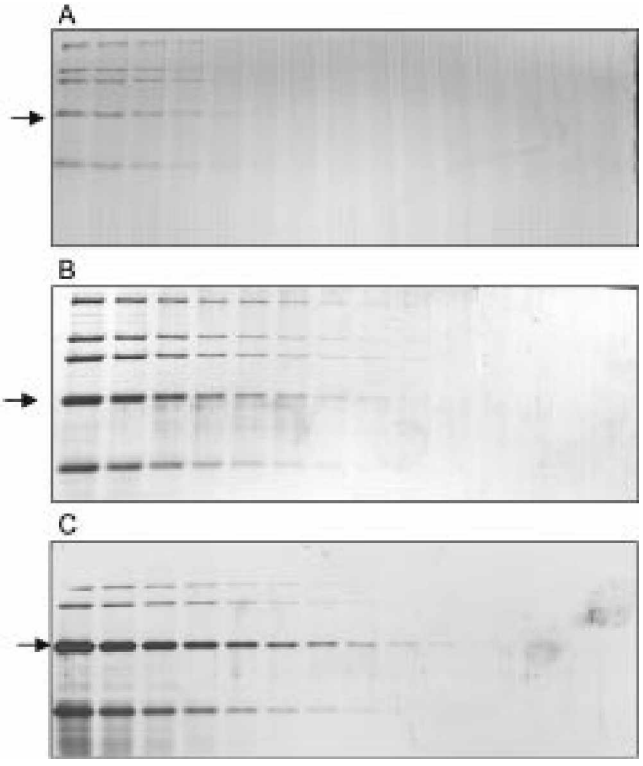

Figure 1. Comparison of Coomassie brilliant blue (CBB-G250)-based stainings with an acidic silver staining of protein bands in SDS-PAGE. Gels were stained overnight with the followings after electrophoresis was performed in 0.75-mm 10% acrylamide gels using the discontinuous buffer system of Laemmli (9) for 2 hours with 100 V at ambient temperature, followed by protein fixation in 30% ethanol containing 2% phosphoric acid: (A) 0.08% w/v CBB G-250 in 2% (w/v) phosphoric acid, 8% w/v ammonium sulfate and 20% methanol; (B) 0.02% CBB-G250 in 2% (w/v) phosphoric acid, 5% aluminum sulfate and 10% ethanol; (C) an acidic silver staining, according to the supplied instructions of Silver Stain Plus Kit from Amersham Pharmacia Biotechnology. Each lane contains approximately equal amount of rabbit skeletal muscle myosin (molecular mass 200 kDa), *Escherichia coli* β-galactosidase (116 kDa), rabbit muscle phosphorylase b (97 kDa), bovine serum albumin (66 kDa), and chicken egg white ovalbumin (45 kDa). Bovine serum albumin bands are indicated by arrows. 250 ng/band proteins were loaded at the most left and 2-fold diluted to the right side consecutively.

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protein bands in the gel treated with 5% aluminum sulfate prior to the addition of CBB-G250 (Fig. 1B) are much darker indicating more CBB-G250 dyes bound to protein bands. Protein bands as low as 1 ng/band are detected in Figure 1B indicating an order of magnitude improvement in sensitivity. The result of an acidic silver staining is shown in Figure 1C for a comparison. As anticipated, intensities of silver stained protein bands varied drastically depending on the proteins. Compared to the bands stained with CBB-G250 in the presence of 5% aluminum sulfate (Fig. 1B), silver staining was more sensitive only for bovine serum albumin detection among the molecular marker proteins employed for this study. Other proteins were visualized much more efficiently with the modified CBB-G250 staining method. It was also found that the band intensities obtained by this modified method has a linear relationship with the amount of proteins ranging from 0.25 μg to 2.0 ng measured in this experiment. The excellent linear dynamic response of protein band intensities is demonstrated in Figure 2.

Not only the modified staining method improved the sensitivity of protein detection in SDS-PAGE considerably, but also the staining was accomplished much faster. Figure 3 shows the time course of protein staining with CBB-G250 in the presence of aluminum ions and ethanol. The time to achieve half maximum staining was only 20 minutes, and moreover, only after 2 hours of incubation in the staining solution, about 90% staining to its maximum level was accomplished. In the case of colloidal CBB-G250 staining method, it was reported that at least 10 hours of incubation was necessary for 90% completion of the staining.[6]

In conclusion, we found a simple way to improve protein detection greatly by incorporating aluminum sulfate and ethanol in the process of SDS-PAGE staining with CBB-G250. The aluminum (III)-based staining in this study shows superior sensitivity that detects as low as 1 ng/spot routinely with little sensitivity variation depending on proteins. In addition, the staining of protein bands in SDS-PAGE requires only 2 hours for completion without needs for destaining afterwards. We believe this modified staining method can be applicable as a sensitive and fast CBB-G250 based protein detection method in SDS-PAGE.

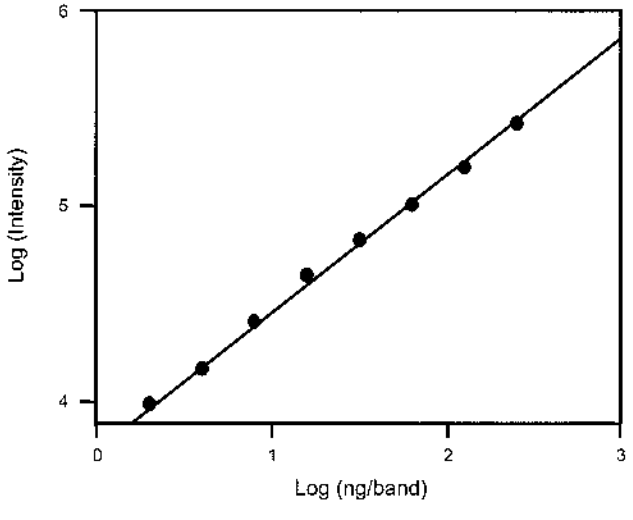

Figure 2. Linear relationship between protein amounts and the band intensities obtained by the modified Coomassie brilliant blue (CBB-G250) protein staining. The intensities of bovine serum albumin bands shown in Fig. 1B are depicted as a function of protein amounts (ng/band). Intensity values are in an arbitrary unit.

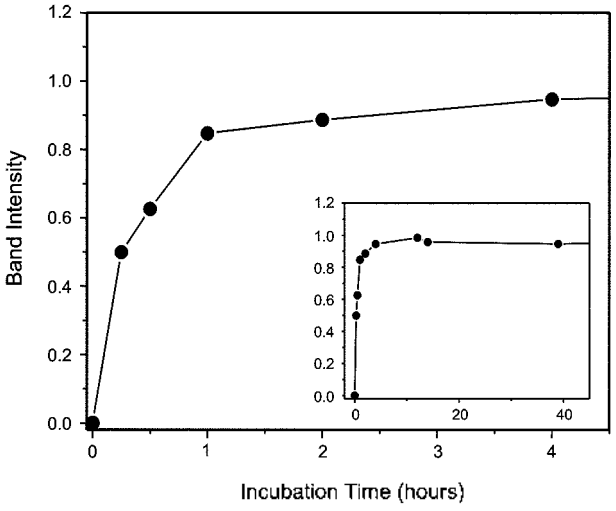

Figure 3. A time-course of protein band staining in SDS-PAGE with Coomassie brilliant blue dye (CBB-G250). Solid circles indicate the band intensities of 250 ng *Escherichia coli* β-galactosidase at various times, and the band intensities are normalized to the intensity obtained after 5 days. The inset shows the time-course at longer time scale up to 40 hours.

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