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Full Length Research Paper

An efficient and rapid method for protein detection with an example of sulfide-quinone reductase expressed in *Escherichia coli*

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Conventional Coomassie brilliant blue (CBB) staining is widely used in protein detection as it is inexpensive and reproducible. Conversely, it is also very time-consuming and cumbersome. In this study, CBB(R-250)-microwaved-water (CMW) was used to detect the sulfide-quinone reductase (SQR) protein expressed in *Esherichia coli* by staining and destaining in under 30 min. The CMW method has similar detection capabilities as the conventional CBB method. However, compared with the CBB protocol, it did not produce pungent odors, and was more efficient and rapid, which saved more than 4 to 10 h. This method is an acceptable alternative for the preliminary detection of protein expression.

Key words: CMW method, Coomassie brilliant blue (R-250), sulfide-quinone reductase, microwave irradiation, tap water.

INTRODUCTION

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) is one of the most popular methods for protein analysis and detection (Gotoa et al., 1999; Kawsar et al., 2008; Liau and Lin., 2008; Walker, 2002; Wilson, 1983). There are many protein staining protocols after SDS-PAGE, such as Coomassie brilliant blue staining (CBB), amido black staining (Heukeshoven and Dernick., 1985) and silver staining (Chevallet et al., 2006). However, the CBB staining is the most common method and overcomes the low sensitivity of amido black staining and the false positives that occur frequently with the silver staining (Wang et al., 2006). CBB (R-250) staining is widely used for protein detection as it is inexpensive and highly reproducible (Patton, 2002). Nonetheless, this process usually takes 1 to 2 h and the destaining commonly requires four or more hours. Additionally, acetic acid and isopropyl alcohol involved in this process emanate pungent odors. In order to save the time required for this procedure, we attempted to stain the gel using a microwave and destaining with 100°C water. Using this method, we detected the sulfide-quinone reductase (SQR) protein (GenBank ID: CAA66112.1) expressed in *Escherichia coli* in under 30 min. For this experiment, we compared the time to detect the same protein using the conventional CBB (R-250) protein staining and destaining processes with the CMW protocol. The results show that the bands of SQR protein produced by the two methods were very clear, but the CMW method was much more efficient and saved more than 4 to 10 h of processing time.

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MATERIALS AND METHODS

Two 10% polyacrylamide gels and the CBB R-250 staining fluid (0.1% (w/v) Coomassie brilliant blue (R-250), 25% (v/v) isopropanol, 10% (v/v) (glacial) acetic acid and 65% (deionized water) used in this study were prepared according to the manufacturer's instructions (TaKaRa Biotechnology, Dalian, China). The SQR gene from *Rhodobacter capsulatus* DSM 1710 was induced by isopropyl β-D-1-thiogalactopyranoside (IPTG) and expressed in *E. coli* cells. The 10 µL of total protein was mixed with 10 µL denaturing solution (250 mM Tris-HCL (pH 6.8), 10% (w/v) SDS, 0.5% (w/v) bromophenol blue, 50% glycerine, and 5% 2-mercaptoethano which was heated for 5 min at 100°C and snap-

cooled on ice. The samples were then subjected to PAGE (Mini-PROTEAN Tetra system and Power Pac universal 500 W electrophoresis system) (BIO-RAD, USA) using a gel (80 mm wide×73 mm high×1.0 mm thick) in 1×SDS (Genview, USA) at 80 V for 45 min and then at 130 V for 60 min. Afterwards, the two gels were placed in separate Petri dishes and washed three times with tap water.

For the second step, one gel was covered completely by the CBB R-250 staining fluid and then irradiated by microwave (Galanz, Guangdong Province, China) twice for 10 s each. At this time, the stain was finished. After the gel was naturally cooled at room temperature (RT), it was placed in a beaker (1000 ml) and 100 ml of boiling water was added to the beaker. The beaker was then placed in a pot of 100°C water for 15 to 20 min until the background of the gel became clear and the bands could be seen distinctly. During this process, the boiled water in the beaker was replaced every 4 min. After, the gel was again naturally cooled, an image of the gel was captured and analyzed using the Tanon-2500 Gel Image System (Tanon, Shanghai, China).

Simultaneously, the other gel was stained and destained using the conventional CBB method. Briefly, the gel was covered with the same staining fluid used in the aforementioned procedure and shaken (TS-2 Orbital Shaker, Kylin-Bell Lab Instruments, Jiangsu, China) at 75 rpm for 90 min. The staining fluid was poured off and the gel was washed with tap water three times and destained [10% (v/v) acetic acid, 5% (v/v) ethanol, and 85% dH_2O] using a shaker for 12 h (over nights) or until the background appeared clear. During this process, the solution was replaced two or more times depending on the color of the liquid. The gel was then analyzed as previously mentioned.

RESULTS AND DISCUSSION

In this study, we used two different methods to detect the SQR protein (50 kDa) expressed in *E. coli*. The results (Figure 1) show that the SQR protein could be detected by both methods and there was virtually no difference between the intensity of the protein bands. The most valuable characteristic of the CMW method was the greatly reduced amount of time spent on the staining and destaining processes. Using the CMW method, approximately 20 min was required for the detection of the protein bands. Compared with the conventional method, the CMW protocol saved 4 to 12 h or so. Moreover, boiled water instead of an irritant or pungent solution was used for destaining in the CMW method, which eliminated exposure to harmful odors. Additionally, the CMW method was completed at a much lower cost and was more efficient than the conventional method. Characteristics of the two methods are described in Table 1.

In the CMW method, the staining was carried out using a microwave oven. Staining and destaining procedures by microwaving and using various solutions have also been reported to shorten the processing time. However, some other complex dye solutions have also been involved, and the saved time also need up to 35~90 min in processing time (Gao et al., 2003; Marchetti et al., 2009). In addition, only a few studies detailing a destaining procedure using boiled tap water have been reported (Wang et al., 2006; Yasumitsu et al., 2010). Compared with these processes, the advantages of the CMW method are a less processing time, lower cost and reduced exposure to harmful chemicals and less potential pollution to the environment.

In this study, the CMW method was used for the rapid detection of the SQR protein but not for qualitative analysis. Several studies on nanogram-sensitive CBB staining have already been reported (Georgiou et al., 2008; Wang et al., 2007; Yasumitsu et al., 2010). Highly-sensitive colloidal CBB G250 staining (Neuhoff et al.,1988) and sensitization-mediated CBB R250 staining (Wang et al., 2007) were used to detect protein bands at 0.1 and 8 ng, res-pectively. However, these methods required long processing times and contained many complex steps. The former requires at least 15 h for staining and the latter takes approximately 8 h. Another study on highly sensitive CBB staining methods using CBB R-250 as the staining dye has also been reported (Yasumitsu et al., 2010). This method was inexpensive and the sensitivity of the detected protein was about 1.4 ng, but the usage of methanol and ammonium sulfate was necessary. Also, the processing time was approximately 40 min longer than that required by the CMW method. In comparison, the CMW method detected proteins with a sensitivity of about 10 to 20 ng in under 30 min, which included 15 to 20 min for the destaining procedure. Recently, some commercially available CBB G-250 staining reagents have been reported to detect protein bands of appro-ximately 10 ng; however, the reagents were proprietary, where the ingredients and their concentrations are not known (Yasumitsu et al., 2010). Also, the CBB stain kit produced by Nacalai Tesque is very expensive.

Some minute details should not be overlooked in the CMW method. Before staining, it is necessary to wash the gels with tap water, which will be helpful to produce better bands and lower background colorization caused by the electrophoresis buffer. Transient washing of three to five times is sufficient for the follow-up work. It should be further noted that cooling the gels naturally is essential for the procedure. Time required for conventional staining is dependent upon the thickness and concentration of gels. In general, 0.7 mm thick gels are stained for 60 min, and those of 1.0 mm thickness require more than 90 min. However, the thickness of gels was not a concern in the CMW method. The shorter staining and destaining time requirements are mainly due to heating or the high temperature, which accelerates the CBB molecular diffusion during the procedure and further accelerates the destaining reaction. Throughout the destaining process, the saturated solutions reached a state of dynamic equilibrium, which limited the amount of Coomassie stain that dissolved and necessitated frequent water changes every 4 to 5 min to achieve a clear background as soon as possible.

In summary, this study provides a much simpler method to stain gels, which required 20 s of irradiation in a microwave oven and 20 min to destain gels using boiling tap water, which replaced the traditional CBB R-250 destaining

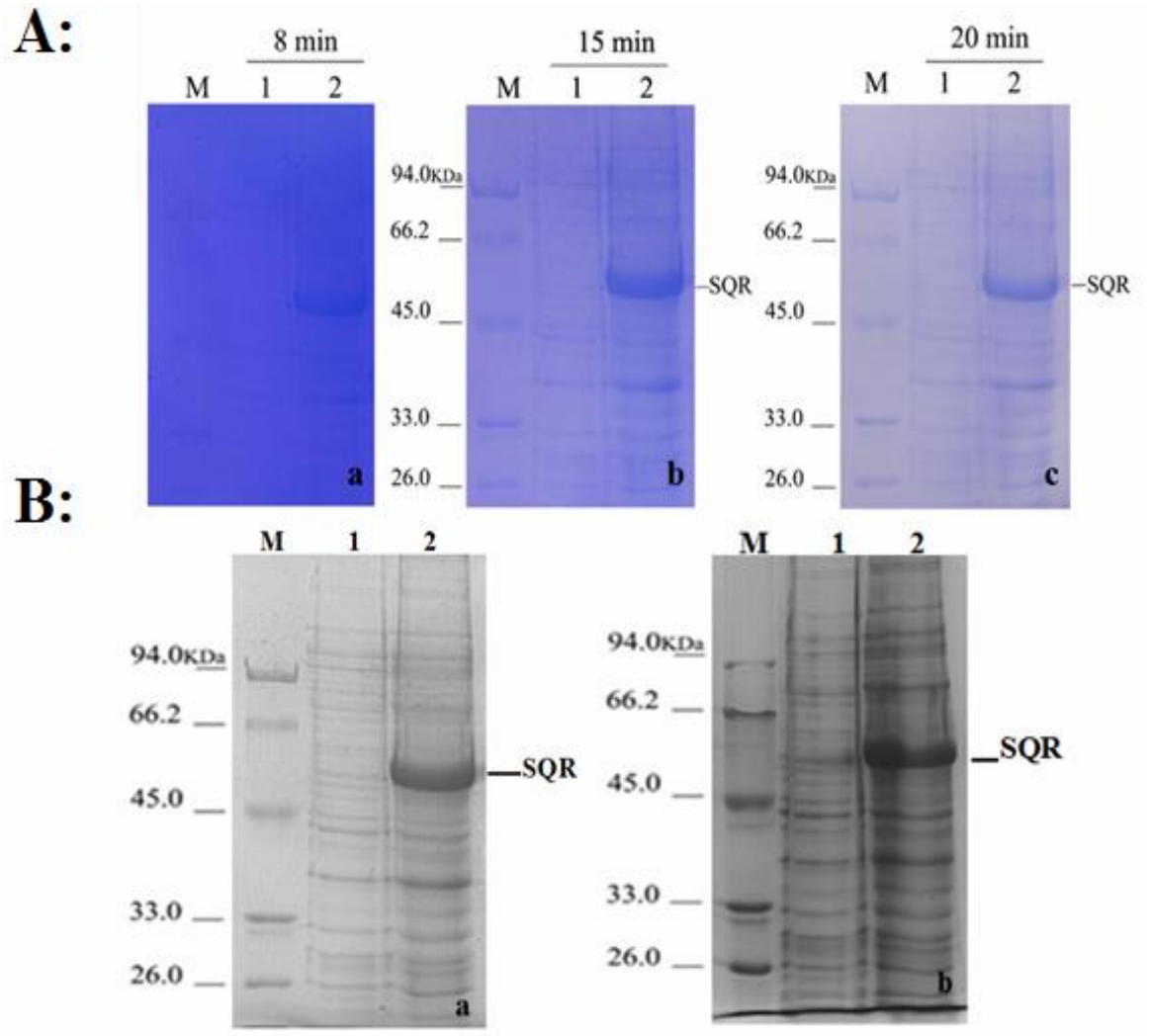

Figure 1. The SDS-PAGE analyses of the SQR protein with an apparent molecular mass of 50 KDa by the CMV method and the conventional CBB method. M, Protein molecular marker; 1, the control vector; 2, the SQR protein sample. A, The destaining process of the SQR protein by the CMW method. The gel was distained for 8, 15 and 20 min, respectively (a, b, c); B, the SQR protein detected by the CMW method and the conventional CBB method; a, the SQR protein detected through the CMW method; b, the SQR protein detected by the conventional CBB method.

Table 1. A comparison between the conventional method and the CMV method.

Characteristic	Conventional method	CMV method
Staining time	≈1 h	20 s
Destaining time	≈4 to 12 h	≈15 to 20 min
Total time	≈5 to 13 h	≈21 min
Background	Clear	Clear
Odor	Long-term, volatile, pungent	Less volatile
Cost	High	Low
Summary	Cumbersome and pungent	Rapid and efficient

solution. This process obtained similar results and a clearer background with providing a more efficient, rapid and cost-effective method compared to conventional CBB staining. This study shows that the CMW method provides

an excellent alternative for preliminary detection of protein expression.

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