Running Colour Electrophoresis and Silver Nitrate Doublestaining of Polyacrylamide Gel

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A running colour electrophoresis of protein in sodium dodecylsulfate (SDS)-polyacrylamide gel (PAG) has been developed and the coloured gel was restained with silver nitrate and hydroquinone (HQ) within 20min. Trichloroacetic acid (TCA) was found to be a powerful destaining reagent and reproducibility of this double staining procedure was excellent.

Key words: Color protein, Silver stain, Gel shrinkage, Polyethylene glycol.

Introduction

It is very important to know a protein component in our biomembrane field. As a sensitive method for the determining protein molecular weights, the techniques of SDS-PAG have rapidly grown in popularity and are now well documented. However, the drawback with these techniques is the indirect staining method. In the running colour electrophoresis described in the present paper, protein bands in the gel were stained directly. These methods can be carried out with a satisfactory. They visualize protein quantities in the order of 100ng for the running colour electrophoresis and 100pg for the silver restaining procedure. The running colour electrophoresis was found to be very useful for preparative PAG.

Materials and Methods

The electrophoretic methods based on the standard procedure described by Weber and the 12.5% gel used here have been recommended for electrophoresis of proteins with a molecular weight (MW) below 100,000. Four proteins; BSA: 68,000 in MW, OA: 45,000, Chy: 25,000 and Cyt: 12,500 were purchased from Böhringer Mannheim, Germany.

The other reagents were obtained from Daiichi Pure Chem. Co. Ltd.,(Tokyo 130). The gels may be of arbitrary acrylamide concentration. Mini-slabs, 50mm in length and 1mm in thickness were used. The proteins were dissolved with SDS-solution and loaded in wells with a width of 4mm formed by a teflon comb.

The running colour procedure used here serially has been tabulated Table 1 and the conventional CBB stain was shown in Table 2 with the destaining method.
Step 1: Running colour electrophoresis

600μl of 0.1% CBB was added to 50ml of running buffer and the resulting coloured solution was placed in the running chamber. After loading protein into the wells formed by the comb, the material was run by electrophoresis. Coloured protein bands could be discriminated during electrophoresis. Several colours; CBB R-250,2 CBB G-250, Safranin O, Bromcresol purple, Eosin, Bromophenol blue, Haematoxylin and Amido-black were tested. CBB R-250, G-250 and Safranin O produced the best staining.

Step 2: Restaining in silver nitrate

The stained gel was placed in 0.1% AgNO₃ for 15 min at 80°C. The silver nitrate solution was prepared as follows: 5ml of 2% AgNO₃, 5ml ethanol and 5g PEG-2000 in MW were dissolved in H₂O and the solution made up to 100ml. During this step, the stained gel shrinks slightly.

Step 3: Development in HQ

Next the gel was transferred to a developmental solution made up of 0.008% HQ for 2min at 80°C. The developmental solution was prepared by dissolving 0.2g of HQ and 50ul of 4% NaOH in 2.5ml of H₂O. The bands stained rapidly into a red-black colour within 2min. During this step, the stained gel changed to a pale brown.

Step 4: Stocking in PEG-2000

Placed the silver restained gel into 100% PEG-2000 for 5min at 80°C. The gel shrank to approximately half the original gel size in this step. Shrinkage resulting from immersion in PEG-2000 was reversible by immersing in water. Immersing in water led to swelling and enlarging of the gel to its original size or more.
Step 5: Destaining

Transferred the above overstained gel to 20% TCA and incubated for approximately 2 min or more at room temperature. During this period the gel swells gradually and destains to give a pale red-yellow in back ground. Following this, the gel was reimmersed in the PEG-2000 solution of step 4. The gels stained doubly with CBB and silver can be permanently stocked in the PEG-2000 of step 4.

Results and Discussion

Reproducibility of the double staining method was excellent. On the whole the method was quick and easy to perform. This double staining method involving direct staining by running colour electrophoresis and restaining with AgNO$_3$ took less time than the previously reported method$^{1-3,9-13}$.

Sensitivity of the running colour electrophoretic method was estimated using different concentrations of protein in test solutions on the same gel. As shown in Fig. 1, the running colour electrophoresis was capable of detecting 100 ng quantities of proteins. This sensitivity was higher than that of the commonly used CBB stain method$^{1-3,9-13}$ and the band coloration was paler than that of the conventional CBB in Table 2.

Different colours were tested as described in step 1 and it was possible to stain the protein bands with different colours. One of the factors leading to good staining of the running protein bands in the SDS-gel is suggested to be the result of the protein molecules present in the gel matrix being encapsulated or coated by the respective stains. This was achieved in the present method by using an optimum concentration of 1.2 $\times$ 10$^{-3}$% stain. Use of R-250, G-250 of CBB and Safranin O having many conjugated valent bonds in the molecular configuration$^{15}$ gave the best results. Concentration over 4.8 $\times$ 10$^{-3}$% led to overstaining of the running colour electrophoretic method.

The present running colour method was useful for preparative gel electrophoresis$^{14}$, because of simultaneous visibility of the stained protein band during electrophoresis (in preparation).

SDS-PAGE is generally used to separate proteins on the basis of MW$^{1,2}$. In these experiments, it is protein dye complexes which are being separated. It may well be that the stoichiometry of these complexes will depend on the amino acid composition of the protein. Hence, the MW of the complex may not closely reflect that of the protein. A series of the control experiments investigating this possibility with Tables 1 and 2 made this a better piece of work.

The sensitivity of the silver restaining method was compared to those of others$^{10,11}$. In this case as well sensitivity was found to be highest for 100 pg to 0.5 ng of protein.

The optimum concentration for the best sensitivity at high temperatures was 0.1% AgNO$_3$ in step 2 and 0.008% HQ in step 3. When the concentration of silver nitrate in the staining solution was increased to 0.12% or higher a silver mirror image was generated. This could be prevented by incubating the gels for shorter periods. Hence it was important to decrease the incubation period, concentration of less than 0.08% or lower need a longer agitation period for good staining.

TCA solution was found to be a poweful destaining reagent for all overstained gels at room temperature. Examination of the destaining ability of several other reagents revealed MCA and acetic acid in the carboxyl group also to possess good destaining potential. The order of their destaining abilities was found to be TCA $>$ MCA $>$ acetic acid. The reason why these reagents$^{14}$ are good destainers is not clear at present. One of the main thesis is the identification of advantages and a need for the simple double stain with using the destainer. Consequently, application of the procedure to an overstaining that warrants double staining would greatly improve the contribution.
Fig. 1 Running colour electrophoresis for protein detection. Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 contain 5μg, 1μg, 0.5 μg, 0.1μg, 50ng, 10ng, 5ng, 1ng, 0.5ng, 100pg, 50pg respectively, calibration of right ordinate means 1mm per one division and all of them are the same gel through the serial experiment. From 5μg to 0.1μg of protein bands were coloured with blue of CBB-G 250 during electrophoresis.

Fig. 2 Influx silver nitrate solution into gel. Gel was transparent except the staining colour of Fig. 1 during the double staining procedure at step 2 and the colour bands were faithfully restained at step 3.

Fig. 3 Developemental HQ in an alkaline solution. A low concentration of HQ was reacted to the influxed AgNO₃ into gel of Fig. 2 and deposited on a protein a band with red-black. 100pg of protein band were restained. Background noise was sometime overstained with a silver mirror image, however, a high concentration of TCA by the repeated treatment of step 5 was always destained the noise.

Fig. 4 Shrunken gel of Fig. 3 by PEG. Notice the gel size at the same scale of Figs. 2 and 3 and a shrunken gel with colour band was not changed during one year.

References


