Continuous-Elution Electrophoresis for Purification of the Baculovirus-Expressed Coronavirus Structural Proteins

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Summary

Three major structural proteins of the porcine coronavirus, transmissible gastroenteritis virus (TGEV), membrane (M), nucleocapsid (N), and spike (S), were expressed in a baculovirus expression system. A method for rapid purification of the virus-specific proteins from cellular components was developed by using continuous-elution polyacrylamide gel electrophoresis (CE-PAGE). This approach might be a valuable preparative tool prior to immunization studies, monoclonal antibody production, subunit vaccine preparation, quantification of protein expression and for other experimental scenarios where purified viral proteins are required.

Introduction

TGEV causes diarrhea and vomiting in pigs of all ages and produces 90–100% mortality in 1–2 week old sero-negative pigs (Bohl *et al.*, 1972). The economic impact of TGEV on the U.S. pork industry was estimated as $200 million per year (Saif and Wesley, 1992). A possible vaccine includes the three purified recombinant TGEV structural proteins embedded in immuno-stimulating complexes (ISCOMs), microcapsules or other carriers.

Materials and Methods

The Sf9 insect cells containing the TGEV proteins were harvested from 150 cm^2 stationary cell culture flasks (1.8 x 10^7 cells) and resuspended in 1 ml of phosphate buffer saline (PBS), pH 7.3, mixed with 4 parts of sample buffer (0.06 M Tris, pH 6.8; 10% glycerol; 2% (w/v) SDS; 5% mercaptoethanol and 0.05% (w/v) bromophenol blue), heated at 95 °C for 4 min and stored at 20 °C until used for preparative electrophoresis.

A Mini Prep Cell (Bio-Rad Laboratories, Hercules, CA) system was used to perform continuous-elution polyacrylamide gel electrophoresis (CE-PAGE) with conditions modified for each TGEV protein to be purified. For the TGEV M-protein, the sample (0.5 ml, corresponding to 18 x 10^5 cells) was layered onto a 4% (1.5 cm) polyacrylamide stacking gel with a 10% (8 cm) polyacrylamide separating gel. Proteins were electrophoresed under denaturing conditions at constant voltage (200 V) for 3.5 h with a flow rate of 0.05 ml/min. After 3.5 h, fractions were collected in 5 min intervals (0.25 ml/fraction) with a total of 30 fractions. For the TGEV N-protein electrophoresis was performed as described above, but for 4.5 h prior to collection of 30 fractions. For the TGEV S-protein; the sample (0.5 ml, corresponding to 18 x 10^5 cells) was layered onto a 4% (1.5 cm) polyacrylamide stacking gel with a 6% (8 cm) polyacrylamide separating gel in the Mini Prep Cell gel tube assembly. Electrophoresis was performed as described above for 8.5 h prior to collection of the 30 fractions.

Selected protein fractions were examined by electrophoresis in analytical polyacrylamide slab gels and stained with Coomassie® blue (Wu and Welsh, 1996). The fractions of desired molecular weight were pooled and concentrated using Centricon devices (Amicon, Beverly, MA) with a 10 kDa cutoff for the M-protein, and a 30 kDa cutoff for the N- and S-proteins. Virus specificity of the M-, N-, and S-proteins and conformational stability of the N-protein and epitopes A and D of the S-protein were confirmed by immunoblotting using porcine hyperimmune antiserum to TGEV (Stott, 1989) or a TGEV neutralizing monoclonal antibody specific for epitope A of the S-protein (25C9) and non-neutralizing monoclonal antibodies specific for epitope D of the S-protein (44C11) and N-protein (25H7) (Lanza *et al.*, 1995). The protein concentration was estimated with spectrophotometer readings at 280 nm.

Results

By extrapolating the molecular size to prestained protein standards or to native coronavirus proteins from virus (Wesley *et al.*, 1991) cell culture adapted TGEV (Miller strain), the recombinant TGEV M-protein (28–31 kDa) was expected to be eluted within fraction range 9–13 (Figure 1A). Fractions 9–13 were pooled and concentrated. The presumed M-protein was electrophoresed and detected by Coomassie blue stain (Figure 1B, lane 5) and its virus specificity was confirmed by western blot (Figure 1C, lane 5). After concentration of fractions 9–13, the final protein concentration was 0.4 mg/ml or 5% of the initial solubilized cell lysate protein.

The recombinant TGEV N-protein (47 kDa) was expected to be within fraction range 25–27 (Figure 2A). These fractions were pooled and concentrated. The concentrated sample was analyzed as described for the recombinant TGEV M-protein (Figure 2B, C). The resulting purified N-protein concentration was 0.5 mg/ml, or 8% of the initial baculovirus lysate.

The recombinant TGEV S-protein (150 kDa) was expected to be within fraction range 20–30 (Figure 3A). Fractions 20–30 were pooled and concentrated. The concentrated S-protein sample was further analyzed as described for the M- and N-proteins (Figure 3B, C). In addition to the major band of 150 kDa, a minor, virus-specific band of 100 kDa was detected on both polyacrylamide gels and nitrocellulose membranes (Figure 3B, C, lane 5). The resulting purified S-protein concentration was 0.15 mg/ml or 2% of the initial baculovirus lysate.

Discussion

The production of subunit viral vaccines requires the purification of the immunogens of interest from other cellular proteins. The CE-PAGE represents an approach whereby proteins are electrophoretically separated based on their molecular size and collected in fractions.

The yields of purified M-, N- and S-proteins corresponding to the 18 x 10^5 Sf9 insect cells (0.5, 0.4 and 0.15 mg/ml, respectively) are reflective of the levels of recombinant protein expected (2–8%) and in agreement with data published on the efficiency of the baculovirus expression system (Luckow *et al.*, 1988). Elution of small volumes of purified, concentrated proteins (0.2–0.5 ml) reflects the capacity of the Mini Prep Cell system, but can be scaled up when larger amounts are required by using the same conditions and a Model 491 Prep Cell.

By western blotting of the purified proteins with porcine hyperimmune antiserum to TGEV (S, M, N) in western blot and the ELISA reaction with monoclonal antibodies (S, N), we demonstrated that immunogenic and neutralizing epitopes were preserved on the purified proteins. On both polyacrylamide gels and nitrocellulose membranes, a minor 100 kDa protein band in addition to the major 150 kDa protein was detected for the TGEV-S protein (Figure 3B, C, lane 5). This most likely reflected the presence of an immature, posttranslationally non-modified form of S-glycoprotein as previously reported (Shoup *et al.*, 1997) or cleavage product. Similarly, a minor protein band in addition to the full size recombinant TGEV-M protein was detected by western blot (Figure 1C, lanes 3 and 5).

The results from this study suggest that CE-PAGE can be a useful semi-preparative tool to purify baculovirus-expressed TGEV viral proteins.

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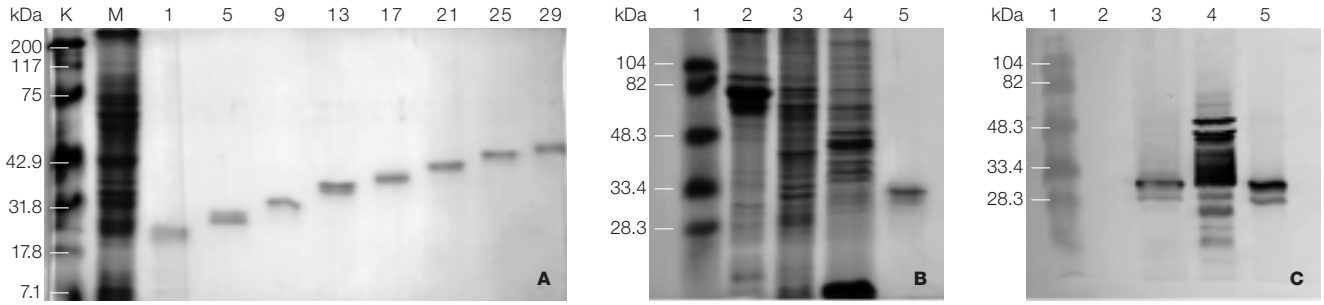

Fig.1. A. Mini Prep Cell purification profile. Coomassie stained 12% SDS PAGE. Lane K= Kaleidoscope prestained protein standard (Bio-Rad), Lane M= baculovirus lysate with TGEV M-protein (starting material), (Lanes 1–29)= Mini Prep Cell fractions; B) Lane 1= low range prestained protein standards, Lane 2= Wild type AcNPV baculovirus protein lysate, Lane 3= TGEV M-protein transformed baculovirus lysate, Lane 4= TGEV viral lysate, Lane 5= CE-PAGE purified, pooled and concentrated fractions 9–13; C) Nitrocellulose membrane with TGEV-hyperimmune serum immunoprecipitated proteins from gel 1-B (Lanes 3–5).

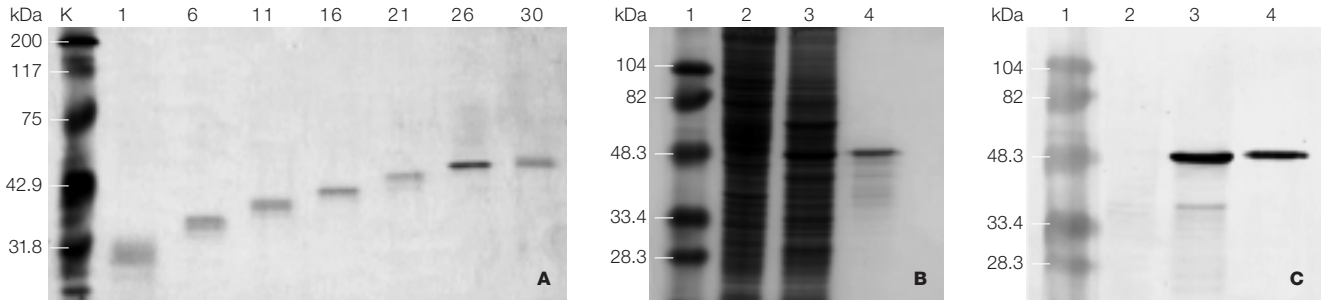

Fig 2. A. Coomassie stained 12% PAGE of Lane K= Kaleidoscope prestained protein standard, (Lanes 1–30)= Mini Prep Cell fractions; B. Lane 1= low range prestained protein standard, Lane 2= wild type AcNPV baculovirus/ysate, Lane 3= baculovirus expressed TGEV N-protein, Lane 4= CE-PAGE purified, pooled and concentrated fractions 25–27; C. Nitrocellulose membrane with TGEV-hyperimmune serum immunoprecipitated and visualized proteins from gel 2B (lanes 3–4).

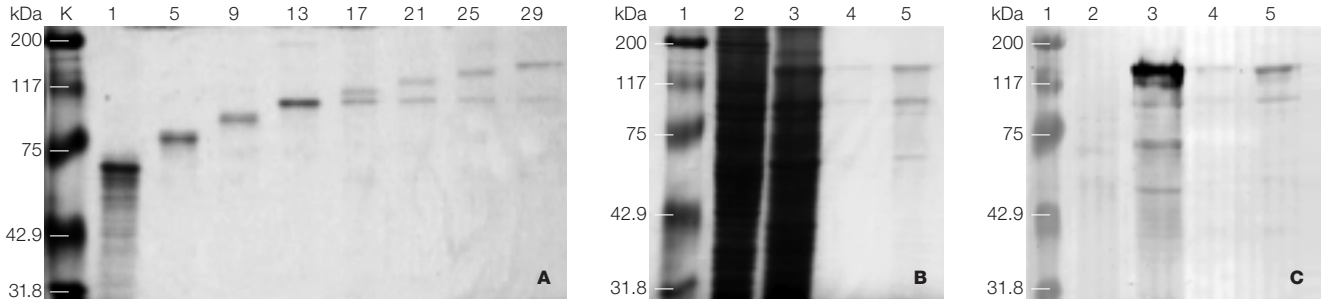

Fig 3. A. Coomassie stained 10% SDS PAGE of Lane K= Kaleidoscope prestained protein standard, (Lanes 1–29)= Mini Prep Cell fractions; B. Lane 1= kaleidoscope prestained protein standard, Lane 2= wild type AcNPV baculovirus/ysate, Lane 3= the baculovirus expressed TGEV S-protein, Lane 4= fraction 29 the baculovirus expressed TGEV S-protein, Lane 5= CE-PAGE purified, pooled and concentrated fractions 20–30; C. Nitrocellulose membrane with TGEV-hyperimmune serum immunoprecipitated and visualized proteins from gel 3B (lanes 3–5).

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