Journal of Experimental Microbiology and Immunology (JEMI) Vol. 14: 48-50

Determination of the Relative OmpA Expression and Membrane Integration in an OmpA-Deficient Escherichia coli Strain Complemented with a Plasmid Containing an OmpA Gene.

Jolina Carson and Alec Lee
Department of Microbiology and Immunology, University of British Columbia

The outer membrane protein A (OmpA) of E. coli, is thought to be necessary for F-factor conjugation. A previous study demonstrated that a wild type E. coli strain and an OmpA-deficient strain, each complemented by the pCR2.1-TOPO plasmid containing a cloned ompA gene, had no significant differences in conjugation efficiency upon induction of the ompA gene on the plasmid. This investigation assessed whether the poor conjugation efficiency was due to lack of integration of the OmpA into the outer membrane in the complemented strain. The wild-type and complemented mutant strains were lysed and outer membranes extracted to separate cytosolic and membrane samples. SDS-PAGE was used to separate proteins in these samples to identify the presence or absence of OmpA. Results from this investigation suggest OmpA was not integrated into the uninduced complemented mutant. The presence or absence of the 40 kDa OmpA protein was difficult to discern in cytosolic samples because of poor gel replicates and the lack of a distinctive OmpA identifier such as an antibody.

OmpA is a transmembrane protein that occurs at approximately 100,000 copies per cell making it a major outer membrane protein of *E. coli* and crucial to cell structure and integrity (4). Moreover, OmpA is also needed for key interactions such as the tight binding of recipient to donor cells during F factor conjugation (5). Previous work by Chambers *et al.* (2) showed that complementing both wild type and OmpA$^-$ *E. coli* strains with the pCR2.1-TOPO plasmid (3), which contains an *ompA* gene, did not affect conjugation efficiency when the *ompA* gene on the plasmid was induced. The current investigation attempted to determine if OmpA was expressed but not integrated into the outer membrane of *E. coli*, which could explain the poor conjugation efficiency previously observed (2). This study used established *E. coli* cell strains C149 (wild type) and C156 (OmpA$^-$) complemented with the *ompA*-containing pCCK06-1 plasmid developed by Chambers *et al.* (2) to determine if OmpA expression could be found in the membrane versus the cytosol. The pCCK06-1 plasmid contains a P_{lac} promoter which allows IPTG induction of the *ompA* gene (2). C149 and C156 strains with the pCCK06-1 plasmid were cultured, followed by cell lysis using the French Press method. An outer membrane extraction was subsequently carried out and cytosolic and membrane samples were ran on SDS-PAGE gel for identification of OmpA presence in the two samples. We observed that OmpA was found integrated into the membrane of *E. coli* strain C149 with induced pCCK06-1 plasmid. A definitive conclusion concerning the membrane of the C156 strain and all cytosolic samples could not be made due to several encountered difficulties described.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The pCCK06-1 plasmid is a pCR2.1-TOPO plasmid (Invitrogen; Catalog No. K4500-01) containing an *ompA* gene in the P_{lac} insertion site. *E. coli* strains containing the pCCK06-1 plasmid were obtained from the MICB 421 bacterial strain collection in the Department of Microbiology and Immunology at the University of British Columbia. The wild-type strain C149 with pCCK06-1 was designated CCFQ071 and the mutant strain C156 with pCCK06-1 was designated CCFQ072. Table 1 describes complete genotypes of the two parental *E. coli* strains used in previous conjugation.

Culture conditions. *E. coli* CCFQ071 and CCFQ072 strains were grown in Luria-Bertani (LB) broth containing 100 μg/mL ampicillin (Sigma; Catalog No. A-9518). Two batches of each culture of 400 mL each were incubated at 37°C in a shaking incubator. IPTG at 280 μg/mL, (Sigma; Catalog No.5529UA), was added to one batch of each culture to induce *ompA* expression from the pCCK06-1 plasmid.

Cell lysis. Both batches of *E. coli* CCFQ071 and CCFQ072, induced with IPTG and uninduced, were centrifuged on a Beckman Model J2-21 centrifuge (Beckman Coulter, Inc., Fullerton, CA) using a SLA-1500 rotor for 25 minutes. Supernatant was removed and pellet was resuspended in 10 mL of Tris HCl, pH 8.0 (Sigma; Catalog No. T3253), 1mM EDTA (Fisher Scientific; Catalog No. S-311), 20% sucrose (Fisher Scientific; Catalog No. BP220-1), and 1mg/mL Lysozyme. Each batch was subsequently added to French Press machine for two cycles at 15,000 psi to lyse cells and the lysate was stored at -20°C.

TABLE 1. Genotypes of C149 and C156 parental *E. coli* strains

Strain	Phenotype	Genotype	Strain with Plasmid
C149	Wild-type	F⁻ *pro*C-24 *aro*A-357 *his*-53 *pur*E-41 *ilv*-277 *met*-65 *lac*Y-29 *xyl*-14 *rps*L-97 *cyc*B-2 *tsx*-63 lambda	CCFQ071
C156	OmpA	F⁻ *pro*C-24 *aro*A-256 *his*-53 *pur*E-41 *ilv*-277 *met*-65 *lac*Y-29 *xyl*-14 *rps*L-97 *cyc*B-2 *tsx*-63 lambda⁻	CCFQ072

Protein assay. A modification of the Lowry protein assay for detergent-solubilized proteins includes the presence of SDS in reagent A and was used as previously described (6).

Outer membrane extraction. Each culture was centrifuged using a Beckman Model J2-21 Centrifuge (Beckman Coulter, Inc., Fullerton, CA) using a JA-20 rotor at 2500 x g for 25 minutes at 10°C to remove unbroken cells. Supernatant was collected and subsequently centrifuged using a JA-20 rotor at 35,000 x g for 20 minutes. Pellet was collected and washed twice with 10 mM Tris HCl, pH 8.0, then resuspended in 4 mL of 2% Triton X 100 (Bio-Rad; Catalog No. 161-0407) and 10 mM Tris HCl, pH 8.0. Resuspended pellets were then stored at room temperature for 30 minutes and stored at 4° C.

Membrane and cytosolic sample preparation. Cytosolic samples and Bio-Rad Low-range molecular weight standard (Bio-Rad; Catalog No. 161-0304) were boiled in a heat block at 100° C for 10 minutes prior to loading onto SDS-PAGE gel. Membrane samples were incubated at 37° C for 30 minutes prior to loading.

Outer membrane buffer displacement. Prior to analysis on SDS-PAGE, the Triton-X100 buffer used for solubilization was displaced with SDS buffer (8 mM TrisHCl, pH 8.0 with 2% SDS). We used Millipore Microcon Centrifugal filter device, 10,000 MWCO (Microcon YM-10; Catalog No. YM-10:42406). Each membrane sample was placed into filtering device and spun at 14,000 x g for 30 minutes at 25° C and volume reduced from 500 μL to 150 μL. After adding 350 μL of SDS buffer the centrifugation step was repeated three more times for a final dilution of the initial Triton-X100 buffer to 2.7% of its initial concentration. Membrane samples were subsequently prepared using this filtrate as described above.

SDS-PAGE. Gels (5% for stacking gel and 10% for separating gel) to run cytosolic and membrane samples were prepared using protocol described previously (1). The stacking gel was 2 to 3 cm in length. The gels were run at 60 V for approximately 45 minutes followed by 140 V for approximately 6 hours. After electrophoresis, the gels were stained in 0.1% Coomassie Blue solution (40% methanol, 10% acetic acid) for 30 minutes, and destained in 15 minute intervals for 1 hour. Gels were photographed using AlphaImager software (v. 4.1.0; Alpha Innotech Corporation).

RESULTS

Using the standard curve generated from the Bio-Rad Low Molecular Weight Standard, the uninduced CCFQ072 had the absence of a band at 40 kDa, approximately in the range of expected OmpA migration (4,5). Additionally, the membrane sample from the induced strain of CCFQ071 had other differences compared to those of the uninduced strain of CCFQ071 and both samples of the CCFQ072 strain lacked bands at 40 kDa and 54 kDa and showed the presence of a band at 62 kDa.

The presence or absence of the 40 kDa band is difficult to discern in the cytosolic samples (lanes 3 through 6) given the much higher abundance of protein bands. Hence a direct comparison cannot be made between the bands seen in the cytosolic lanes and bands seen in membrane lanes. Observing the gel, there were no obvious differences in the banding patterns that would indicate the overwhelming presence of OmpA in the cytosolic sample of the induced CCFQ strain versus the cytosolic samples from the other strains.

DISCUSSION

The absence of a protein band in the uninduced CCFQ072 strain and its presence in the induced CCFQ072 samples of the strain indicated that OmpA expression was likely being induced by IPTG. It is important to note that the presence of OmpA in any of the samples could not be definitively determined as no

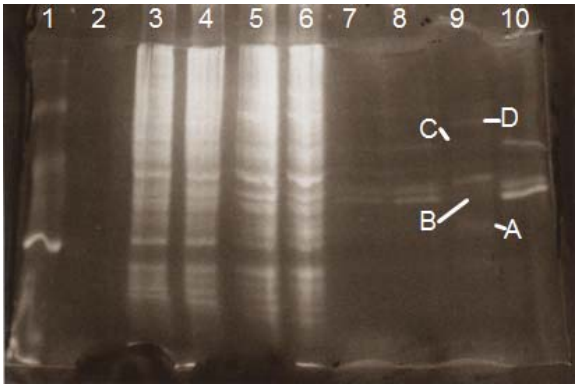

FIG 1. 10% SDS-PAGE Gel with Cytoplasm and Membrane Samples. (A) indicates the location of the 36 kDa region of the C149 Membrane Induced sample showing the presence of a protein band. (B, C, and D) indicate the lack of the 40 kDa protein, lack of the 54 kDa protein, and presence of the 62 kDa protein respectively. Lane 1 contains Bio-Rad Low-range molecular weight standard, lane 2 is empty, lane 3 contains the cytosolic fraction of uninduced CCFQ071, lane 4 contains the cytosolic fraction of induced CCFQ071, lane 5 contains the cytosolic fraction of uninduced CCFQ072, lane 6 contains the cytosolic fraction of induced CCFQ071, lane 7 contains the membrane fraction of uninduced CCFQ071, lane 8 contains the membrane fraction of induced CCFQ072, lane 9 contains the membrane fraction of uninduced CCFQ072, and lane 10 contains the membrane fraction of induced CCFQ072.

selective and specific technique was used to detect OmpA. Hence we can only speculate that the band at 40 kDa is indeed OmpA.

The sample from the uninduced CCFQ072 strain had a number of differences besides the presence of the 40 kDa protein band. These other differences include the presence of a band at 36 kDa, the lack of a band at 54 kDa, and the presence of another band at 61.6 kDa (see Figure 1). The identity of these bands is unknown. The bands were unexpected and may indicate a problem with the samples. However, it is possible that the induction of the pCCK06-1 plasmid resulted in expressional changes in the bacteria, leading to the differences seen on the gel. Compensation for the lack of OmpA is possible, which may explain the unknown bands. However, the bands at their corresponding sizes are not, to our knowledge, described in literature. Repetition of this experiment is required to confirm these results and explanation.

Because the outer and inner membranes were not separated during the membrane extraction, it is impossible to tell if the OmpA was present in the inner or outer membranes. If OmpA was present in the inner membrane but not the outer membrane, it supports the prediction of Chambers, *et al.* (2) that OmpA is not being properly integrated into the outer membrane of the CCFQ072 strains. If indeed OmpA is present in the outer membrane as predicted, the poor conjugation efficiency observed by Chambers, *et al.* (2) requires an alternative explanation. There was no direct evidence that OmpA was being expressed but remained in the cytoplasm of the bacteria, hence no conclusion can be made about the relevance of our cytoplasm results to the predictions of *Chambers, et al.* (2)

Though the gel used to obtain the data was of mediocre quality, it was by far the best gel of many that were run. The problems encountered with running the gel were never fully solved. After displacement of the membrane sample buffers from a Triton-X100 buffer to an SDS-buffer using microfiltration, the membrane samples dramatically improved when run on the SDS-PAGE gel. Further problems were seen with the low-range molecular weight standard, and with the temperature of the gels, indicating improper buffer conductivity and perhaps ion depletion, but this was not tested.

FUTURE EXPERIMENTS

Future studies need to improve the resolution of the bands and optimize conditions to unambiguously identify the OmpA band and distinguish it from a mutant that lacks a complementing plasmid. It would also help to run an analysis on SDS page of the fractions from the whole cell, the inner membrane and the outer membrane. Some improved resolution might be accomplished by substituting silver staining for Coomassie Blue staining since this will allow smaller samples to be loaded into the wells.

ACKNOWLEDGMENTS

We thank Dr. William Ramey of the Microbiology and Immunology Department at the University of British Columbia for laboratory resources and guidance during these experiments. We also thank Susan Farmer of the R. E. W. Hancock Lab at the University of British Columbia for guidance and laboratory resources.

REFERENCES

1. **Ausable, M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1988. Current protocols in molecular biology. John Wiley & Sons, Inc., Media, PA; Vol. 1 and 2.
2. **Chambers, C., B. Chang, K. Fehrmann, and G. Quan.** 2007. Investigating the sex life of *Escherichia coli*: The effect of *Omp*A expression on Hfr conjugation efficiency. J Exp. Microbiol. Immunol. **11**: 98-102.
3. **Chan, C., T. Chiu, and R. Kang.** 2006. Construction of a plasmid that increases the level of *ompA* gene expression in *Escherichia coli* for the study of its effect on bacterial conjugation. J. Exp. Microbiol. Immuno. **9**:102-107.
4. **Koebnik, R.** 1999. Structural and functional roles of the surface-exposed loops of the β-barrel membrane protein OmpA from *Escherichia coli*. J. Bacteriol. **181**: 3688-3694.
5. **Manoil, C. and J. P. Rosenbusch.** (1982). Conjugation-deficient mutants of *Escherichia coli* distinguish classes of functions of the outer membrane OmpA protein. Mol. Gen Genet. **187**: 148-156.
6. **Sandermann, H. J. and L Strominger.** 1972. Purification and properties of C 55-isoprenoid alcohol phosphokinase from Staphylococcus aureus. J. Biol. Chem. **247**:5123-5131