JOURNAL OF BACTERIOLOGY, Dec. 1997, p. 7638–7643 Vol. 179, No. 24
0021-9193/97/$04.00+0

Temperature-Sensitive Lesions in the *Francisella novicida valA* Gene Cloned into an *Escherichia coli msbA lpxK* Mutant Affecting Deoxycholate Resistance and Lipopolysaccharide Assembly at the Restrictive Temperature

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Received 14 August 1997/Accepted 1 October 1997

The *valAB* locus of *Francisella novicida* has previously been found to be highly similar at the deduced amino acid level to *msbA lpxK* of *Escherichia coli*. Both ValA and MsbA are members of the superfamily of ABC transporters, and they appear to have similar functions. In this study we describe the isolation of a temperature-sensitive *valAB* locus. DNA sequence analysis indicates that the only changes to the ValAB deduced amino acid sequence are changes of S453 to an F and T458 to an I in ValA. *E. coli* strains defective in *msbA* and expressing temperature-sensitive ValA rapidly ceased growth when shifted from a permissive temperature to a restrictive temperature. After 1 h at the restrictive temperature, cells were much more sensitive to deoxycholate treatment. To test the hypothesis that ValA is responsible for the transport or assembly of lipopolysaccharide, we introduced *gseA*, a Kdo (3-deoxy-D-manno-octulosonic acid) transferase from *Chlamydia trachomatis*, into a strain with a temperature-sensitive *valA* allele and a nonfunctional *msbA* locus. These recombinants were defective in cell surface expression of the chlamydial genus-specific epitope within 15 min of a shift to the nonpermissive temperature. Also, there was enhanced association of the epitope with the inner membrane after a shift to the nonpermissive temperature. Thus, we propose that ValA is involved in the transport of lipopolysaccharide to the outer membrane.

We have recently discovered a genetic locus in *Francisella novicida* that contains an apparent operon consisting of two open reading frames, *valAB* (13). *F. novicida* strains thought to harbor mutations in *valAB* are defective in growth in macrophages and exhibit increased sensitivity to serum and deoxycholate, thus suggesting compromised outer membrane integrity. These two genes show high identity at the deduced amino acid level to the *Escherichia coli* genes *msbA* and *lpxK*, respectively.

Interestingly, the *msbA* gene was originally identified as a multicopy suppressor of the *E. coli htrB* gene (8). Mutants having lesions in *htrB* exhibit an unusual phenotype whereby growth and viability of mutants at temperatures above 32.5°C in rich media are impaired. The gene product of *htrB* has been shown to be a Kdo (3-deoxy-D-manno-octulosonic acid)-dependent acyl transferase involved in lipopolysaccharide (LPS) biosynthesis (3). We have previously shown that, similar to *msbA*, *valAB* can suppress mutations in *htrB*, which suggests that both gene products may have similar functions related to LPS expression. Recent work has demonstrated that *lpxK* (previously called *orfE*) encodes a lipid A 4′-kinase (6).

The presumptive gene products ValA and MsbA are members of the superfamily of ATP binding cassette (ABC) transporters. ABC transporters are a large family of integral membrane proteins that are responsible for the uptake or efflux of a wide variety of both proteinaceous and nonproteinaceous substrates (4). In several bacterial systems, the involvement of ABC transporters in the export of capsular polysaccharide and the translocation of LPS O antigen across the cytoplasmic membrane has been clearly demonstrated (2, 18). Interestingly, recent evidence suggests a role for *msbA* in the translocation of LPS or its precursors across the cytoplasmic membrane (19). Given the similarity between *msbA* and *valA*, our objective in this study was to determine if *valAB* are involved in LPS transport.

Here we demonstrate that *valAB* can suppress the lethal phenotype of an *msbA lpxK* mutation in *E. coli*. Furthermore, through the use of a temperature-sensitive allele of *valA*, we show that *E. coli* cells defective in both *valA* and *msbA lpxK* exhibit increased sensitivity to the detergent deoxycholate and impaired cell surface expression of the LPS epitope synthesized by GseA, a *Chlamydia trachomatis* Kdo transferase. Thus, we hypothesize that ValA is involved in transport of LPS to the outer membrane.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are described in Table 1. *E. coli* W3110, DH5α, DK1, and JM109 were used as host strains and were cultured in Luria-Bertani (LB) (20) broth supplemented with 30 μg of kanamycin sulfate (Km) per ml, 100 μg of ampicillin (Ap) per ml, 10 μg of chloramphenicol (Cm) per ml, and 30 μg of tetracycline (Tet) per ml as required. For induction of expression of cloned genes, the medium was supplemented with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) (Sigma).

DNA manipulation and analysis. Standard recombinant techniques (20) were used except where described. *E. coli* strains were transformed according to the procedure developed by Hanahan (7). PCR was performed with *Taq* polymerase (Perkin-Elmer) according to the manufacturer's instructions. *msbA* and *lpxK* were amplified with the primers 5′ CGGGATCCAGTGGTTGGCGTGCCA 3′ and 5′ CGGGATCCCGTAACTAGTTGCCAGA 3′ to initiate amplification at the 5′ and 3′ ends of the operon, respectively. Generalized transduction with phage P1 was performed as previously described (14).

NTG mutagenesis. Temperature-sensitive mutations in the appropriate genes were induced by using *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) mutagenesis as previously described (14). Briefly, *E. coli* strains harboring plasmids containing the genes to be mutated were grown at 37°C in LB to exponential

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Reference or source
Strains		
W3110	Wild-type strain	Laboratory strain
DH5α	Φ-F80*lac*ZΔM15 *endA1 recA1 hsdR17 supE44 thi1 gyrA96 relA1* Δ(*lacZYA-argF*)*U169*	Bethesda Research Laboratories
DK1	Δ(*srl-recA*)*306 araD139* Δ(*ara leu*)7697 Δ*lacX74 galU-galK hsdR strA mcrA mcrB*	12
MLK1088	W3110 *msbA*::Δ*cam zbj-1230*::Tn*10*(pK-Cla)	9
MCL30	Hfr P045 *thi-1* Δ(*srl-recA*)*306*::Tn*10*	E. Ishiguro, University of Victoria
MKM1	W3110 *msbA*::Δ*cam*(pMM1)	This study
MKM2	W3110 *msbA*::Δ*cam* Δ(*srl-recA*)*306*::Tn*10*(pMM1)	This study
MKM50	W3110 *msbA*::Δ*cam*(pMM2)	This study
MKM55	W3110 *msbA*::Δ*cam*(pMM3)	This study
MKM5023	W3110 *msbA*::Δ*cam*(pMM2, pMM23)	This study
MKM5523	W3110 *msbA*::Δ*cam*(pMM3, pMM23)	This study
Plasmids		
pBGS18	Km^r	21
pCM.301	Temperature-sensitive pSC101 replicon	22
pGEM-T	Ap^r	Promega
pK-Cla	pREG153::3.8-kb *Kpn*I-*Cla*I *msbA kpxK* Ap^r	9
pMMB66HE	IncQ *lacI*^{q} *bla*(Ap^r) *ptac rrnB*	15
pKEM14-5	pTZ18U::5-kb *Sal*I *valAB* fragment	13
pMM1	pCM.301::*msbA lpxK*	This study
pMM2	pBGS18::5-kb *Sal*I-*Sal*I *valAB* Km^r	This study
pMM3	pBGS18::5-kb *Sal*I-*Sal*I temperature-sensitive allele of *valA* Km^r	This study
pMM23	pMMB666HE::mTn*10*::*gseA*	This study

phase. Cells were then washed three times and resuspended in 0.1 M citrate buffer (pH 5.5). NTG dissolved in citrate buffer was added to a final concentration of 50 μg/ml, and the mixture was incubated at 37°C for 30 min. The mutagenized cells were then washed three times in phosphate buffered saline (PBS), resuspended in LB, and grown overnight at 30°C.

Deoxycholate sensitivity assay. The susceptibility of temperature-sensitive *valAB* mutants to deoxycholate was evaluated according to the following procedure. Cultures of bacteria were aliquoted into 1.5-ml centrifuge tubes, washed three times, and resuspended in PBS. One hundred microliters of bacteria at a concentration of approximately 10^6 CFU per ml was added to wells of a 96-well microtiter plate. The indicated concentrations of sodium deoxycholate (Sigma) were added to the bacteria. Killing of bacteria was determined by measuring CFU on LB agar from control wells (PBS) and from sodium deoxycholate wells after 30 min of incubation.

Temperature shift growth assay. The growth kinetics of various strains at 30°C and 42°C were assayed by growing cultures in LB media supplemented with the appropriate antibiotics at 30°C with agitation. Every hour, turbidity readings were taken with a Klett-Summerson meter to measure cell mass. When the cultures reached early logarithmic phase at 2 h, one flask for each strain was maintained at 30°C while another flask was shifted to 42°C. Turbidity measurements were then continued every hour post-temperature-shift for 6 h.

Immunofluorescence microscopy. Expression of the *Chlamydia* genus-specific epitope by strains MKM5023 and MKM5523 was detected according to the following procedure. Both strains were grown as described in the temperature shift growth assay. However, 15 min following the temperature shift to 42°C, the media of selected cultures were supplemented with 1 mM IPTG. Five-milliliter aliquots were taken 2 h post-IPTG addition, pelleted by centrifugation at 8,000 × *g* for 15 min, and resuspended in 500 μl of fresh 4% formaldehyde in PBS and allowed to sit for 1 h at room temperature. Cells were then pelleted again and resuspended in 500 μl of PBS. Formaldehyde-treated cells were allowed to air dry on microscope slides. Cells were fixed by immersion in acetone at −20°C for 20 min and allowed to air dry. Normal goat serum in PBS containing 5% fetal bovine serum (HyClone) was added to the acetone-fixed cells for 30 min. Slides were then washed three times in PBS plus 5% fetal bovine serum for 5 min each. The primary antibody was mouse immunoglobulin G (IgG) monoclonal antibody CHL-888 (Pharmingen), reactive with the chlamydial genus-specific LPS epitope, and the secondary antibody was a fluorescein isothiocyanate-conjugated goat $F(ab')_2$ anti-mouse IgG. Both antibodies were added at 1:50 dilutions and washed as outlined above. To prevent photobleaching, microscope slides were treated with the antifade reagent 1,4-diazabicyclo[2.2.2]octane according to the manufacturer's procedure (Molecular Probes). Slides were viewed at ×1,000 with a Zeiss Axioscope microscope equipped with epifluorescence and camera. Excitation light was passed through a filter that transmits light in the 450- to 490-nm range. Fluorescent images were recorded on Kodak Kodacolor ISO 100 film exposed for 125 s.

Bacterial inner and outer membrane purification and detection of the *Chlamydia* genus-specific epitope. Cultures of MKM5523 (200 ml) were grown at 30°C to an optical density at 600 nm of 0.5 to 0.7. Cells were then either shifted to 42°C or maintained at 30°C for 15 min and then grown for a further 2 h in the presence of 1 mM IPTG. Equal amounts of cells were harvested, and inner and outer membranes were separated by sucrose density gradient centrifugation of the total membrane fraction, as described previously (17). Fractions (400 μl) were collected, and 50 μl of each fraction was used to assay NADH oxidase activity (17). Enzyme-linked immunosorbent assays (ELISAs) were used to determine the levels of the chlamydial genus-specific epitope in 200 μl of each fraction. The primary antibody was mouse anti-*Chlamydia* genus-specific epitope CHL-888 or EVI-HI (a kind gift of H. Caldwell), and the secondary antibody was an alkaline phosphatase-conjugated sheep anti-mouse IgG + IgM (Caltag). Absorbence at 405 nm of hydrolyzed *p*-nitrophenyl phosphate (Sigma) was determined with a Biotech microplate reader.

RESULTS AND DISCUSSION

In order to study the function of *valAB*, we chose to perform experiments in an *E. coli* genetic background, where we could perform many genetic manipulations. Given that *msbA lpxK*—and presumably *valAB*—are essential to the viability of the cell, we decided to construct conditional lethal mutations in *valAB*.

To provide the proper background to study *valAB*, we cloned *msbA lpxK* into pCM.301, an ampicillin-resistant plasmid that is temperature sensitive in its replication. *msbA lpxK* was amplified from plasmid pK-Cla via PCR. After being cloned into the pGEM-T vector, the insert was removed by digestion with *Bam*HI and subcloned into the *Bam*HI site of pCM.301 to yield plasmid pMM1.

Plasmid pMM1 was transformed into W3110. Subsequently, bacteriophage P1 transduction from strain MKL1088 was used to introduce an allele of *msbA* containing a chloramphenicol resistance cassette into the chromosome of W3110(pMM1). As well, a *recA* locus that is interrupted by mini-Tn*10* was introduced by P1 transduction from strain MCL30 in order to prevent homologous recombination between the chromosomal copy and the plasmid-borne copy of *msbA*. The *msbA*::Ω*cam recA*::Tn*10*(pMM1) strain, named MKM2, was unable to grow

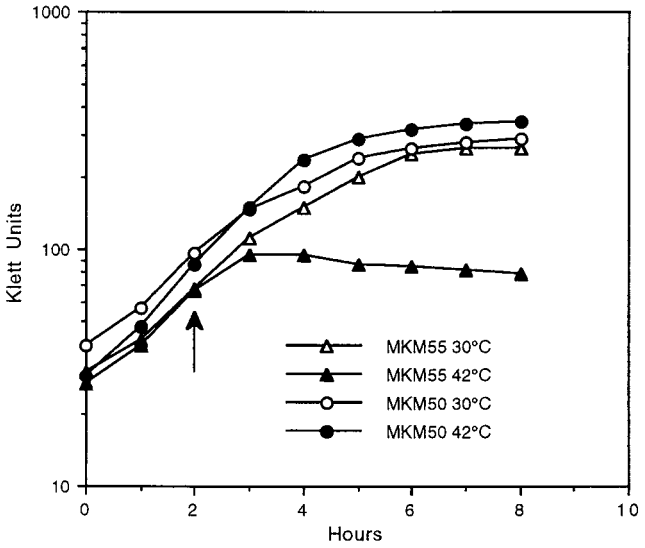

FIG. 1. Growth characteristics of *E. coli* MKM50 and MKM55 at 30°C and 42°C. All bacterial cultures were initially grown at 30°C. Every hour the cell density was measured with a Klett-Summerson meter. After 2 h (indicated by the arrow), selected cultures were shifted to 42°C.

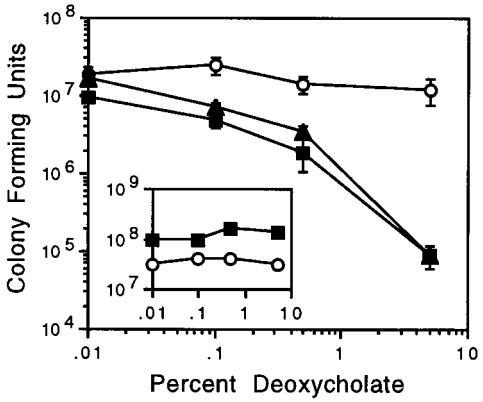

FIG. 2. Deoxycholate sensitivity of *E. coli* MKM55 and MKM50 (inset). All bacterial cultures were treated with deoxycholate for 0.5 h at 30°C and then shifted to 42°C for the following periods of time. MKM55: 0 h (circles), 1 h (triangles), and 1.5 h (squares); MKM50 (inset): 0 h (circles) and 1.5 h (squares). Results are expressed as the averages of three determinations ± standard errors.

at 42°C due to the loss of the temperature-sensitive partition plasmid encoding *msbA lpxK*.

A 5-kb *Sal*I-*Sal*I fragment containing *valAB* was subcloned from pKEM14-5 into the kanamycin-resistant plasmid pBGS18 to form pMM2. DK1(pMM2) was mutagenized with NTG, after which the plasmid was isolated and used to transform MKM2. One thousand transformants were picked to duplicate agar plates that were incubated at 30° and 42°C. At the restrictive temperature, pMM1 (encoding *msbA lpxK*) is lost, and the cells must rely on *valAB*, encoded by pMM2, for survival. Hence, cells containing temperature-sensitive alleles of *valAB* should not be viable at 42°C. Two colonies were found to be stably temperature sensitive for growth, and plasmid pMM3, from one of these temperature-sensitive strains, was chosen for further study.

The DNA insert in plasmid pMM3 was subjected to DNA sequence analysis. Three nucleotide changes were found in the *valAB* locus (accession no. L17003). One change, a C-to-T transition at bp 430, was 53 bp upstream of the translational start of ValA. As well, two changes in the *valA* cistron were found; both were C-to-T transitions that changed S543 to an F (bp 1841) and T458 to an I (bp 1856). These amino acid changes were next to the Walker B ATP binding motif, which spans amino acids 461 to 498. By analogy with other ABC transporter proteins (9), the C terminus (amino acids 284 to 572) of ValA lies on the cytoplasmic side of the inner membrane.

Plasmid pMM3 was transformed into W3110 to yield a Km^r, Am^s strain, and the chloramphenicol-interrupted *msbA* locus from strain MKL1088 was introduced into the chromosome as described above. The resulting strain, named MKM55, was unable to grow on LB agar plates at 42°C. Further, this strain ceased growth in broth culture 1 h after a shift from 30°C to 42°C (Fig. 1). A control strain, named MKM50, harboring a wild-type copy of *valAB* continued growth at 42°C.

E. coli cells that have defects in the outer membrane, especially alterations in LPS composition, are often sensitive to detergents such as deoxycholate. Hence, we tested the relative sensitivity to deoxycholate of strains harboring a temperature-sensitive *valA* locus. Figure 2 shows that strain MKM50, harboring a wild-type version of *valA*, was resistant to a 30-min exposure to 5% deoxycholate, regardless of whether it was grown at 30°C (data not shown) or 42°C. Also, strain MKM55, harboring a temperature-sensitive lesion of *valAB* and an interrupted *msbA* locus, was insensitive to 5% deoxycholate when grown at 30°C, the permissive temperature (data not shown). However, when strain MKM55 was grown at 42°C it was significantly more sensitive to 5% deoxycholate than was strain MKM50.

To test the hypothesis that ValA may be involved in the transport of LPS to the outer membrane, we introduced a clone of *gseA* into strains MKM55 and MKM50 (16). The *Chlamydia* gene *gseA* encodes a Kdo transferase responsible for the addition of three Kdo residues to a lipid A precursor (1, 5). These three Kdo residues constitute the *Chlamydia* genus-specific epitope located within the inner core of the LPS molecule and may be readily detected on the surfaces of *E. coli* cells expressing a clone of *gseA*. A 2.3-kb *Eco*RI-*Eco*RI fragment from pFEN212 was subcloned into the *Eco*RI site of the expression vector pMMB66HE and subsequently transformed into MKM55 and MKM50 to yield strains MKM5523 and MKM5023. Thus, strains MKM5523 and MKM5023 harbor a defective *msbA lpxK* locus, an IPTG-inducible subclone of *gseA*, and either a temperature-sensitive or wild-type allele, respectively, of *valA*.

IPTG-induced expression of the *Chlamydia* genus-specific epitope by both strains was determined at the restrictive and permissive temperatures by immunofluorescence microscopy with antibody reactive with the chlamydial genus-specific LPS epitope (Fig. 3). Cultures of experimental and control strains were grown at 30°C and either maintained at 30°C or shifted to 42°C during mid-logarithmic-phase growth. Fifteen minutes after the shift, IPTG was added to induce expression of the chlamydial epitope, and the ratio of immunofluorescent cells (expressing the epitope) to nonfluorescent cells was interpreted as an estimate of the transport of core LPS.

Strain MKM5523, harboring the temperature-sensitive *valA* locus, exhibited 10-fold-lower expression of the *Chlamydia* genus-specific epitope after a shift to 42°C than cultures maintained at 30°C and similarly showed approximately 10-fold-lower expression than the control strain, MKM5023, at both the permissive and nonpermissive temperatures (Fig. 3). In addition to the use of standard error as a criterion for the

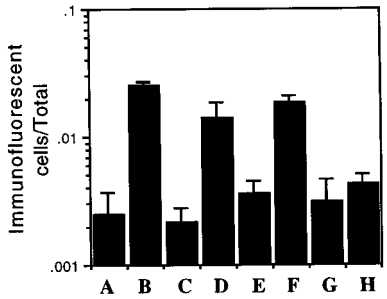

FIG. 3. Assembly of the chlamydial LPS epitope in *E. coli* strains harboring a temperature-sensitive *valA* locus. Cultures were grown to mid-logarithmic phase at 30°C, and select cultures were shifted to 42°C. Fifteen minutes post-temperature-shift, IPTG was added to the appropriate cultures; after 2 h, samples were collected and reacted with monoclonal antibody specific for the genus-specific LPS epitope of *Chlamydia*. Cells reacting with monoclonal antibody were detected with a fluorescein isothiocyanate-conjugated secondary antibody and counted. The values represent the number of fluorescent cells divided by the total number of cells in the cultures. A, MKM5023 maintained at 30°C; B, MKM5023 maintained at 30°C after IPTG induction; C, MKM5023 post-shift to 42°C; D, MKM5023 post-shift to 42°C after IPTG induction; E, MKM5523 maintained at 30°C; F, MKM5523 maintained at 30°C after IPTG induction; G, MKM5523 post-shift to 42°C; H, MKM5523 post-shift to 42°C after IPTG induction. Data are expressed as means ± standard errors of the ratio of fluorescent cells to the total number of cells per field. Cells were counted from a minimum of 10 fields of view for each parameter, which included a minimum of 500 cells viewed by bright-field microscopy in two experiments. For statistical purposes, each field of view was chosen in an unbiased manner and treated as a sample of the population.

validity of the results, we also employed the Mann-Whitney U test to examine the significance of the induction values. When expression of the chlamydial epitope between different cultures was compared by the Mann-Whitney U test, the *P* values were as follows: A versus B, 0.036; C versus D, 0.003; E versus F, 0.0003; G versus H, 0.452. These results confirm that there is a significant increase in the expression of the chlamydial genus-specific epitope between the IPTG-induced and noninduced cultures of strain MKM5523 at 30°C and of control strain MKM5023 at both temperatures. Conversely, there was no significant increase in epitope expression for strain MKM5523 at 42°C. Therefore, in an *E. coli* strain harboring a nonfunctional *msbA lpxK* locus and a temperature-sensitive allele of *valA*, expression of the *Chlamydia* genus-specific epitope at the cell surface is decreased at the nonpermissive temperature, thus suggesting that ValA may be involved in the transport of LPS to the cell surface.

We also measured the association of the chlamydial genus-specific epitope with the inner membrane, as an indicator of impaired translocation across the inner membrane at the restrictive temperature. Membranes from strain MKM5523 grown at the restrictive and permissive temperatures were prepared and separated on sucrose gradients according to the method of Osborn et al. (17). NADH oxidase activity was used as a marker for the inner membrane, and the amount of chlamydial epitope was determined by ELISA. The results of these assays are shown in Fig. 4. In two separate experiments there is increased association of the chlamydial epitope with the NADH oxidase fraction in membranes isolated from cultures grown at the restrictive temperature relative to membranes isolated from cultures grown at the permissive temperature. These results suggest the accumulation of LPS in the inner membrane at the restrictive temperature in ValA temperature-defective mutants.

We have previously demonstrated that in *F. novicida*, mutants thought to contain a lesion in the *valAB* locus exhibit increased sensitivity to serum and are defective in growth in macrophages (13). Since LPS is located in the outer membrane of gram-negative bacteria, it is essential for bacterial growth and survival and often confers protection against bactericidal agents such as serum complement and detergents. Therefore, if the transport of LPS to the outer membrane is reduced or abolished in the absence of *valA*, then such a mutant may be expected to show increased sensitivity to membrane-permeating agents. Indeed, in this study we demonstrate that the expression of a temperature-sensitive *valA* locus in *E. coli* can suppress the lethal phenotype of a mutation in the *E. coli* homolog *msbA*. At the permissive temperature, these cells are resistant to the detergent deoxycholate. However, at the nonpermissive temperature, ValA is inactivated and the cells become sensitive to deoxycholate. Furthermore, these mutants exhibit decreased cell surface expression of the *Chlamydia* genus-specific LPS epitope at the nonpermissive temperature and increased association of the epitope with the inner membrane. Collectively, this evidence suggests that ValA is required for optimal expression of LPS at the bacterial cell surface.

The role of ABC transporters in the export of LPS O antigen and capsular polysaccharide has previously been demonstrated. For example, capsular polysaccharides in *E. coli* K1 and K5 are synthesized at the inner face of the cytoplasmic membrane and subsequently require the action of a two-component ABC transporter system (KpsT and KpsM) for transport across the cytoplasmic membrane (2, 10, 11, 18). The presence of an ATP binding protein is believed to couple the energy of ATP hydrolysis to the transport of the polysaccharide. Similarly, LPS O antigen export in *Yersinia enterocolitica* serotype O:3 and *Klebsiella pneumoniae* serotype O1 requires a two-component ABC transporter system encoded by the *rfb* locus (2, 23). In *Yersinia*, mutations in these genes result in the intracellular accumulation of O antigen, thus suggesting a role for these ABC transporter systems in the transport of O antigen across the cytoplasmic membrane. Similarly, *E. coli* K-12 cells harboring a clone of the *Klebsiella rfb* locus deleted in the genes for the ABC transporter system (*rfbA* and *rfbB*) accumulate cytoplasmic O antigen but retain expression of rough LPS molecules on their cell surfaces (2).

Significantly, recent evidence provided by Polissi et al. suggests a role for the *E. coli msbA* gene in the translocation of LPS or its precursors across the cytoplasmic membrane (19). Those authors demonstrated that the LPS precursor *N*-acetyl [^{3}H]-glucosamine (GlcNAc) accumulates in a strain in which the levels of MsbA and LpxK have been reduced by dilution of a recombinant plasmid with the only functional genes encoding these proteins. They were unable to demonstrate accumulation of GlcNAc in inner membranes in strains with a temperature-sensitive MsbA.

In this study we evaluated the *E. coli* cell surface expression of the *Chlamydia* genus-specific LPS epitope encoded by *gseA* in the presence of temperature-sensitive ValA. Since we demonstrate that *E. coli* strains simultaneously lacking functional ValA and MsbA exhibit decreased cell surface expression of an epitope located within the inner core region of LPS, it is unlikely that ValA is involved in the transport of O antigen. Instead, ValA may be required for the transport of lipid A molecules linked to core polysaccharide across the cytoplasmic membrane. Conversely, ValA may be involved in the transport of another unknown component essential for the export or synthesis of a complete LPS molecule.

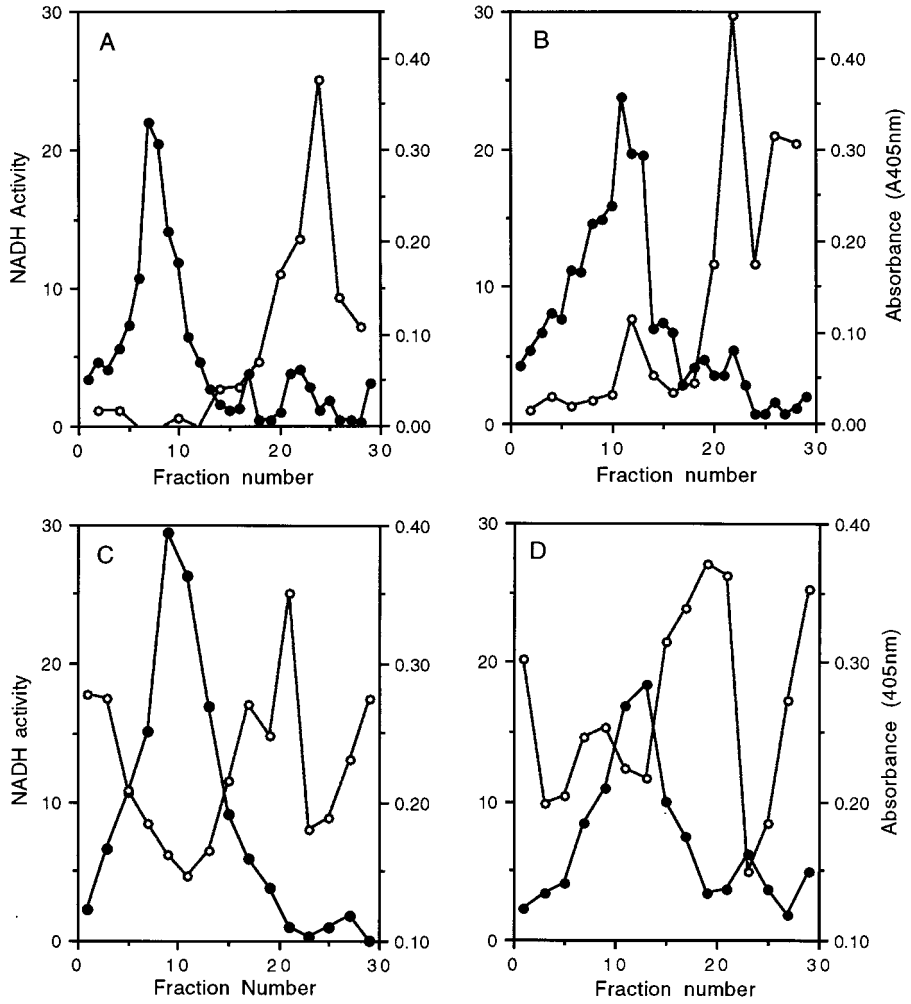

FIG. 4. Association of the chlamydial genus-specific LPS epitope with NADH oxidase activity in sucrose density gradient profiles. Strain MKM5523 was grown at 30°C (A and C) or 42°C (B and D). Two separate experiments (represented in panels A and B and in panels C and D) are shown. NADH activity (closed circles) is expressed as millimoles per minute oxidized by 50 μl of each fraction. Fraction 1 represents the top of the gradient. Relative amounts of the chlamydial genus-specific LPS epitope were determined in 200 μl of each fraction by ELISA (open circles), with absorbance at 405 nm.

ACKNOWLEDGMENTS

S. Cowley and M. McDonald contributed equally to the experiments presented in this manuscript.

This work was supported by a grant from the Medical Research Council (MT11668) and from the Natural Sciences and Engineering Council to F.E.N.

We thank M. Karow, C. Miller, and E. Ishiguro for strains and plasmids and H. Caldwell for monoclonal antibody.

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