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Sequence Analysis of the 17-Kilodalton-Antigen Gene from *Rickettsia rickettsii*

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DNA obtained from the Sheila Smith strain of *Rickettsia rickettsii* was digested to completion with the restriction endonucleases *Bam*HI and *Sal*I and ligated with the plasmid vector pUC19. The ligation mixture was used to transform *Escherichia coli*. A total of 465 bacterial clones were screened for antigen production with hyperimmune rabbit serum. One of the reactive clones, containing a recombinant plasmid designated pSS124, was solubilized and subjected to immunoblot analysis and revealed expression of a 17-kilodalton protein reactive with anti-*R. rickettsii* serum that comigrated with an antigen from *R. rickettsii*. A 1.6-kilobase *Pst*I-*Bam*HI fragment from pSS124 was subcloned and continued to direct synthesis of the 17-kilodalton antigen. The nucleotide sequence was determined for this 1.6-kilobase subclone, which encompassed the gene encoding the polypeptide as well as flanking regions containing potential regulatory sequences. The open reading frame consisted of 477 nucleotides that specified a 159-amino-acid protein with a calculated molecular weight of 16,840. The deduced amino acid sequence contained a hydrophobic sequence near the amino terminus that resembled signal peptides described for *E. coli*. The carboxy terminus was hydrophilic in nature and probably contained the exposed epitopes.

Rickettsia rickettsii, the etiologic agent of Rocky Mountain spotted fever (RMSF), is currently the rickettsial disease of greatest public health concern in the United States. Since 1975, an average of over 1,000 cases per year have been reported in this country, with the mortality rate ranging from 3 to 8% (4). Although RMSF has been well recognized and studied for decades, the immune response against rickettsial infection is still not completely understood. Specifically, the role and nature of rickettsial antigens responsible for eliciting the immune response during infection are not well defined.

Previous studies have shown that many spotted fever-group rickettsiae, including *R. rickettsii*, contain six major proteins, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (6, 20). However, the immunogenicity of these proteins was not determined. Anacker et al. (2) demonstrated antibodies in sera from patients convalescing from RMSF targeted to surface polypeptides of 130,000 molecular weight (130K) and 170K. Immunoblot analysis of various *R. rickettsii* strains has revealed antigens with apparent molecular weights ranging from 15,000 to 165,000 when reacted with immune guinea pig sera (3). Recently, Williams et al. resolved seven different antigens by radioiodination and immunoprecipitation of *R. rickettsii* with immune guinea pig serum (27). Two of these surface proteins had molecular weights of 16,500 and 17,500, similar in molecular weight to the possible modified and unmodified forms, respectively, of the cloned antigen proposed in this report. Little additional information is available on the biochemical nature of *R. rickettsii* polypeptides at the molecular level, primarily because studies of specific rickettsial antigens have been impeded by difficulties in obtaining purified antigens in sufficient quantities for analysis.

Molecular cloning techniques have aided the study of obligate intracellular bacteria that are difficult to cultivate and manipulate in the laboratory, such as *Chlamydia trachomatis* and *Rickettsia prowazekii* (1, 12, 19, 28). In particular, studies of chlamydial and rickettsial protein antigens and their genetic bases have been enhanced by the molecular cloning and expression of specific protein antigens in *Escherichia coli* host systems (13, 24). In this study we used molecular cloning techniques to demonstrate the expression, in *E. coli*, of a putative surface protein antigen from *R. rickettsii*. We also describe the nature of the protein and the gene responsible for directing its synthesis.

MATERIALS AND METHODS

Reagents. Restriction enzymes and T4 DNA ligase were obtained from New England Biolabs, Beverly, Mass. The cordycepin 3′-end-labeling kits, calf intestinal alkaline phosphatase, polynucleotide kinase, Klenow fragment of DNA polymerase I, and all ^{32}P-labeled nucleotide triphosphates were purchased from New England Nuclear Corp., Boston, Mass. Proteinase K, 4-chloro-1-napthol, Tween 20, 3,3′-diaminobenzidine, isopropyl-β-D-thiogalactopyranoside, DNase I, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, and ampicillin were purchased from Sigma Chemical Co., St. Louis, Mo. Peroxidase-conjugated goat anti-rabbit immunoglobulin was obtained from United States Biochemical Corp., Cleveland, Ohio. All organic solvents for chemical sequencing were purchased from Aldrich Chemical Co., Milwaukee, Wis.

Bacterial strains. *E. coli* TB1 (Δ*lac pro ara thi strA* φ80 d*lacZ* ΔM15 *hsdR*) and TB1(pUC18)(pUC19) were gifts from S. Hollingshead of Emory University, Atlanta, Ga. *E. coli* strains were grown in Luria-Bertani (LB) broth and on LB agar at 37°C overnight. When appropriate, ampicillin was

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added at a final concentration of 100 μg/ml. *R. rickettsii* Sheila Smith was grown in Vero E-6 cells with minimal essential medium supplemented with L-glutamine and 2% fetal bovine serum (FBS). Rickettsiae were cultivated for 3 to 4 days at 33°C in T-150 flasks and subcultured into new flasks of the same size. Rickettsiae were harvested when growth was sufficient and purified by renografin density centrifugation as previously described (26).

Antiserum. Polyvalent antiserum to *R. rickettsii* was produced in female New Zealand white rabbits. Whole purified rickettsiae were gamma-irradiated with 2×10^6 rads from a cobalt source, suspended in an equal volume of Freund incomplete adjuvant, and injected into the footpad. The animals were bled 6 weeks later, and the serum was collected. The fluorescent antibody assay was used to determine titers against *R. rickettsii*.

Construction of recombinant plasmids containing rickettsial DNA. Rickettsial DNA was isolated from viable *R. rickettsii* Sheila Smith by methods described previously (22). Plasmid vector DNA was harvested from *E. coli* TB1 and purified by alkaline lysis and cesium chloride-ethidium bromide density gradient centrifugation (16). Purified rickettsial DNA was digested to completion with the restriction endonucleases *Bam*HI and *Sal*I overnight at 37°C. Plasmid vector pUC19 DNA was also digested with both *Bam*HI and *Sal*I. The doubly digested rickettsial and plasmid vector DNAs were ligated at 13°C for 20 h with T4 DNA ligase. The ligation mixture was used to transform $CaCl_2$-treated *E. coli* TB1 by standard procedures (16). Recombinant plasmids were detected by formation of colorless bacterial colonies on LB agar containing ampicillin, isopropyl-β-D-thiogalactopyranoside, and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

Antibody screening of recombinants. *E. coli* strains containing recombinant plasmids were grown overnight in microtiter plates containing 100 μl of LB broth with ampicillin and subsequently transferred to prewetted nitrocellulose filters with a 96-well manifold (Schleicher & Schuell, Keene, N.H.). The nitrocellulose dot-blots were exposed to chloroform vapor for 1 h and then placed on Whatman 3MM paper soaked with TBS (50 mM Tris, pH 8.0, 150 mM NaCl) containing 1% Triton X-100 and 10 mg of lysozyme per ml for 20 min. The filters were then transferred to 3MM paper soaked with TBS containing 10 mM $MgCl_2$ and 50 μg of DNase per ml and incubated for 45 min. The filters were washed once with TBS and incubated for 45 min in TBS containing 20% FBS. The blots were incubated with polyvalent rabbit antiserum to *R. rickettsii* (diluted 1:100 in TBS containing 20% FBS) for 2 h at room temperture. The filters were washed three times with TBS alone and then with TBS containing 0.1% Nonidet P-40 and were subsequently incubated in goat anti-rabbit immunoglobulin G (IgG) conjugated with horseradish peroxidase diluted 1:1,500 in TBS containing 20% FBS. The blots were washed, and the color was developed by adding 50 mg of 4-chloro-1-naphthol dissolved in 10 ml of methanol and diluted to 100 ml with deionized water together with 150 μl of 30% hydrogen peroxide.

Immunoblots. Immunoblots were performed by a modified method of Towbin et al. (25). Briefly, whole cell samples containing 50 μg of protein were solubilized at 37 or 100°C with 2% SDS and 4% 2-mercaptoethanol and electrophoresed on 11% discontinuous SDS-PAGE gels (15). Samples were electrotransferred to nitrocellulose at 60 V overnight. Filters were washed with phosphate-buffered saline (PBS) containing 0.3% Tween 20 and then incubated for 1 h in PBS-Tween 20 with 10% FBS. Filters were incubated for 3 h at room temperature with rabbit anti-*R. rickettsii* serum diluted 1:100 in PBS-Tween and subsequently washed repeatedly in PBS-Tween and reacted for 1 h with goat anti-rabbit IgG horseradish peroxidase conjugate. After extensive washes with PBS-Tween and PBS alone, the color was developed with 25 mg of 3,3′-diaminobenzidine in 100 ml of deionized water and 30 μl of 30% hydrogen peroxide.

Preparation of outer membrane proteins from the *E. coli* clone. Outer membranes (Sarkosyl insoluble) from *E. coli* TB1 containing the recombinant plasmid pSC2 were isolated by the method of Filip et al. (7). The Sarkosyl-soluble fraction containing inner membrane proteins was saved for comparison with the insoluble pellet. The resulting insoluble pellet was suspended in PBS with a tissue homogenizer with a Teflon pestle. For each of the fractions (pelleted total membranes, Sarkosyl insoluble, and Sarkosyl soluble), 25 μg was solubilized and subjected to immunoblot analysis as described above.

Characterization of pSS124 insert DNA. Plasmid DNA was prepared by a rapid alkaline lysis procedure (16). Restriction endonuclease mapping, agarose electrophoresis, and Southern blotting were performed by standard procedures (16). Subcloning was accomplished by isolating specific restriction endonuclease fragments and ligating these fragments to appropriately cleaved pUC19 plasmid vector DNA. In this manner, a new plasmid (pSC2) containing the 1.6-kilobase (kb) *Pst*I-*Bam*HI fragment of pSS124 was constructed. The plasmid pBS1 was constructed by ligating the 4.5-kb insert fragment from pSS124 into plasmid vector pUC18. This resulted in a subclone with the insert reversed relative to the *lacZ* gene promoter of the plasmid vector (29). *E. coli* TB1 strains containing these derivatives of plasmid pSS124 were examined for expression of the 17,000-molecular-weight antigen (17K antigen) by immunoblot analysis.

End labeling and DNA sequencing. DNA fragments to be sequenced were 3′-end labeled by the addition of [^{32}P]cordycepin via terminal transferase or by the addition of the appropriate α-^{32}P-deoxyribonucleotide triphosphate by fill-in reactions catalyzed by the Klenow fragment of DNA polymerase I. End labeling at the 5′ end was performed by [γ-^{32}P]ATP by using polynucleotide kinase after calf intestinal phosphatase treatment of the DNA. Labeled DNA fragments were subcut with restriction endonucleases to yield uniquely labeled fragments that were purified by gel electrophoresis. Sequence analysis of the labeled DNA was performed by the base-specific chemical cleavage method described by Maxam and Gilbert (17).

RESULTS

Detection of rickettsial antigens. A total of 465 colonies containing presumptive recombinant plasmids were screened with rabbit anti-*R. rickettsii* serum by the dot-blot procedure. Two recombinant clones reacted strongly with the antibody probe.

Analysis of gene products from recombinant plasmids. The two reactive clones were examined by SDS-PAGE and immunoblot analysis. Both were found to produce an antigen reactive with anti-*R. rickettsii* serum but not with normal rabbit serum. The reactive antigen from both clones was estimated to have an apparent molecular weight of 17,000. One of the clones, TB1(pSS124), was studied in greater detail. Figure 1 shows an immunoblot of *E. coli* TB1 containing recombinant plasmid pSS124 (lane B). An antigen migrating at approximately 17K and reactive with anti-*R. rickettsii* serum was clearly visible on this blot and was seen

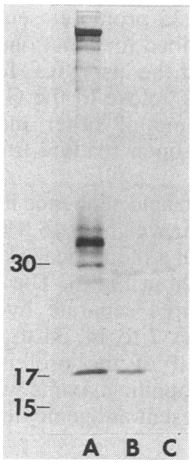

FIG. 1. Immunoblot of *R. rickettsii* and recombinant plasmid- or vector-containing *E. coli*. Proteins were separated on SDS-PAGE gels, transferred to nitrocellulose, and then allowed to react with rabbit anti-*R. rickettsii* hyperimmune serum. Color was developed as described in the text. Lanes: A, *R. rickettsii*; B, *E. coli* TB1 containing recombinant plasmid pSS124; C, *E. coli* TB1 containing plasmid vector pUC19. The locations of molecular size standards (in kilodaltons) are indicated at left.

to comigrate with an antigen of the same size from *R. rickettsii* (lane A). *E. coli* containing plasmid vector pUC19 alone did not react with immune sera in this molecular weight range (lane C). The weaker band(s) in the 36K range (Fig. 1 and 2) were found with both immune and normal sera, indicative of a nonspecific reaction.

Localization of the cloned antigen. The *E. coli* cells harboring the recombinant plasmid pSC2 were fractionated to determine the intracellular location of the cloned antigen. Figure 2A shows an immunoblot of the pelleted membranes from *E. coli* TB1(pSC2) (lane 1). The 17K antigen is clearly visible in this fraction, which is composed of both inner and outer membranes. However, when the inner membranes were solubilized with the detergent Sarkosyl, the 17K protein was found in the insoluble (outer membrane) pellet (lane 2). Both of these fractions contained large amounts of the 17K antigen compared with the control Sarkosyl-soluble inner membrane fraction (Fig. 2B, lane 1). Thus, the antigen contained the necessary signal sequences to be exported to

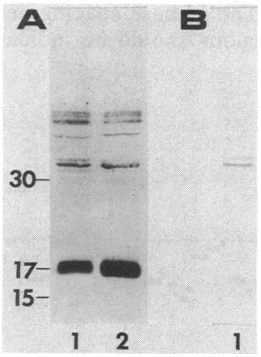

FIG. 2. Immunoblot of fractionated subclone. *E. coli* TB1 containing plasmid pSC2 was fractionated and solubilized as described in the text. (A) Lane 1, pelleted membranes; lane 2, outer membrane fraction (Sarkosyl insoluble). (B) Lane 1, Sarkosyl-soluble fraction. Sizes are indicated (in kilodaltons).

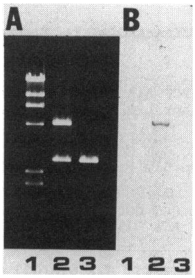

FIG. 3. (A) Agarose gel electrophoresis of plasmid DNA digested with the restriction endonucleases *Bam*HI and *Sal*I and stained with ethidium bromide. Lane 1, Molecular weight standards of lambda phage DNA cleaved with *Hin*dIII. Sizes (top to bottom): 23.1, 9.4, 6.6, 4.4, 2.3, 2.0, and 0.6 kb. Lane 2, Recombinant plasmid pSS124. Lane 3, Plasmid vector pUC19. (B) Southern blot of gel shown in panel A after hybridization with ^{35}S-labeled *R. rickettsii* DNA.

the outer membrane (Sarkosyl insoluble) when expressed in *E. coli*.

Characterization of pSS124 insert DNA. Isolated plasmid DNA from *E. coli* containing pSS124 and vector pUC19 was digested to completion with the restriction endonucleases *Bam*HI and *Sal*I and electrophoresed on an agarose gel (Fig. 3A). Two bands were seen when pSS124 was cleaved with these enzymes (lane 2). The smaller vector band comigrated with linearized pUC19 (lane 3) and was 2.7 kb in size. The larger insert band had an estimated size of 4.5 kb. When the cleaved plasmid DNA was transferred to nitrocellulose and probed with ^{35}S-labeled *R. rickettsii* DNA, only the larger 4.5-kb insert band hybridized (Fig. 3B, lane 2). Thus, the insert from plasmid pSS124 is rickettsial in origin.

A partial restriction map for the rickettsial DNA insert of pSS124 is shown in Fig. 4. The gene encoding the 17K antigen was localized to the 1.6-kb *Pst*I-*Bam*HI fragment by subcloning. This fragment was located distal to the *lacZ* promoter. The plasmid containing this 1.6-kb fragment, designated pSC2, was tranformed into *E. coli*, and production of the 17K antigen in its entirety continued, as determined by immunoblot analysis (Fig. 2A, lanes 1 and 2). An additional plasmid, designated pBS1, produced by reversing the insert of pSS124 relative to the *lacZ* promoter, continued to direct synthesis of the 17K protein in *E. coli* (data not shown). This result suggests that plasmid pSS124 may contain an internal rickettsial promoter recognized by *E. coli* RNA polymerase regardless of its orientation with respect to the plasmid promoter.

Analysis of nucleotide sequence. Both strands of the insert from plasmid pSC2 were sequenced by the Maxam and Gilbert (17) chemical modification method. Sequencing re-

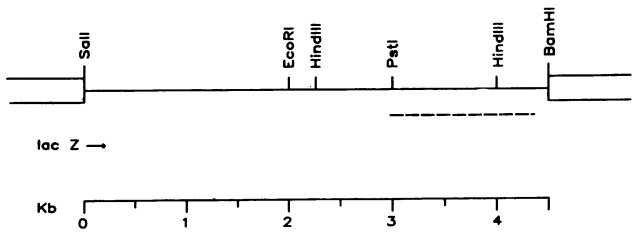

FIG. 4. Partial restriction map for the rickettsial insert of recombinant plasmid pSS124. The *lacZ* region of the plasmid vector pUC19 is shown for purposes of orientation. The subcloned region containing the 17K protein gene is indicated by the dashed line.

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1                                                                   65
CTAGTTTTAGCTTTACAAAATTCTAAAAACCATATACTTATTAATTATATATTAATTAGAGAGAA
          -35                    -10                        S/D

66                                                                 114
 T TAT ATG AAA CTA TTA TCT AAA ATT ATG ATT ATA GCT CTT GCA ACT TCT
       MET Lys Leu Leu Ser Lys Ile MET Ile Ile Ala Leu Ala Thr Ser

115                                                                163
ATG TTA CAA GCC TGT AAC GGT CCG GGC GGT ATG AAT AAA CAA GGT ACA G
MET Leu Gln Ala Cys Asn Gly Pro Gly Gly MET Asn Lys Gln Gly Thr

164                                                                212
 GA ACA CTT CTT GGC GGT GCT GGC GGC GCA TTA CTT GGT TCT CAA TTC GG
Gly Thr Leu Leu Gly Gly Ala Gly Gly Ala Leu Leu Gly Ser Gln Phe Gly

213                                                                261
 T AAG GGC AAA GGA CAG CTT GTT GGA GTA GGT GTA GGT GCA TTA CTT GGA
   Lys Gly Lys Gly Gln Leu Val Gly Val Gly Val Gly Ala Leu Leu Gly

262                                                                310
 GCA GTT CTT GGT GGA CAA ATC GGT GCA GGT ATG GAT GAA CAG GAT AGA A
Ala Val Leu Gly Gly Gln Ile Gly Ala Gly MET Asp Glu Gln Asp Arg

311                                                                359
 GA CTT GCA GAG CTT ACC TCA CAG AGA GCT TTA GAA ACA GCT CCT AGT GG
Arg Leu Ala Glu Leu Thr Ser Gln Arg Ala Leu Glu Thr Ala Pro Ser Gly

360                                                                408
 T AGT AAC GTA GAA TGG CGT AAT CCG GAT AAC GGC AAT TAC GGT TAC GTA
   Ser Asn Val Glu Trp Arg Asn Pro Asp Asn Gly Asn Tyr Gly Tyr Val

409                                                                457
ACA CCT AAT AAA ACT TAT AGA AAT AGC ACT GGT CAA TAT TGC CGT GAG T
Thr Pro Asn Lys Thr Tyr Arg Asn Ser Thr Gly Gln Tyr Cys Arg Glu

458                                                                506
 AC ACT CAA ACA GTT GTG ATA GGC GGG AAA CAA CAA AAA GCA TAC GGT GA
Tyr Thr Gln Thr Val Val Ile Gly Gly Lys Gln Gln Lys Ala Tyr Gly Asp

507                                                                555
 T GCA TGC CGC CAA CCT GAC GAA CAA TGG CAA GTT GTG AAT TGA TAG ACA
   Ala Cys Arg Gln Pro Asp Glu Gln Trp Gln Val Val Asn TER TER

556                                                                620
AAACGTCATTGCGAGGAAAATTACGAAGTAATTGCCGAAGCAATCTCAGGAATTTTATTATTTCA
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FIG. 5. Complete nucleotide sequence of the cloned 17K antigen gene. Sequences were determined in both directions by the Maxam and Gilbert method (17). The deduced amino acid sequence is shown. Potential −10 and −35 promoter sequences and a possible signal peptide are underlined. The presumptive ribosome-binding site is underlined and marked S/D (Shine-Dalgarno).

vealed that a protein of 159 amino acid residues could be translated between the ATG triplet at position 70 to 72 and the TGA stop codon at position 547 to 549 (Fig. 5). The start codon was preceded by the polypurine-rich sequence AGAGAGA at position 58 to 64. This region was similar in polypurine base composition and position, with respect to the first downstream AUG, to the typical ribosomal-binding site observed for *E. coli* (9). Upstream of the open reading frame at positions 12 to 17 and 33 to 38 were sequences resembling the −10 and −35 regions of promoters described for *E. coli* (10). The presumed −10 region differed by one base at the marginally conserved fifth position from the *E. coli* consensus sequence, TATAAT. Likewise, the potential −35 region differed by a G-to-T transition at the third base of the consensus sequence TTGACA. The spacing between the potential −10 and −35 promoter sequences was 15 bases, the lower limit described for functional promoters in *E. coli* (10). The sequence of the insert was found to a have a G+C content of about 30%, close to the G+C content described for the entire genome of other members of the genus *Rickettsia* (18). The open reading frame itself had a G+C content of 43%.

The deduced amino acid sequence indicated a protein with a calculated molecular weight of 16,840. This deduced amino acid sequence yielded a theoretical pI of 8.9. The hydrophobicity profile is shown in Fig. 6. The N-terminal half of the protein contained three separate hydrophobic regions located around residues 7 to 14, 34 to 44, and 55 to 71. The carboxy-terminal half of the protein was hydrophilic in nature. Several hydrophilic maxima were also evident in this region and may represent antigenic determinants exposed at the surface.

DISCUSSION

R. rickettsii is a member of a large group of organisms that are obligate intracellular gram-negative bacteria. Because of their fastidious growth requirements, many aspects of rickettsial infection, pathogenesis, and immunity are poorly understood. Recent studies have provided evidence that the immune response to rickettsial surface antigens plays a significant role in protection from disease (2). To examine the relative roles of *R. rickettsii* surface antigens, as both diagnostic and vaccine candidates, more closely, we undertook the molecular cloning and expression of these potentially important proteins.

The present report describes the cloning, sequencing, and expression, in *E. coli*, of a 17K antigen of *R. rickettsii*. A 1.6-kb fragment of the original 4.5-kb clone (pSS124) was subcloned and found to retain the ability to express the 17K protein. This 1.6-kb fragment was sequenced in its entirety, and an open reading frame of 477 nucleotides was identified that was capable of encoding a protein with a calculated molecular weight of 16,840. The polypurine-rich sequence found five bases upstream of the putative AUG initiator methionine codon was similar to ribosome-binding sites of *E. coli* (9). Although the position of this possible ribosome-binding site relative to the AUG initiator codon is allowable for initiating translation in *E. coli*, determining its role in rickettsial translation awaits further investigation. Additionally, the sequences closely resembling the −10 and −35 consensus sequences for *E. coli* promoters were separated by 15 bases of A+T-rich sequences, in agreement with the 15- to 20-base spacing described for functional *E. coli* promoters (10). The 15-base spacing between the predicted −10 and −35 regions should be indicative of a weak pro-

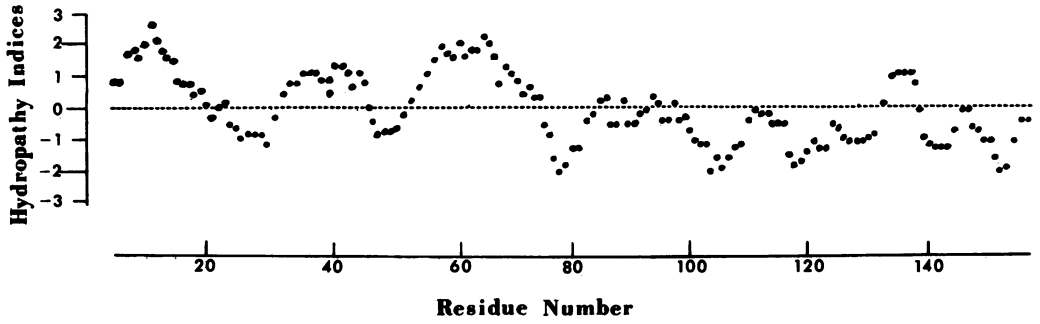

FIG. 6. Hydrophobicity profile of the 17K antigen. The plot was constructed by the method of Kyte and Doolittle (14).

moter, consistent with the low levels of expression seen for this gene in both the *E. coli* clone and *R. rickettsii*.

The cloned rickettsial DNA directed synthesis of the 17K antigen regardless of its orientation with respect to the *lacZ* gene promoter of the plasmid vectors pUC18 and pUC19. This result suggests that transcription initiation for the rickettsial insert DNA is autonomous, since expression of the 17K antigen was independent of orientation with respect to any possible plasmid promoter. Although it is tempting to suggest that the identified −10 and −35 sequences observed directly upstream of the open reading frame represent a true rickettsial promoter, formal proof awaits mapping of the 5′ end of the transcript from *R. rickettsii* to determine the precise start of transcription. These studies are presently under way in our laboratory.

As started earlier, we are primarily interested in cloning the surface antigens of *R. rickettsii* to assess their usefulness as diagnostic and vaccine candidates. Several lines of evidence suggest that the 17K protein is, in fact, a surface protein of *R. rickettsii*. Radioiodination of *R. rickettsii* surface proteins followed by immunoprecipitation by antiserum from infected guinea pigs resulted in the identification of seven potential surface antigens (27). Among these were two proteins with molecular weights of 16,500 and 17,500. A similar doublet in this size range has been identified in immune precipitates of *R. rickettsii* antigens with human convalescent antiserum to RMSF (3). In our hands, the 17K antigen derived from both *R. rickettsii* and the *E. coli* clone at times resolved as a doublet. The reason(s) for the doublet formation is presently unclear, but may have to do with posttranslational modification of the 17K antigen. The amino-terminal region of the deduced primary structure of the 17K protein contained all the characteristic features of a signal peptide for membrane insertion (23). Specifically, the extreme amino terminus contained two positively charged lysyl residues (residues 2 and 6, Fig. 5). This region was followed by seven hydrophobic or uncharged amino acids with a tendency to form an alpha helix. The tripeptide Leu-Gln-Ala (Fig. 5, residues 17 to 19) conformed favorably with consensus sequences which target *E. coli* signal peptidase to the cleavage site (21). In the case of the 17K antigen, a cysteine residue followed the tripeptide consensus sequence and may serve as a residue for lipid addition (11, 21).

Taken to its logical conclusion, the processing of the amino-terminal region of the 17K protein would occur as described for the prolipoprotein outer membrane protein (Lpp) of *E. coli* (21). In fact, the first 20 amino acids of Lpp and the predicted counterpart of this region in the 17K protein are essentially identical in terms of charge, hydropathicity, and overall structure.

The most compelling evidence for the possible surface location of the 17K protein is the identification of the cloned expressed rickettsial antigen within the outer membrane (Sarkosyl-insoluble fraction) of *E. coli*. This presumed compartmentalization verifies the predictions about the signal sequences within the deduced amino terminus of the 17K protein. Moreover, given the overall similarities between the rickettsiae and gram-negative bacteria, it is likely that processing of the 17K protein is similar, if not identical, in both *E. coli* and *R. rickettsii*. Further experiments with highly specific labeled antiserum may be needed to conclusively show that the 17K protein is a surface antigen.

Further analysis of the predicted structure for the 17K protein by hydropathicity indices (14) and two algorithms for secondary structure analysis (5, 8) predicted that the carboxy-terminal half of the molecule is largely hydrophilic and replete with beta-sheet characteristics. This suggests that residues 80 to 159 contain the exposed domains of the 17K antigen. This possibility is being explored by the construction of synthetic oligopeptides that will be used to raise specific antisera to the predicted exposed regions of the protein. These will serve as valuable tools in assessing the exposed epitopes of the protein and will aid in the purification of this antigen.

In addition to providing valuable information as to the possible mechanisms of gene regulation and control in the rickettsiae, the nucleic acid sequences for the 17K antigen and flanking regions could provide a valuable tool for diagnosing rickettsial diseases. Since all members of the genus *Rickettsia* are susceptible to tetracycline, a DNA or immunological probe that can identify a febrile exanthemous illness as being rickettsial in origin would be invaluable for early diagnosis and treatment of the rickettsioses. In future studies, we intend to evaluate the feasibility of cloning and expressing other antigens of the rickettsiae that may have diagnostic and immunological significance. We also intend to determine the role of the 17K antigen in eliciting protective immunity and to conduct detailed studies of rickettsial genes and regulatory regions.

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