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# Evidence for Proteolytic Cleavage of the 120-Kilodalton Outer Membrane Protein of Rickettsiae: Identification of an Avirulent Mutant Deficient in Processing

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**The 120-kDa rickettsial outer membrane protein (rOmpB) is encoded by a gene with the capacity to encode a protein of approximately 168 kDa. The carboxy-terminal end of the molecule is apparently cleaved to yield 120- and 32-kDa products. Both polypeptides are surface exposed and remain associated with the outer membrane of intact rickettsiae. All species of rickettsiae examined display similar cleavage of rOmpB. Comparison of diverse species of rickettsiae demonstrate a conserved N terminus of the 32-kDa fragment, with a predicted procaryotic secretory signal peptide immediately upstream of the proposed cleavage site. Coprecipitation of the 120-kDa rOmpB protein and the 32-kDa peptide by monoclonal antibodies specific for the 120-kDa portion of the molecule suggests that the two fragments remain noncovalently associated on the surface of rickettsiae. Analysis of an avirulent mutant of *Rickettsia rickettsii* revealed reduced amounts of the 120- and 32-kDa fragments, but with a correspondingly larger rOmpB protein that displayed properties expected of the putative precursor. This avirulent mutant grows intracellularly but fails to cause the lysis of infected cells that is typical of *R. rickettsii*. DNA sequence analysis of the region of the gene encoding the cleavage site of the avirulent strain revealed no difference from the sequence obtained from virulent *R. rickettsii*. The 168-kDa putative precursor of the avirulent strain of *R. rickettsii* was not extracted from the surface by dilute buffers, as is the 120-kDa protein of virulent *R. rickettsii* or *R. prowazekii*. These latter results suggest that the 32-kDa C-terminal region of the molecule may serve as a membrane anchor domain.**

*Rickettsia rickettsii*, the etiologic agent of Rocky Mountain spotted fever, possesses two large immunodominant surface protein antigens with estimated molecular masses of 190 and 120 kDa, termed rickettsial outer membrane proteins A and B (rOmpA and -B), respectively (14). The rOmpB protein is the most abundant surface protein on rickettsiae and has been of interest because of its surface location, strong immunogenicity, and reactivity with monoclonal antibodies (MAbs) that protect mice against lethal rickettsial challenge (2, 4, 5, 38). Antigenically, rOmpB displays species- and serotype-specific antigenic properties but also displays group- and genus-specific reactivities (3).

Ultrastructural analyses of the rickettsial outer membrane indicate a regularly arrayed surface structure (23, 24), which has been interpreted as evidence for a paracrystalline surface array, or S layer, on rickettsiae (29). The abundance of rOmpB, its amino acid composition, and the release of the rOmpB homolog from typhus group rickettsiae by hypotonic shock have been taken as evidence supporting a structural role for rOmpB in the proposed S layer (10).

The rOmpB protein of *R. rickettsii* is encoded by a gene with the capacity to code for a protein of approximately 168 kDa (14, 15). A polypeptide of this size is not detected on purified rickettsiae, but N-terminal amino acid sequencing of a 32-kDa, heat-modifiable, outer membrane protein revealed that it is encoded by the 3′ end of the rOmpB gene, suggesting that the rOmpB protein may be processed from a large precursor to yield the mature 120-kDa rOmpB protein and the 32-kDa peptide (14). The rOmpB homolog of *R. prowazekii* is similarly encoded by a large open reading frame, and cyanogen bromide (CNBr) fragments corresponding to the deduced C-terminal domain could not be produced from the 120-kDa protein (9).

In this report, we demonstrate that this apparent processing step occurs in all species of spotted fever or typhus group rickettsiae examined and that the putative cleavage site is conserved among diverse species of rickettsiae. In support of the proposed processing is the demonstration of an avirulent mutant of *R. rickettsii* (12) that displays reduced amounts of the 32-kDa heat-modifiable fragment typical of all other rickettsiae examined and exhibits a correspondingly large rOmpB protein, as would be predicted from an inability to cleave the 168-kDa precursor protein.

## MATERIALS AND METHODS

**Organisms.** Rickettsiae were propagated in Vero cells with M199 medium and purified by Renografin density gradient centrifugation. Strains used were *R. rickettsii* R, *R. rickettsii* Iowa (12), *R. conorii* Kenya tick typhus, *R. parkeri* maculatum, *R. rhipicephali* 3-7♀6, *R. montana* M5/6, *R. akari* Kaplan, *R. australis* Philips, *R. sibirica* 246, *R. prowazekii* Breinl, *R. typhi* Wilmington, and *R. canada* 2678.

Plaque titration was performed with Vero cells basically as described by Cory et al. (11). Rickettsiae were diluted in brain heart infusion broth (BHI) and plated on confluent monolayers of Vero cells in six-well trays. After 1 h of incubation at 34°C, the cultures were overlaid with Leibovitz (L15) medium (GIBCO) containing 5% fetal bovine serum (HyClone), 10% tryptose-phosphate broth (GIBCO), and 0.5% agarose. After 5 to 7 days of incubation at 34°C, 150 μl of 1% neutral red solution was added to each well and incubation continued overnight.

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Intrinsic radiolabeling of *R. rickettsii* with $^{14}$C-amino acids was accomplished by treating infected monolayers with emetine (2.5 μg/ml) in RPMI 1640 medium containing 1/10 the normal concentration of amino acids for 4 h. This medium was removed and replaced with fresh medium containing 1/10 the normal concentration of amino acids, emetine (2.5 μg/ml), 1% fetal bovine serum, and a $^{14}$C-amino acid mixture (1 μCi/ml) (New England Nuclear). The cultures were incubated for an additional 48 h prior to harvest. Cells were dislodged from the glass and collected by low-speed centrifugation, and the supernatant was saved. Cells were disrupted by brief sonication at 40 W with a Braun-Sonic 2000 ultrasonic homogenizer and again pelleted by low-speed centrifugation. The supernatants were pooled with the culture medium, and the rickettsiae were pelleted by high-speed centrifugation. The pellets were resuspended in K36 buffer and pelleted through a 30% (vol/vol) Renografin pad at 18,000 × *g* for 30 min. The pellets were washed once, resuspended in BHI, aliquoted, and stored frozen at −70°C.

**SDS-PAGE.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done as described by Laemmli (19). For two-dimensional SDS-PAGE, rickettsial lysates prepared without heating were electrophoresed and the lane was cut from the gel. The excised polyacrylamide strip was sealed in a plastic bag with 5 ml of Laemmli sample buffer and heated to boiling for 5 min. The gel strip was removed from the bag and laid over a polyacrylamide gel for electrophoresis in the second dimension.

**N-terminal amino acid sequence analysis.** Whole-cell lysates of purified rickettsiae were heated to boiling for 5 min and subjected to SDS-PAGE on 10% acrylamide gels. Electrophoretic transfer to PVDF membrane (Immobilon; Millipore) for amino acid sequencing was performed with 25 mM $NaPO_4$ (pH 7.2) for 2 h at 32 V and 1.0 A. Protein adhering to the membrane was stained with 0.1% amido black 10B–45% methanol–10% acetic acid in water for 10 min and destained by extensive rinsing in water. Bands of interest were excised, and the N-terminal sequence was determined with an Applied Biosystems model 470A Protein Sequencer equipped with an on-line PTH-Analyzer (20).

**Radioiodination.** Purified whole organisms were $^{125}$I labeled by using the lactoperoxidase procedure (21).

**Immunochemical techniques.** MAb 13-2 was purified and concentrated from hybridoma culture supernatants on protein A-Sepharose columns. The specificity of this antibody has been described previously (3, 4).

Immunoblotting was performed essentially as described by Batteiger et al. (7) except for the use of 25 mM sodium phosphate (pH 7.4) as the transfer buffer. Transfer was for 1.5 h at 25 V and 1.0 A. Antibody-reactive protein bands were detected with $^{125}$I-protein A (New England Nuclear) and autoradiography.

Radioimmunoprecipitations were modified from the procedure described by Swanson and Barrera (33). $^{125}$I-labeled *R. rickettsii* were pelleted, resuspended in Hanks balanced salt solution (HBSS; GIBCO) containing 1% Zwittergent 3-14 (Calbiochem) with or without 1 mM dithiothreitol (DTT), and incubated for 1 h with constant mixing at room temperature. Iodoacetamide was added to a final concentration of 5 mM, and the extracts were centrifuged at 15,000 rpm for 10 min in a Microfuge 12 (Beckman). A 200-μl portion of extract was mixed with 200 μl of MAb 13-2 (100 μg/ml) or of an irrelevant negative-control MAb directed against the *Chlamydia trachomatis* major outer membrane protein and was incubated for 1 h at 4°C. To this mixture, 40 μl of protein A-Sepharose 4B-CL (Sigma) in 50 mM $NaPO_4$–150 mM NaCl (pH 7.4) (PBS) was added, and the mixture was incubated for 1 h at room temperature. The suspension was centrifuged at 5,000 rpm for 5 min in the Beckman Microfuge 12 and washed twice with HBSS–1% Zwittergent 3-14. One milliliter of absolute ethanol was added to the washed pellet, and the ethanol supernatant was discarded after centrifugation. The pellet was evaporated to dryness in a boiling water bath prior to the addition of Laemmli sample buffer (4% SDS, 8% 2-mercaptoethanol, 0.125 M Tris-HCl [pH 6.8], 10% glycerol, and bromphenol blue) for SDS-PAGE.

**DNA sequencing of PCR-amplified DNA.** The polymerase chain reaction (PCR) was utilized to amplify DNA encoding the region of the rOmpB protein encompassing the proteolytic cleavage site. Purified rickettsial DNA (0.5 to 1 ng) was subjected to 35 cycles of PCR by using a Perkin Elmer/Cetus thermal cycler and GeneAmp PCR reagent kit (Perkin Elmer/Cetus, Norwalk, Conn.) according to the manufacturer's instructions. Custom-synthesized primers based on the *R. rickettsii* R rOmpB gene sequence (15), GTGCGGCCAT AACTAATGTA in the 5′-to-3′ direction and ACCGATTAC GACACCGGTGG for the opposite strand, were used to amplify an approximately 240-bp product.

The amplified product was precipitated with isopropanol, washed twice with 70% ethanol, dried, and resuspended in 10 mM Tris-HCl–1 mM EDTA, pH 7.5. DNA sequencing of the PCR products was done with the Sequenase Version 2.0 sequencing kit (U.S. Biochemical Corp., Cleveland, Ohio), with modifications to the manufacturers instructions as described previously (34).

## RESULTS

**Conservation of the 32-kDa heat-modifiable polypeptide among rickettsiae.** The rOmpB protein of *R. rickettsii* is encoded by a gene with the capacity to code for a protein of approximately 168 kDa (14, 15), but this putative precursor is believed to be processed to yield the mature 120-kDa rOmpB protein and a 32-kDa fragment (14). Comparison of SDS-PAGE profiles of various spotted fever group and typhus group rickettsiae solubilized either at room temperature or at 100°C demonstrate a heat-modifiable polypeptide of approximately 32 kDa among all rickettsiae analyzed (Fig. 1). N-terminal amino acid sequence analysis of this polypeptide from some of the more diverse rickettsial species, including members of both the spotted fever and typhus groups, confirms the identity of this peptide and demonstrates conservation of the N terminus (Fig. 2).

**Processing of rOmpB.** SDS-PAGE of unheated samples, followed by excision of the lane and boiling prior to running in a second dimension, revealed that the 32-kDa peptide, termed the β peptide, migrated off of the diagonal, as is typical of other bacterial heat-modifiable proteins (22, 32). These results further indicate that the 32-kDa peptide did not appear as a result of the cleavage of rOmpB during heat denaturation (Fig. 3).

**Surface exposure and association of rOmpB and the β peptide.** Surface radioiodination profiles demonstrate that rOmpB and the β peptide are both exposed on the outer surface of rickettsiae (Fig. 4B). Although the data presented above suggest that these polypeptides appear to be cleaved as they exist on the native rickettsial surface, they seem to remain in association with each other since they are coprecipitated by a MAb which reacts with only the 120-kDa rOmpB protein by immunoblotting (Fig. 4C and D). This

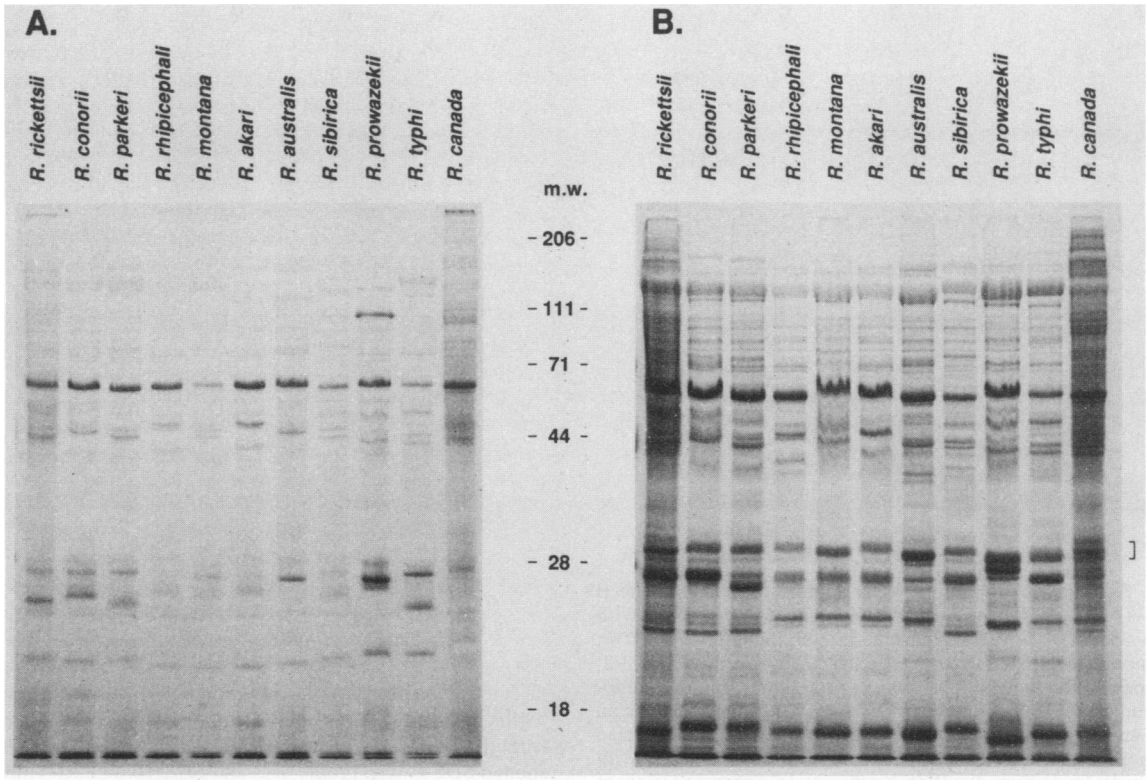


FIG. 1. Coomassie brilliant blue-stained SDS-PAGE profile of proteins from rickettsial species solubilized at room temperature (A) or by heating to boiling for 5 min (B). The approximate location of a heat-modifiable outer membrane protein encoded by the 3′ region of the rOmpB gene is indicated by a bracket. Molecular weights (in thousands) are indicated.

association has been observed previously (1), although its significance was not fully understood. The association does not appear to be through disulfide bridging, since reduction and alkylation of the lysates prior to radioimmunoprecipitation did not affect the coprecipitation.

**Identification of an avirulent mutant deficient in processing the rOmpB precursor.** An avirulent mutant of *R. rickettsii*, known as the Iowa strain, was described by Cox (12). This strain was passaged 248 times in eggs and was found to have lost virulence towards guinea pigs. Comparison of the SDS-PAGE profile of the Iowa strain with that of the virulent R strain of *R. rickettsii* revealed that the 32-kDa β peptide was absent, or greatly reduced in amount, and that the rOmpB protein was correspondingly larger, as would be predicted if the rOmpB precursor was not cleaved (Fig. 5). Although neither the 32-kDa β peptide nor the 120-kDa rOmpB protein were discerned in stained gels of the Iowa strain, immunochemical techniques detected small amounts of these prod-

| | |
|---|---|
| *R. rickettsii* "R" | GDEAIDNVAYGIXAKPFYT |
| *R. conorii* | ....V..... |
| *R. rhipicephali* | ....V.....T..... |
| *R. australis* | ..A.V..... |
| *R. prowazeki* | ....--E.S..V.....N |
| *R. typhi* | .....Q.S..V.....N |

FIG. 2. N-terminal amino acid sequence of 32-kDa β peptide. Periods indicate identical amino acids, and hyphens indicate gaps inserted for optimal alignment.

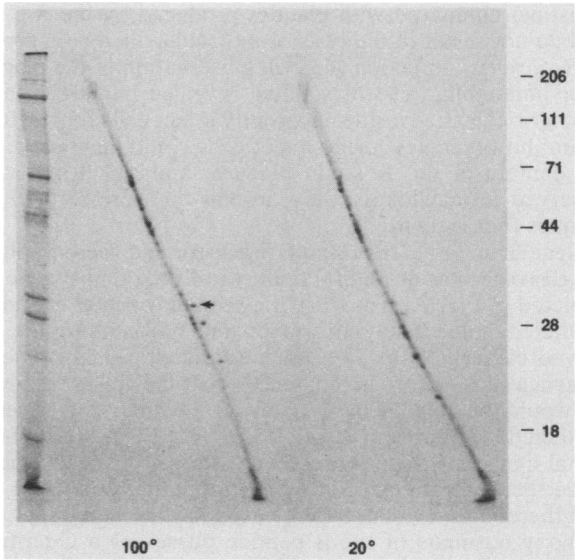


FIG. 3. Two-dimensional SDS-PAGE demonstrating the heat modification of the 32-kDa β peptide. Excised gel strips were either heat denatured (100°C) or untreated (20°C) prior to reelectrophoresis in the second dimension. The profile of a heat-denatured *R. rickettsii* lysate is shown on the left. The β peptide is indicated by an arrowhead. Molecular weights (in thousands) are indicated.

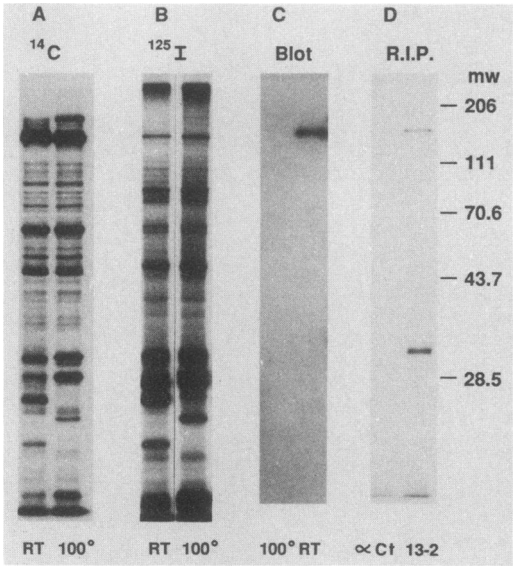


FIG. 4. Association of the 120-kDa rOmpB protein and the β peptide. (A) Extracts of *R. rickettsii* intrinsically labeled with $^{14}$C-amino acids; (B) extracts of surface-radioiodinated *R. rickettsii*; (C) immunoblot against *R. rickettsii* extracts by using MAb 13-2, which is specific for the 120-kDa rOmpB protein; (D) radioimmunoprecipitation (R.I.P.) of *R. rickettsii* extracts with MAb 13-2 or an irrelevant antibody (αCt). Proteins were extracted from rickettsiae for immunoprecipitation in the presence of DTT and blocked with iodoacetamide to disrupt disulfide bonds. Note coprecipitation of the 120-kDa rOmpB protein and β peptide by the antibody, which is specific for the 120-kDa mature protein. Molecular weights (in thousands) are indicated.

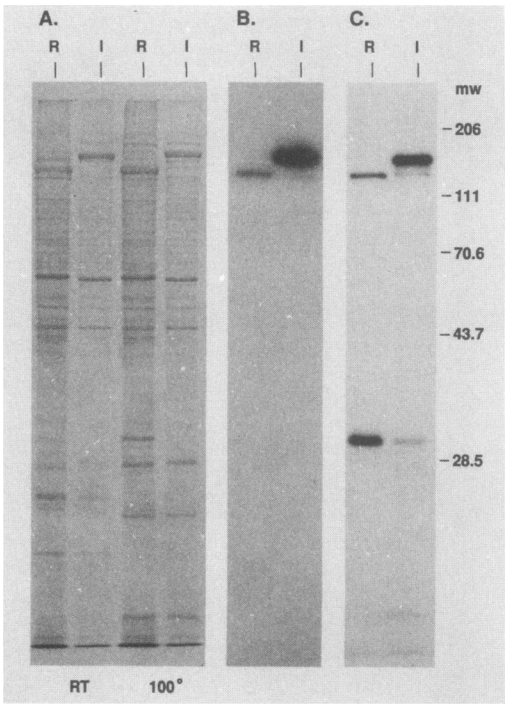


FIG. 5. Comparison of the virulent R strain (R) of *R. rickettsii* with the avirulent Iowa strain (I). (A) Coomassie brilliant blue-stained SDS-PAGE profile of R and Iowa solubilized either at 100°C or at room temperature (RT). (B) Immunoblot of R and Iowa with MAb 13-2. The Iowa lane was overloaded to demonstrate the presence of a small amount of the 120-kDa product in addition to the apparent precursor form of the molecule. (C) Radioimmunoprecipitation of R and Iowa with MAb 13-2, again demonstrating the presence of lesser amounts of the cleavage products in the Iowa strain. Molecular weights (in thousands) are indicated.

ucts relative to the majority of the uncleaved precursor form of rOmpB (Fig. 5B and C).

The avirulent nature of *R. rickettsii* Iowa is reflected in its interaction with Vero cells. Plaques of the Iowa strain are indistinct compared with plaques produced by the R strain and do not result in the lysis of infected cells that is typical of *R. rickettsii* infection (Fig. 6). Observation of the plaques after immunofluorescent staining revealed numerous intracellular rickettsiae inside apparently intact cells for the Iowa strain; however, few or no intact cells could be observed in the centers of the R strain plaques, and rickettsiae were observed intracellularly only around the periphery of the plaques (not shown).

**Gene structure of the rOmpB precursor and conservation of the cleavage site.** Structural features of the rOmpB gene are depicted in Fig. 7. A procaryotic secretory signal peptide is predicted immediately upstream and adjacent to the observed cleavage site. Two other secretory signal sequence cleavage sites are predicted. One is near the amino terminus, as would be expected of a leader peptide; the other is predicted between amino acids 1290 and 1291, but no functional significance can presently be ascribed to these regions, since the N terminus of rOmpB is apparently blocked (14) and there is no evidence for cleavage at the other site. The carboxy terminus of the β peptide possesses a C-terminal phenylalanine residue and β-sheet structure that appears to be highly conserved among outer membrane proteins of gram-negative bacteria (31). Amplification of DNA by the PCR and sequence analysis of the region of the gene encompassing the predicted signal peptide and first 20 amino acids of the β peptide revealed a very high degree of conservation among spotted fever group rickettsiae and complete identity between the virulent and avirulent strains of *R. rickettsii*. The typhus group rickettsias, *R. prowazekii* and *R. typhi*, were distinct from the spotted fever group but were still highly conserved, although they differed from each other.

**Possible function of the β peptide as an anchor domain.** The 120-kDa protein homolog of *R. prowazekii* is dissociated from the surface by water or dilute buffers (13). We examined the solubility of the rOmpB protein from *R. rickettsii* R and Iowa under similar conditions and compared this extraction with that observed for *R. prowazekii* (Fig. 8). The

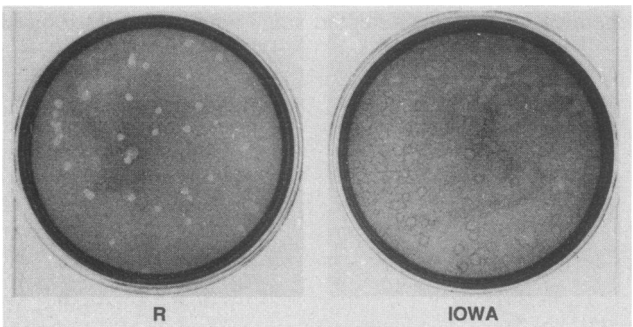


FIG. 6. Comparison of plaques produced by *R. rickettsii* R and Iowa.

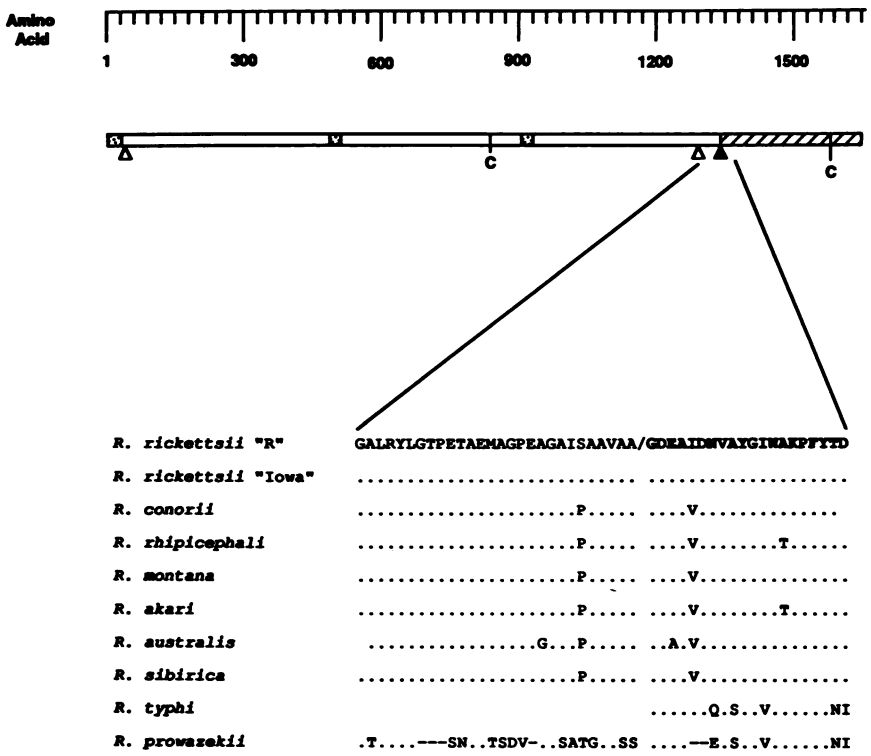


FIG. 7. Gene structure of *ompB* and sequence of the predicted secretory signal peptide and N terminus of the β peptide. The β-peptide domain is cross-hatched. Secretory signal peptide cleavage sites predicted by the method of von Heijne (35) are indicated by arrowheads. Where confirmation of actual cleavage is unavailable, the arrowheads are open; the closed arrowhead indicates the site of cleavage of the precursor molecule. The stippled areas indicate putative transmembrane domains predicted by the method of Klein et al. (16) or Rao and Argos (28). The location of the two cysteine residues is also shown. The deduced amino acid sequences derived from the sequences of PCR-amplified DNA overlapping the predicted signal peptide and the N terminus of the β peptide are compared. The N termini of the typhus group rickettsiae *R. typhi* and *R. prowazekii* were determined by amino acid sequencing. The *R. prowazekii* sequence corresponding to the signal peptide region of spotted fever group rickettsiae was from the sequence deduced by Carl et al. (9).

120-kDa rOmpB protein was the predominant component extracted by incubation with 10 mM Tris-HCl–10 mM EDTA at 45°C. The efficiency of extraction of the 168-kDa precursor from *R. rickettsii* Iowa was much less than the efficiency of extraction of the mature 120-kDa portion of the molecule from either of the virulent species of rickettsia examined, suggesting that covalent linkage to the putative anchor domain hindered extraction.

## DISCUSSION

The 120-kDa rOmpB protein is encoded by a large open reading frame with the capacity to code for a protein of approximately 168 kDa (14). The molecular mass of the mature protein has been variously estimated from its migration in SDS-PAGE as 133 (1), 135 (6), or 120 kDa (2); our own estimates from SDS-PAGE place the molecular mass at approximately 135 kDa, but, in keeping with the most recent terminology for this protein, we have referred to it as the 120-kDa rOmpB protein. We have proposed the term rOmpB (14) to avoid the confusion in nomenclature introduced by differences in gel systems. Regardless, a significantly smaller protein is observed than would be predicted from the DNA sequence. N-terminal amino acid sequencing of a 32-kDa, heat-modifiable outer membrane protein revealed that it is encoded by the 3′ end of the rOmpB gene. On the basis of our estimates of the molecular masses of rOmpB and the β peptide as 135 and 32 kDa, respectively, the total mass of the two fragments approximate the size of the product predicted from the gene sequence. For technical reasons, including the long doubling time of rickettsiae and the relatively small numbers of organisms per infected cell, we have not yet detected the putative precursor in virulent *R. rickettsii*; thus, we cannot formally rule out the possibility that transcriptional or translational reinitiation at that site produces the two separate products. However, we believe that a more likely explanation for the appearance of these two polypeptides is the proteolytic processing of a larger precursor; we also believe that this processing, occurring in vivo, reflects the disposition of these proteins on the native rickettsial surface. Our reasoning is as follows. (i) The gene consists of a single open reading frame with neither transcriptional terminators nor consensus controlling elements for either transcription or translation in the immediate upstream region of the gene encoding the N terminus of the β-peptide. (ii) SDS-PAGE followed by boiling and reelectrophoresis in a second dimension did not indicate that cleavage during heat denaturation produces smaller 120- and 32-kDa products below the diagonal; instead, the 32-kDa β peptide moved upward off of the diagonal, typical of heat-modifiable outer membrane proteins such as *Escherichia coli* OmpA (17, 22) or the gonococcal Opa proteins (32). (iii) A mutant strain of *R. rickettsii* exhibits an apparently unprocessed form of the protein, since it lacks the 32-kDa β peptide and possesses a correspondingly larger rOmpB protein that is reactive with MAbs specific for the *R. rickettsii* 120-kDa rOmpB protein.

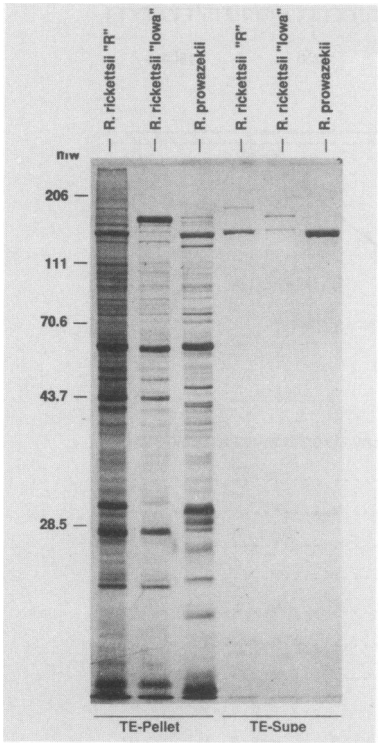


FIG. 8. Comparison of extraction of rOmpB from *R. rickettsii* R and Iowa and from *R. prowazekii*. Extraction was done with 10 mM Tris-HCl–1 mM EDTA, pH 7.5, at 45°C for 30 min essentially as described previously (13). The rickettsiae were pelleted and extracted a second time, and the pellets and supernatants were analyzed by SDS-PAGE with Coomassie brilliant blue staining. TE, Tris-EDTA; Supe, supernatant.

This apparent proteolytic processing of rOmpB is characteristic of every species of rickettsia examined, including spotted fever group rickettsia pathogenic for man (*R. rickettsii*, *R. conorii*, *R. sibirica*, *R. akari*, and *R. australis*), avirulent or weakly pathogenic spotted fever group rickettsia (*R. rhipicephali*, *R. montana*, and *R. parkeri*), virulent typhus group rickettsia (*R. prowazekii* and *R. typhi*), and a nonpathogenic member of the typhus group (*R. canada*). The avirulent Iowa strain of *R. rickettsii* was the only organism detected that displayed a reduced ability to process the rOmpB precursor and thus displayed a molecule with a size expected for the putative rOmpB precursor. The Iowa strain is reported to be a laboratory-derived mutant that initially caused mild infections in guinea pigs but, upon extensive passage in eggs, exhibited reduced virulence, caused only inapparent infections, and could not be recovered from infected animals (12). Our observations demonstrate that this strain also displays reduced pathogenicity towards cultured cells. Typically, plaques produced by *R. rickettsii* result from the lysis of infected cells (36). Plaques produced by *R. rickettsii* Iowa are indistinct; cells in the plaque itself retain the vital dye neutral red and do not lyse or display obvious changes in morphology despite having large numbers of intracellular rickettsia. The kinetics of association of the Iowa strain with host cells has not been examined in detail; however, the ratios of PFU to rickettsial particles do not differ substantially. The defect in the Iowa strain seems instead to moderate lysis of the infected cell. The decreased ability of the Iowa strain to process rOmpB correlates with decreased virulence, but it must be emphasized that genetic systems have not yet been developed for any procaryotic obligate intracellular parasite, and attempts to correlate structure with function or pathogenesis must therefore remain purely speculative. Interestingly, a protease inhibitor has been reported to inhibit plaque formation by *R. rickettsii* (37). Although the site of action of this inhibitor has not been identified, it will be worthwhile to determine whether cleavage of the rOmpB precursor is a process that is blocked.

On the basis of observations of a regularly arrayed surface structure on *R. akari* and *R. prowazekii* (23, 24), rickettsiae have been proposed to possess a surface or S layer (29). The structure and organization of the 120-kDa rOmpB protein on the surface of *R. rickettsii* remains undefined, however. On the basis of surface iodination (1, 5) and their presence in outer membrane preparations of *R. prowazekii* (30), both the 120- and 32-kDa fragments of rOmpB are surface exposed. Precipitation of the 120-kDa protein and the 32-kDa β fragment by MAb specific for the 120-kDa rOmpB protein implies that the two fragments retain a relatively stable, although noncovalent, association with each other, possibly stabilized by divalent cations. The β fragment exhibits a 10-amino-acid C-terminal domain characteristic of bacterial outer membrane proteins (31). Lack of cleavage of the β peptide from the rOmpB protein results in a precursor that is not released from the outer surface under conditions which cause the release of the mature 120-kDa rOmpB protein from virulent *R. rickettsii*. The diminished release of rOmpB from the Iowa strain under these conditions is consistent with the proposal that the C-terminal domain of the precursor protein may serve as a membrane anchor.

There are several examples of in vivo and in vitro proteolysis of bacterial surface layer proteins (8, 18, 25, 27). In most of these cases, multiple proteolytic fragments are observed. Our results indicate the presence of only a single, specific cleavage site on the rickettsial rOmpB protein that generates two apparently stable products. The rOmpB structure shares several interesting features with that of the immunoglobulin A (IgA) protease of *Neisseria gonorrhoeae*, in which the β peptide functions in the secretion of the amino-terminal IgA protease domain of the molecule (26). In the case of the *N. gonorrhoeae* IgA protease, the cleavage is believed to autocatalytically release soluble IgA protease and leave the β fragment associated with the outer membrane (26). It will be of great interest to determine whether such an export function exists for the *R. rickettsii* β peptide. If the *R. rickettsii* β peptide is indeed analogous in function, it would suggest a unique system in which the carboxy-terminal domain of the precursor would direct export of the 120-kDa, N-terminal domain of rOmpB, which may then be cleaved by unknown mechanisms (either autocatalytically or by signal peptidase or other protease) but then remain in association with the outer surface via interaction with the membrane-bound β peptide.

Recognition of the 32-kDa β peptide as a cleavage product of the 168-kDa *ompB* gene product would explain the discrepancy in size between the observed protein product and that predicted by the gene sequence (14) and the failure of others to detect cyanogen bromide fragments predicted from the homologous gene sequence of *R. prowazekii* (9). The identification of an avirulent mutant of *R. rickettsii* defective in processing of the precursor should prove useful in studies of the structure and organization of the mature protein on the rickettsial surface as well as in studies of the role of rOmpB in rickettsial pathogenesis.

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