Molecular Cloning of the Major Outer Membrane Protein of Chlamydia trachomatis

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Received 21 March 1984/Accepted 31 May 1984

A gene library of Chlamydia trachomatis (serovar L1) DNA has been prepared in the phage vector λ1059. From this bank, 20 recombinant phage-expressing components which reacted with serum from a patient with a C. trachomatis (L1) infection were chosen. Selective expression and radiolabeling of phage polypeptides in irradiated Escherichia coli demonstrated that one of these clones encoded a polypeptide doublet with an apparent molecular weight similar to that of the C. trachomatis (L1) major outer membrane protein. Both species of this cloned doublet (40 and 41 kilodaltons) could be immunoprecipitated by serum from a patient with a C. trachomatis (L1) infection but not by normal human serum. Components of this apparent molecular weight were not precipitated from irradiated E. coli infected with vector phage λ1059 by either of these sera. Comparison of the Staphylococcus aureus-V8 protease peptide maps of these two cloned polypeptides and chlamydial major outer membrane protein extracted from elementary bodies showed all three polypeptides to produce peptide fragments of 15.5, 13.8, and 11.5 kilodaltons. Due to the indentical apparent molecular weights of the fragments produced from the 40- and 41-kilodalton cloned polypeptides, these were concluded to be different conformational forms of the same molecular species. These cloned components were indistinguishable from C. trachomatis (L1) major outer membrane protein.

Members of the species Chlamydia trachomatis are obligate, intracellular, bacterial parasites of eucaryotic cells. They have long been known to be responsible for trachoma, the single largest cause of infectious human blindness, and more recently have been strongly implicated in genital tract infections which in frequency, severity, and syndrome range at least parallel gonococcal infections (23).

A major immunogen of the host response to such infections is the chlamydial major outer membrane protein (MOMP) (17). This surface-located (5, 20) polypeptide can induce antibody capable of neutralizing chlamydial infectivity in vitro (6) and contains epitopes which contribute to the species, sub-species, and type-specific (7) definition of the 15 currently known serovars of this species. For many other bacterial pathogens, such antigenic diversity of surface components provides a potentially important mechanism for evasion of the host immune response (3, 24, 25).

The MOMP is also the most abundant polypeptide species of the infectious (elementary body [EB]) and reproductive (reticulate body) forms of the organism (5, 13). This substantial molar contribution of MOMP to the composition of the outer membranes of these parasites, which lack readily detectable quantities of muramic acid (1, 9, 16), has led to the proposal that MOMP, in conjunction with other outer membrane components, contributes significantly to the structural integrity of the rigid form of these pathogens (5, 13, 18).

Other possible biological functions of MOMP are at present unclear. On the basis of the general similarities between MOMP and outer membrane proteins of other gram-negative bacteria, it has been proposed (5) that MOMP may function as a porin, channeling nutrients toward the chlamydial cytosol. This raises the possibility that alterations in MOMP conformation, with concomitant changes in porin activity, may be involved in differentiation of the impermeable, metabolically inactive EB form to the permeable, metabolically active reticulate body form of the organism. Newhall and Jones (18) have reported the presence of disulfide-linked oligomeric complexes of MOMP in EB, and Hatch et al. (13) have described disulfide-linked homo- and hetero-oligomers of MOMP which are abundant in the EB but almost completely absent in the reticulate body. Hence, the possible conformational changes described above could involve reduction of disulfide bonds, leading to formation of a parasite membrane permeable to host nutrients. In favor of this proposal, Sarov and Becker (21) have reported that treatment of the EB with reducing agents led to enhanced incorporation of nucleotides into RNA.

It is clear that MOMP is a major and complex chlamydial antigen, that it may play an important structural role in the EB, and that it may possibly be involved in the regulation of the complex life cycle of these parasites. For these reasons the MOMP of C. trachomatis was chosen as the subject of this study.

Previously, progress in studies of the antigenic and biological properties of isolated chlamydial constituents has been severely hampered by the difficulty of producing large quantities of denatured components from these fastidious pathogens. In an attempt to circumvent these problems, we prepared a library of chlamydial chromosomal and plasmid DNA in a bacteriophage vector and obtained expression of this information in Escherichia coli. The specific aim of the present project was to select recombinant phage which expressed the MOMP of C. trachomatis and to determine the serological and biochemical relatedness between this component and the analogous chlamydial component obtained from the EB.

MATERIALS AND METHODS

Organism. C. trachomatis L1/440/Bu was grown in HeLa-229 cells and purified as previously described (15). Seed stocks of this strain were kindly provided by J. Schachter.
Preparation of the *C. trachomatis* gene library in A1059.

Preparation of the *C. trachomatis* gene library in λ1059 was as described by Wenman and Lovett (27). Briefly, purified chlamydial DNA was partially digested with Sau3A1 to create fragments of ca. 15 kilobases which were ligated to BamHI-cleaved vector phage DNA (λ1059) (14) with T4 ligase, packaged in vitro (2), and used to transfect *E. coli* Q359. This bacterial strain is permissive only for recombinant phage in which the central region of the λ1059 DNA spanning the two internal BamHI sites has been replaced by foreign DNA (14).

**Preparation of *E. coli* Q358 immunoabsorbant.** One volume of packed Sepharose 4B (Pharmacia Fine Chemicals) was washed three times with 5 volumes of water. The packed beads were then suspended in five volumes of CNaBr (5% [wt/vol]) and stirred for three min while the pH was maintained at 10.0 with 20% (wt/vol) NaOH. The slurry was immediately added to 15 volumes of 0.1 M NaHCO₃ (pH 9.0), and the beads were collected on a scinttered glass filter and washed with a further 5 volumes of 0.1 M NaHCO₃ (pH 9.0). These activated beads were suspended in 1 volume of 0.1 M NaHCO₃ (pH 9.0), 10 g (wet weight) of sonically disrupted *E. coli* Q358 cells suspended in 5 volumes of 0.1 M NaHCO₃ (pH 9.0) was added, and the mixture was stirred gently for 16 h at 4°C. Uncoupled protein was then washed away from the beads with TSA (50 mM Tris-hydrochloride [pH 7.5], 150 mM NaCl, 0.15% [wt/vol] sodium azide) until the absorbance of the supernatant fluid was stable at less than 0.1 at 280 nm.

**Adsorption of sera with *E. coli* Q358 immunoabsorbant.** Three volumes of serum (diluted 1:3 [vol/vol] in TSA) was added to 1 volume of packed immunoabsorbant and rocked gently for 1 h at 25°C. The serum was collected, and the process was repeated twice with fresh immunoadsorbant.

The second adsorption was for 3 h at 25°C, and the third was for 16 h at 4°C. Both normal and infected human sera were adsorbed in this way to remove seroreactivity to *E. coli*.

In situ radioimmunoassay for detection of recombinant phage expressing products reactive with serum from a patient infected with *C. trachomatis* (L₁). The in situ radioimmunoassay for detection of recombinant phage expressing products reactive with serum from a patient infected with *C. trachomatis* (L₁) was performed as described by Wenman and Lovett (27). Briefly, putative antigens released from phage plaques were blotted onto nitrocellulose, the remaining vacant protein binding sites on the membranes were saturated with ovalbumin, and the blots were reacted with infected or normal serum. After thorough rinsing to remove unassociated antibody, bound antibody was detected by reaction with ¹²⁵I-labeled staphylococcal protein A and subsequent direct autoradiography.

**Selective expression of phage polypeptides in UV-irradiated uvrA mutant *E. coli*.** Selective expression of phage polypeptides in UV-irradiated uvrA mutant *E. coli* was as described by Calhoun and Gray (28). *C. trachomatis* (L₁) was grown as described by Calhoun and Gray (28). Bacteriophage L₁ DNA was digested with Sau3A1 to create fragments of ca. 15 kilobases which were ligated to BamHI-cleaved vector phage λ1059 DNA. Ligated DNA was then packaged in vitro and used to transfect *E. coli* Q359.

Plaques produced by ca. 1,000 such recombinant phage were screened for chlamydial antigen production by an in situ radioimmunoassay (27) with a high-titer human serum sample from a serovar L₁ infection and ¹²⁵I-labeled protein A from *S. aureus*. Twenty clones exhibiting various degrees of seroreactivity were selected for further characterization, and their polypeptide coding potential was examined by using the selective expression of phage DNA which occurs in uvrA mutants of *E. coli K*12 after UV irradiation-induced suppression of endogenous protein synthesis (see below and above). On the basis of the apparent molecular weights (AMWs) of polypeptides expressed by the recombinant synthesized polypeptides. After addition of excess unlabeled amino acids, infected cells were pelleted (Microfuge; 3 min, 13,500 × g, 25°C) and prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by solubilization with SDS and β-mercaptoethanol. After resolution of polypeptides, SDS-PAGE gels (8 to 15% gradient) were processed for fluorography by using Fuji-RX film as described by Hames (11).

**Immunoprecipitation of radiolabeled, phage-encoded polypeptides.** Phage-encoded polypeptides were radiolabeled in UV-irradiated *E. coli* as described above. Pelleted *E. coli* cells were then suspended in 200 µl of TES (50 mM Tris-hydrochloride [pH 8.0], 50 mM EDTA, 15% [wt/vol] sucrose), and 50 µl of 2 mg of lysozyme ml⁻¹ was added. After incubation for 5 min on ice, 250 µl of TESNP (50 mM Tris-hydrochloride [pH 8.0], 50 mM EDTA, 15% [wt/vol] sucrose, 0.5% [wt/vol] Nonidet P-40) was added, and the suspension was mixed gently. After a further 2 min of incubation on ice, cellular debris was removed by centrifugation (Microfuge; 2 min, 13,500 × g, 25°C), and the released putative antigens in the supernatant were preadsorbed with 50 µl of 10% (packed volume) Formalin-fixed *Staphylococcus aureus* (Cowan) cells for 60 min at 0°C. After centrifugation (Microfuge; 5 min, 13,500 × g, 25°C) to remove staphylococci, 30 µl of serum (diluted 1:3 [vol/vol] in TSA) was added, and the solution was incubated for 16 h at 4°C. An amount of 250 µl of 10% (packed volume) Formalin-fixed *S. aureus* cells (Cowan) was then added, and the suspension was incubated for 4 h at 0°C. Staphylococci were collected by centrifugation (Microfuge; 5 min, 13,500 × g, 25°C) and washed four times with 1 ml of 0.05% (vol/vol) Nonidet P-40 in TEN (10 mM Tris-hydrochloride [pH 8.0], 100 mM NaCl, 1 mM EDTA). Pelleted staphylococci were then prepared for SDS-PAGE by solubilization in 6.25 mM Tris-hydrochloride–2% (wt/vol) SDS–10% (vol/vol) glycerol–5% (vol/vol) β-mercaptoethanol for 10 min at 100°C.

**SDS-PAGE and Western blotting.** SDS-PAGE and Western blotting were performed as described by Hanfl et al. (12).

*S. aureus*-V8 protease peptide mapping. *S. aureus*-V8 protease peptide mapping was performed as described by Caldwell and Judd (4). Chlamydial MOMP was labeled with ¹²⁵I (4) after excision of the band from a preparative gel. Putative cloned MOMP was labeled with [³⁵S]methionine in irradiated *E. coli* as described above, and appropriate bands were excised from gels before peptide mapping.

**RESULTS**

Preparation of the *C. trachomatis* gene bank and selection of recombinant phage expressing seroreactive components. Purified L₁ DNA was digested with Sau3A1 to create fragments of ca. 15 kilobases which were ligated to BamHI-cleaved vector phage λ1059 DNA. Ligated DNA was then packaged in vitro and used to transfect *E. coli* Q359.

Plaques produced by ca. 1,000 such recombinant phage were screened for chlamydial antigen production by an in situ radioimmunoassay (27) with a high-titer human serum sample from a serovar L₁ infection and ¹²⁵I-labeled protein A from *S. aureus*. Twenty clones exhibiting various degrees of seroreactivity were selected for further characterization, and their polypeptide coding potential was examined by using the selective expression of phage DNA which occurs in uvrA mutants of *E. coli K*12 after UV irradiation-induced suppression of endogenous protein synthesis (see below and above). On the basis of the apparent molecular weights (AMWs) of polypeptides expressed by the recombinant
phage, one clone (designated D-2) was chosen for further study.

The seroreactivity of products expressed by clone D-2 is shown in Fig. 1. Plaques of this phage reacted strongly with serum from a patient with a C. trachomatis serovar L1 infection (infected human serum [IHS]; Fig. 1a) but not with normal human serum (NHS; Fig. 1b). Vector phage (λ1059, lacking a chlamydial DNA insert) did not react strongly with either IHS (Fig. 1c) or NHS (Fig. 1d).

Selective expression of phage-encoded polypeptides in UV-irradiated E. coli. Infection of UV-irradiated E. coli (M159; avrA) bacterial cells led to the appearance of several polypeptides (Fig. 2, lane 3) which were not seen in parallel infections with vector phage (lane 2) or mock infections with no phage (lane 1). These polypeptides were tentatively assumed to be encoded by the chlamydial DNA insert. One of the components, a doublet (labeled with an arrowhead), migrated in SDS-PAGE with an AMW equivalent to that of the C. trachomatis L1 MOMP (40 to 41 kilodaltons [kd]). The high molar ratio of MOMP in chlamydial samples does not always allow resolution of this component into a doublet.

Immunoprecipitation of the 40- to 41-kd doublet by IHS. The antigenicity of this putative recombinant MOMP was examined by reaction with IHS or NHS. Polypeptides coded for by recombinant phage D-2, with vector phage λ1059 as control, were labeled by incorporation of [35S]methionine during infection of irradiated E. coli M159 and released from host cells by gentle lysis with EDTA, lysozyme, and the nonionic detergent Nonidet P-40 at 0°C. Potential antigens were then sequentially reacted with IHS or NHS and formaldehyde-fixed S. aureus (Cowan). Precipitated, [35S]methionine-labeled antigens were subsequently released from the complexes by being boiled in SDS and β-mercaptoethanol and were resolved by SDS-PAGE.

The 40- to 41-kd doublet specified by D-2 was precipitated by IHS but not NHS (Fig. 3). Interestingly, immunoprecipitation altered the molar ratios of the 40- and 41-kd polypeptides. Components of this AMW were not precipitated from polypeptides encoded by vector phage λ1059 by reaction with either serum. Figure 4 shows the immunoreactivity of IHS and NHS with chlamydial polypeptides examined by Western blotting.

S. aureus-V8 protease peptide mapping of cloned and chlamydial products. The degree of relatedness between the chlamydial MOMP and the cloned product was further studied by peptide mapping of these components by using S. aureus-V8 protease, which specifically cleaves polypeptides on the carboxyl side of glutamic and, to a lesser extent, aspartic acid residues. Bands corresponding to the 40- to 41-kd doublet of D-2 and chlamydial MOMP were excised from gels and subjected to V8 protease hydrolysis, and the peptide fragments thus generated were resolved by SDS-PAGE. The peptide fragments generated from the upper and lower bands of the cloned doublet and the chlamydial MOMP were indistinguishable from each other (15.5, 13.8, and 11.5 kilobases; Fig. 5), indicating that there is a high degree of relatedness shared between the chlamydial and cloned products.

FIG. 1. Seroreactivity of clone D-2 and vector phage plaques detected by an in situ radioimmunoassay. (a) D-2 reacted with IHS; (b) D-2 reacted with NHS; (c) λ1059 reacted with IHS; (d) λ1059 reacted with NHS.

FIG. 2. Expression of phage polypeptides in UV-irradiated E. coli. Shown is a fluorograph of phage-encoded polypeptides labeled with [35S]methionine. Samples are E. coli infected with no phage (lane 1), E. coli infected with vector phage λ1059 (lane 2), and E. coli infected with clone D-2 (lane 3). Molecular weight standards are indicated on the right in kd. Putative MOMP is indicated by an arrowhead. Polypeptides were resolved by SDS-PAGE (8 to 15% gradient gel).

DISCUSSION

A gene bank of C. trachomatis (serovar L1) DNA was prepared in the phage vector λ1059. Choice of this vector was influenced by (i) the ease with which phage plaques, in contrast to bacterial colonies, can be screened by an in situ radioimmunoassay (26), (ii) the reported toxicity of outer membrane proteins for E. coli when present on plasmid vectors (10), and (iii) the previously reported expression of a chlamydial antigen in this system (27).
FIG. 3. Immunoprecipitation of phage products by IHS and NHS. Shown is a fluorograph of phage-encoded polypeptides labeled with [35S]methionine and immunoprecipitated with IHS or NHS. Samples are λ1059 polypeptides (lane 1), D-2 polypeptides (lane 2), λ1059 polypeptides immunoprecipitated by IHS (lane 3), D-2 polypeptides immunoprecipitated by NHS (lane 4), λ1059 polypeptides immunoprecipitated by NHS (lane 5), and D-2 polypeptides immunoprecipitated by NHS (lane 6). Molecular weight standards are indicated on the right in kd. The putative MOMP is indicated by an arrowhead. Polypeptides were resolved by SDS-PAGE (8 to 15% gradient gel).

From this library 20 clones exhibiting various degrees of seroreactivity with serum (IHS) from a patient with a C. trachomatis L1 infection were selected. Given the coding potential of the chlamydial genome (ca. 700 polypeptides of average size) and the reported existence of at least 20 chlamydial antigens (22), this frequency of seroreactivity is compatible with the hypothesis that the majority of the chlamydial genome is readily expressed in E. coli. Examination of the polypeptides encoded by these recombinant phage showed several that code for more than one protein which could be immunoprecipitated by IHS (data not shown).

One clone, designated D-2, encoded polypeptides with AMWs of 40 and 41 kd which were not coded for by the vector phage and which could be immunoprecipitated by IHS but not NHS. These two polypeptides had indistinguishable S. aureus-V8 peptide maps (see below), suggesting that they may be different conformational forms of the same molecular species. Although the 40-kd species was the predominant form in polypeptide preparations before immunoprecipitation (Fig. 3, lane 2), this procedure led to preferential precipitation of the 41-kd species (Fig. 3, lane 4). This may have been due either to greater seroreactivity of the 41-kd form or conversion of the 40-kd form to the 41-kd form during the immunoprecipitation process.

Parallel experiments to immunoprecipitate chlamydial MOMP were not attempted, as release of this component from the EB requires exposure of the particles to denaturing agents (SDS, β-mercaptoethanol) (5) which would be expected to inhibit subsequent immunoprecipitation. Rather, the immunoreactivity of the serum with chlamydial components was examined by Western blotting and was shown to be highly reactive with MOMP.

S. aureus-V8 protease peptide maps of the chlamydial

FIG. 4. Seroreactivity of IHS and NHS for chlamydial polypeptides exhibited by Western blotting (8 to 20% gradient gel). Samples are C. trachomatis (serovar L1) reacted with IHS (lane 1) and NHS (lane 2). The MOMP is indicated by an arrowhead.

FIG. 5. Peptide maps generated by S. aureus-V8 proteolytic cleavage of the 40- and 41-kd polypeptides encoded by D-2 and the chlamydial MOMP. Shown is a fluorograph of fragments from 40-kd (lane 1) and 41-kd (lane 2) polypeptides labeled with [35S]methionine and the chlamydial MOMP (lane 3) labeled with 125I.
MOMP and the 40- and 41-kd polypeptide encoded by phase D-2 were all found to be indistinguishable. Although this technique is not capable of determining true identity among proteins, it is capable of determining the degree of relatedness.

The appearance of the intact cloned products as a doubllet may be explained by incomplete reduction of intramolecular disulfide bonds generating a heterogeneous population of molecules with respect to their mobility in SDS-PAGE. An alternative explanation is that the more slowly migrating species may represent a precursor (unprocessed form) of the 40-kd molecule. However, processing of procaryotic membrane proteins has been reported to involve the removal of a leader peptide of ca. 2 kd (19); such a difference in molecular mass should have been readily visible by V8 protease peptide mapping.

The radiochemicals used to label the chlamydial MOMP and the 40- and 41-kd polypeptides encoded by phase D-2 for these peptide mapping experiments were 125I and 35S-methionine, respectively. One possible drawback to this approach was that a peptide(s) generated by proteolysis may have contained only one of the radioisotopes, e.g., it may have contained methionine by not tyrosine (the preferential target of the iodination reaction). Comparison of the peptides visualized showed that all components detected by 125I-labeling were also seen by 35S-methionine labeling. This does not preclude the possibility that fragments lacking both tyrosine and methionine were generated. However, summation of the AMWs of the fragments visualized by both systems showed the total ($15.5 + 13.8 + 11.5 \text{ kd} = 40.8 \text{ kd}$) to closely approach the AMW of the intact molecule (40 to 41 kd).

We have described here the cloning and expression in E. coli of a molecule indistinguishable from a major structural and seroreactive chlamydial component, the MOMP. We believe that this approach may provide valuable tools for future study of the pathogenicity and immunobiology of these organisms, as well as a potentially abundant source of chlamydial constituents for biological, antigenic, and diagnostic analysis. In particular, we hope that comparison of the DNA sequences coding for MOMP from a variety of different serovars will define type-specific and common domains of the molecules and allow investigations of the biological and immunological importance of these regions.

ACKNOWLEDGMENTS

This work was supported in part by a gift from the Cetus Corporation. We gratefully acknowledge the advice of A. M. Walford and the excellent technical assistance of Allyson M. Haislip.

LITERATURE CITED


