Polypeptide Composition of *Chlamydia trachomatis*

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Strains of *Chlamydia trachomatis* representative of 14 serotypes were grown in HeLa 229 cells. HeLa cell susceptibility to chlamydial infection was increased by treating the host cells with DEAE-dextran. Optimal conditions of DEAE-dextran treatment were determined for each serotype of *C. trachomatis* to maximize chlamydial yields. Chlamydial polypeptides were selectively radiolabelled with ^3H -labelled amino acids in the presence of emetine, an inhibitor of HeLa cell protein synthesis. The radiolabelled chlamydiae were purified from host cell components by density gradient centrifugation and their polypeptide composition was determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis. The distribution of the major chlamydial polypeptide of 38 000 to 42 000 daltons amongst the different serotypes correlated closely with the predominant human infections caused by each serotype. Lymphogranuloma venereum agents possessed a unique polypeptide of 118 000 daltons not found amongst trachoma inclusion conjunctivitis strains of chlamydiae. Chlamydial surface polypeptides were selectively radiolabelled with ^{125}I by lactoperoxidase-catalysed oxidation. The major chlamydial polypeptide and polypeptides of 155 000 and 29 000 daltons were thus identified as surface polypeptides of the chlamydial elementary body. It is suggested that the 155 000 dalton polypeptide is a species-specific antigen, the major polypeptide is the principal outer membrane protein, and the 29 000 dalton polypeptide is the type-specific antigen.

INTRODUCTION

Chlamydiae are obligate intracellular bacteria which possess a specialized growth cycle. *Chlamydia trachomatis*, the major source of human infection, possesses a group-specific carbohydrate antigen containing 2-keto-3-deoxyoctulosonic acid (Dhir *et al.*, 1972) and at least 15 type-specific antigens which were demonstrated by micro-immunofluorescence tests (Wang & Grayston, 1970). The importance of type-specific antigens in the pathogenesis of chlamydial infections was shown by the fact that clinically distinct infections were associated with different serotypes of *C. trachomatis* (Schachter & Dawson, 1978). Moreover, experimental vaccination of monkeys and man demonstrated that immunity to ocular infection was type-specific (Grayston *et al.*, 1971). Interestingly, vaccinated subjects who subsequently became infected with chlamydiae of heterologous serotype developed more severe ocular disease than unvaccinated controls (Woolridge *et al.*, 1967), presumably as a result of delayed hypersensitivity reactions to chlamydial group-specific antigens. Clearly, both an understanding of the pathogenesis of chlamydial infections and the development of a trachoma vaccine require the characterization of chlamydial antigens.

The technical difficulties of large-scale growth and purification of *C. trachomatis* have hampered the immunochemical analysis of the surface antigens. The most recent approaches have been to grow chlamydiae in the yolk sac of embryonated eggs (Caldwell *et al.*, 1975; Sacks *et al.*, 1978) or, less commonly, in cell culture (Hourihan *et al.*, 1980), followed by

extraction of both chlamydial and host cell components with detergents and isolation of antigens by immunoaffinity with monospecific antibody. This approach, using an impure starting material and elaborate purification procedures, yields minute amounts of purified antigen. Moreover, the antigens studied were limited by the available monospecific antibodies. An alternative approach is to use purified chlamydiae as a starting point for immunochemical analysis. In this paper, we describe the purification of 14 different serotypes of *C. trachomatis* from large-scale growth in HeLa cells and their polypeptide compositions determined using sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis.

METHODS

Chlamydial strains. The following strains of *C. trachomatis* were kindly provided by Drs S. Darougar and J. Treharne (Institute of Ophthalmology, Judd Street, London WC1H 9QS) as dilutions of infected egg yolk sac: A/SA-1/OT, B/TW-5/OT, C/UW-1/OT, D/IC-Cal-8/ON, E/DK-20/ON, F/MRC-301/GU, G/IOL-238/R, H/UW-4/GC_x, I/UW-12/GU, J/UW-36/GC_x, K/UW-31/GC_x, L₁/440/LN, L₂/434/Bu and L₃/404/LN. After receipt the strains were passaged once or twice through egg yolk sacs and stored in a freezing medium of phosphate-buffered glutamate, pH 7.4, with added sucrose (SPG) at -70 °C (Bovarnick *et al.*, 1950).

Infectivity titration. Serial five-fold dilutions of egg-grown chlamydiae were prepared in Eagle's Minimal Essential Medium containing 10% (v/v) foetal calf serum, L-glutamine and sodium bicarbonate (MEM). The chlamydial dilutions in 1 ml volumes of MEM were centrifuged at 2000 *g* for 1 h at room temperature on to confluent, DEAE-dextran-treated HeLa 229 cell monolayers on 13 mm diameter coverslips. The monolayers were incubated to permit inclusion development, stained with Hoechst 33258 and the numbers of inclusions were counted (Salari & Ward, 1979). The dilution of yolk sac-derived chlamydiae capable of infecting at least 80% of the HeLa cells (generally between 1 in 100 and 1 in 10000) was the dilution used to prepare an inoculum for large-scale tissue culture growth of chlamydiae.

Large-scale growth of chlamydiae. To obviate any possibility of egg yolk contamination of the final chlamydial harvest an inoculum of tissue culture-grown chlamydiae was first prepared. HeLa 229 cells were grown overnight in MEM to confluent monolayers in 260 ml Nunc disposable tissue culture flasks (Gibco Bio-Cult, Paisley, PA3 4EP). The tissue culture medium was then decanted and replaced with MEM containing 1 µg emetine hydrochloride ml⁻¹ (Sigma). After 2 h incubation at 35.5 °C the emetine-containing MEM was discarded, the cells were washed once in Hanks' balanced salt solution (Hanks' BSS), then incubated twice for 5 min at 35.5 °C in two changes of DEAE-dextran (mol. wt 5 × 10⁵; Sigma) in Hanks' BSS. The concentration of DEAE-dextran used was optimal for the chlamydial strain being grown (see Table 1). Lymphogranuloma venereum strains of *C. trachomatis* (serotypes L₁, L₂ and L₃) were incubated at 37 °C without DEAE-dextran. After a further wash in Hanks' BSS, 15 ml of the appropriate dilution of yolk sac-grown chlamydiae in MEM were added to the HeLa cells and the flasks were centrifuged in a micro-titration tray carrier (MSE Instruments, Crawley, Sussex) for 1 h at 2000 *g* at room temperature using an MSE GF8 centrifuge. After 2 h incubation to permit chlamydial adsorption, the cells were washed twice with Hanks' BSS then incubated in MEM containing 1 µg emetine hydrochloride ml⁻¹. After 40 to 60 h, infected cells from each of 12 flasks were harvested into 5 ml of ice-cold chlamydial freezing medium (SPG) using a rubber-covered scraper. The cells were vortex-mixed for 2 min with 3 mm diameter sterile glass beads, then homogenized with a Dounce homogenizer (Wheaton Industries, Millville, N.J. 08332, U.S.A.) so as to break open cells but leave the cell nuclei intact. The nuclei were sedimented by centrifugation at 500 *g* for 5 min at 4 °C and the supernatant, consisting of partially purified chlamydiae, was frozen at -70 °C. After infectivity titration these tissue culture-grown chlamydiae were used as the inoculum for the large-scale growth of further chlamydiae using the same techniques. As a general guide 960 cm² of HeLa cells were inoculated for each strain yielding some 8 to 17 mg purified chlamydiae. All tissue culture cells used for these experiments were routinely screened to ensure the absence of mycoplasma infection by the method of Russell *et al.* (1975).

Selective radiolabelling of chlamydial proteins with ³H-labelled amino acids. The procedure for large-scale growth of chlamydiae was modified when it was necessary to selectively label chlamydial polypeptides with ³H-labelled amino acids. Following chlamydial inoculation and adsorption as described above the MEM was replaced with Ham's F-10 medium (Flow Laboratories) containing 10% (v/v) foetal calf serum and 1 µg emetine hydrochloride ml⁻¹ and incubated for 20 h. The medium was then replaced with fresh F-10 medium containing 1 µg emetine hydrochloride ml⁻¹ and 15 µCi ³H-labelled amino acid mixture ml⁻¹ (TRK 440; The Radiochemical Centre, Amersham). Infected cells were then incubated for a further 20 to 30 h before harvesting in Hanks' BSS and homogenization as described above.

Radiolabelling chlamydial surface proteins with ¹²⁵I. The *C. trachomatis* strains of serotype C, E, G, H, L₂ and L₃ were bulk grown as described above and harvested in ice-cold phosphate buffered saline (PBS) pH 7.2.

After homogenization and low-speed centrifugation the protein concentration of a sample of the crude supernatant of mixed chlamydiae and host cell debris was rapidly determined using a micro-analytical protein assay (Bradford, 1976) with bovine gamma globulin as reference standard. Surface proteins in the crude supernatant were radiolabelled with ^{125}I by lactoperoxidase-catalysed oxidation using a modification of the method of Phillips & Morrison (1971). The suspension of impure chlamydiae was standardized to 8 mg protein ml^{-1} . To each 500 μl of suspension at 30 °C was added 50 μg lactoperoxidase (Sigma) in 50 μl PBS followed by 50 μCi carrier-free $^{125}\text{I}^-$ (The Radiochemical Centre) diluted to 50 μl in PBS. The reaction was started by the addition of 50 μl 0.01 M- H_2O_2 in PBS followed by further additions after 2.5, 5 and 7.5 min incubation at 30 °C. The reaction was terminated at 10 min by the addition of 4 ml 0.01 M-cysteine.HCl in PBS at 0 °C. The ^{125}I -surface-labelled chlamydiae were rapidly separated from labelled host cell material by Trisil density gradient centrifugation at 4 °C and their polypeptide composition was determined by SDS-polyacrylamide gel electrophoresis.

Chlamydial purification. Portions (2 ml) of impure chlamydial suspension were overlaid on a discontinuous gradient of 19 to 60% (w/v) Trisil 440 (Nyegaard & Co., Oslo, Norway). The gradient in each 16 ml centrifuge tube consisted of a base layer of 2 ml 60% (w/v) Trisil 440 overlaid successively with 3 ml, 3 ml and 2 ml of 37.5%, 25% and 19% (w/v) Trisil 440, respectively. After centrifugation at 100000 g for 45 min at 2 °C in an MSE 6 $\times$ 16 ml titanium swing-out rotor, chlamydial elementary bodies were harvested as a sharp band at the boundary between the 25% and 37.5% (w/v) Trisil, washed in PBS and frozen at -70 °C.

Polyacrylamide gel electrophoresis. Purified chlamydiae were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) on a separating gel composed of a linear polyacrylamide gradient (10 to 25%, w/v) essentially as described by Lambden & Heckels (1979). The apparent molecular weights of polypeptides were calculated by the method of Weber & Osborn (1969) using egg lysozyme, trypsin soy bean inhibitor, trypsin, ovalbumin, bovine serum albumin, β -galactosidase and ferritin as reference proteins.

Detection of radiolabelled polypeptides. Individual polypeptide bands from SDS-PAGE gels of ^{125}I -labelled chlamydiae were excised with a razor blade and the radioactivity due to ^{125}I was determined by gamma counting. ^3H -labelled polypeptides in SDS-PAGE gels were impregnated with scintillator and dried according to the method of Bonner & Laskey (1974). Kodak-RP-X-Omat film hypersensitized by a controlled flash of light (Laskey & Mills, 1975) was then exposed to the gel at -70 °C.

RESULTS

Polycation treatment of HeLa cells

Kuo *et al.* (1973) showed that HeLa cell susceptibility to infection with trachoma inclusion conjunctivitis (TRIC) strains of *C. trachomatis* was markedly increased when the cells were briefly washed with polycations. However, optimal conditions of polycation treatment were not determined for all TRIC serotypes. In an effort to maximize the yield for each chlamydial serotype the effect of treating HeLa cells with different concentrations of various polycations was investigated. Treatment with DEAE-dextran (mol. wt 5×10^5) stimulated HeLa cell susceptibility to infection with all TRIC strains of *C. trachomatis* but had no significant effect on infection with lymphogranuloma venereum (LGV) agents (Fig. 1). This stimulatory effect of DEAE-dextran was markedly concentration dependent. Table 1 shows the optimal concentration of DEAE-dextran or poly-L-lysine for each of the strains tested together with the resulting increase in chlamydial growth when compared with control (untreated) HeLa cells. Optimal sensitization of HeLa 229 cells to infection with chlamydiae of serotypes A, B or C was achieved with a lower concentration of DEAE-dextran (30 $\mu\text{g ml}^{-1}$) or poly-L-lysine (10 $\mu\text{g ml}^{-1}$) than was required for chlamydiae of serotypes D to K (45 or 15 $\mu\text{g ml}^{-1}$, respectively). At optimal concentrations, DEAE-dextran (mol. wt 5×10^5) caused a consistently greater increase in chlamydial inclusion count than the other polycations tested and was therefore used for large-scale chlamydial culture.

Effect of emetine

The growth of chlamydiae in HeLa cells was followed by monitoring the chlamydial-dependent incorporation of radioactive amino acids in the presence of emetine, an alkaloid which inhibits the 80S ribosome-dependent host cell protein synthesis without affecting 70S ribosome-dependent chlamydial protein synthesis (Becker & Asher, 1972). In the

Fig. 1. Effect of different concentrations of DEAE-dextran on the susceptibility of HeLa 229 cells to infection with *C. trachomatis*. Confluent monolayers of HeLa 229 cells on 13 mm diameter coverslips were washed twice with the appropriate concentration of DEAE-dextran (mol. wt 5×10^5) in Hanks' BSS. The treated cells were then inoculated with one of the chlamydial strains such that approximately 10% of untreated (control) cells developed inclusions. Following incubation for 48 h at 35.5 °C, chlamydial inclusions in 10 microscope fields were counted and the mean and standard deviation were plotted. Representative results are shown.

The optimal concentrations of DEAE-dextran for potentiating TRIC infection of HeLa 229 cells were 30 $\mu\text{g ml}^{-1}$ for serotypes A, B and C and 45 $\mu\text{g ml}^{-1}$ for serotypes D to K. DEAE-dextran had no effect on the infectivity of LGV strains (serotypes L₁, L₂ and L₃).

presence of emetine, only chlamydial-infected cells incorporated significant amounts of ^3H -labelled amino acids (Fig. 2). Other experiments demonstrated that this incorporation was totally abolished when the anti-chlamydial drug rifampicin at 1 $\mu\text{g ml}^{-1}$ was added to the tissue culture medium immediately following chlamydial adsorption. Maximal incorporation of ^3H occurred about 48 h post-inoculation for both TRIC and LGV strains of *C. trachomatis*, an unexpected finding as TRIC agents are generally accepted as having more extended growth cycles (72 h) than LGV strains. Thus, for large-scale growth of *C. trachomatis*, emetine hydrochloride at 1 $\mu\text{g ml}^{-1}$ was added both to shorten the growth cycle and to facilitate the selective radiolabelling of chlamydial proteins with radioactive amino acids.

Table 1. Optimal concentrations of polycations for potentiating HeLa 229 susceptibility to *C. trachomatis* serotypes A to K and their effect on the number of chlamydial inclusions

The susceptibility to infection with *C. trachomatis* of HeLa cells washed with different concentrations of polycation (dissolved in Hanks' BSS) was determined as described in the legend to Fig. 1. The results show, for each *C. trachomatis* strain, the optimal polycation concentration and the increase in the number of chlamydial inclusions in cells treated with the optimal concentration compared with control (untreated) cells.

<i>C. trachomatis</i> strain	DEAE-dextran (mol. wt 5×10^5)		DEAE-dextran (mol. wt 2×10^6)		Poly-L-lysine (mol. wt 3.4×10^3)	
	Optimal concn ($\mu\text{g ml}^{-1}$)	Chlamydial increase	Optimal concn ($\mu\text{g ml}^{-1}$)	Chlamydial increase	Optimal concn ($\mu\text{g ml}^{-1}$)	Chlamydial increase
A/SA-1/OT	30	$\times 7$	30	$\times 6$	10	$\times 3$
B/TW-5/OT	30	$\times 5$	30	$\times 5$	10	$\times 3$
C/UW-1/OT	30	$\times 10$	30	$\times 8$	10	$\times 5$
D/IC-Cal-8/ON	45	$\times 5$	45	$\times 4$	15	$\times 3$
E/DK-20/ON	45	$\times 4$	45	$\times 4$	15	$\times 2$
F/MRC-301/GU	45	$\times 4$	45	$\times 2$	15	$\times 2$
G/IOL-238/R	45	$\times 2$	45	$\times 1.5$	15	$\times 1.5$
H/UW-4/GC $\times$	45	$\times 3$	45	$\times 3$	15	$\times 2$
I/UW-12/GU	45	$\times 3$	45	$\times 3$	15	$\times 2$
J/UW-36/GC $\times$	45	$\times 3$	45	$\times 2.5$	15	$\times 2$
K/UW-31/GC $\times$	45	$\times 2$	45	$\times 2$	15	$\times 2$

Fig. 2. Growth curve of *C. trachomatis* UW-4 in HeLa 229 cells. Confluent monolayers of HeLa 229 cells were incubated for 2 h in MEM containing emetine hydrochloride ($1 \mu\text{g ml}^{-1}$), an inhibitor of eukaryotic cell protein synthesis. After washing with DEAE-dextran some of the cells were inoculated with *C. trachomatis* UW-4 (O) and others were left uninfected (●). Following chlamydial adsorption, MEM containing ^3H -labelled amino acid mixture plus emetine was added to each well. At appropriate times, cells were washed, lysed and the cumulative incorporation of ^3H -labelled amino acid was measured.

Maximum chlamydial-dependent amino acid incorporation was noted around 48 h post-adsorption. Beyond this time chlamydial-induced cell lysis caused a loss of ^3H from the cells. No significant amino acid incorporation was noted in uninfected cells in the presence of emetine. Thus, emetine facilitates the selective radiolabelling of chlamydial proteins with ^3H -labelled amino acids.

Chlamydial purity

Experiments were performed to assess the ability of Triosil density gradient centrifugation to separate chlamydial elementary bodies from host cell-derived material. Uninfected HeLa 229 cells were radiolabelled by overnight growth in tissue culture medium containing $1 \mu\text{Ci}$

ml⁻¹ each of [1-³H]glucose and ³H-labelled amino acid mixture. After washing, the radio-labelled cells were detached from the culture vessel and mixed with a suspension containing five times their number of non-radioactive HeLa cells which had been infected for 50 h with *C. trachomatis* UW-4. The cell mixture was then homogenized and the chlamydiae were separated by centrifugation on a discontinuous Triosil density gradient. Radioactivity attributable to host cell material contaminating the purified chlamydial elementary bodies was less than 2% of the total radioactivity applied to the gradient. Moreover, there was little loss of chlamydiae during separation. When chlamydiae were selectively radiolabelled with ³H-labelled amino acids and a homogenate of the infected cells was applied to the gradient, more than 90% of the total radioactivity was recovered in the band of purified chlamydial elementary bodies.

To confirm the purity of density gradient-separated chlamydiae, uninfected HeLa cells or HeLa cells infected with one of 14 different *C. trachomatis* strains were incubated in Ham's F-10 medium containing emetine and ³H-labelled amino acid mixture. After purification, the chlamydiae and the uninfected HeLa cells were denatured in SDS plus 2-mercaptoethanol

Fig. 3. Chlamydial polypeptides were selectively radiolabelled with ³H-labelled amino acids in the presence of emetine. The polypeptides were then separated by SDS-PAGE and a fluorogram was prepared from the gel. The figure shows a contact print prepared from the resulting fluorogram.

As expected, in the presence of emetine, only chlamydial polypeptides became radioactive and were able to form a fluorogram. Prominent in the fluorogram of the LGV strains (serotypes L₁, L₂ and L₃) was a polypeptide of 118 000 daltons (118K) not found in TRIC agents (serotypes A to K). The similarity in the polypeptide compositions of the purified chlamydiae revealed either by staining (Fig. 4) or selective radiolabelling and fluorography (Fig. 3) confirmed the absence of significant contaminating host cell polypeptide.

and subjected to electrophoresis on a pore-limiting gradient of 10 to 25% (w/v) polyacrylamide. Slab gels were deliberately overloaded, with 75 μ g protein per sample, to ensure that any significant host cell contamination would become apparent. Molecular weights of the Coomassie blue-stained host cell (HeLa) and chlamydial polypeptides were then compared. Inevitably, with the large number of polypeptides present, the molecular weights of some HeLa and chlamydial polypeptides were coincident. Nevertheless, the polypeptide compositions of chlamydiae and HeLa cells were clearly different and showed that the chlamydiae were free of significant host cell contamination. Confirmation of this came from a fluorogram of the ^3H -labelled polypeptides in the same gel (Fig. 3). In the presence of emetine most of the radioactive amino acid utilized by infected cells became incorporated into the chlamydiae (Fig. 2). Thus the HeLa cell polypeptides (control) did not produce a fluorogram whereas the selectively labelled chlamydial polypeptides did (Fig. 3). The pattern of the chlamydial polypeptides visualized by fluorography was very similar to the pattern obtained by Coomassie blue staining, again confirming the purity of the chlamydial preparations.

Fig. 4. Determination of the molecular weights of chlamydial polypeptides. Purified chlamydiae were treated with SDS and mercaptoethanol and the polypeptides were separated by electrophoresis on a linear gradient of 10 to 25% (w/v) polyacrylamide in the presence of SDS. To improve polypeptide resolution and facilitate molecular weight determination samples of only 25 μ g protein were applied to the gel. Molecular weights were determined by the method of Weber & Osborn (1969) and are expressed as kilodaltons (K). The position of the molecular weight markers is shown on the left hand side of the figure and the molecular weight of some important chlamydial polypeptides on the right.

Table 2. Comparison of the molecular weights of prominent polypeptides from 14 serotypes of *C. trachomatis*

Samples were denatured in 2% (w/v) SDS plus 5% (v/v) 2-mercaptoethanol (pH 6.8, 100 °C, 5 min), then electrophoresed in the presence of 2% (w/v) SDS on a gradient of 10 to 25% (w/v) polyacrylamide. Molecular weights were determined by the method of Weber & Osborn (1969). The major polypeptide for each strain is asterisked. All results are expressed in kilodaltons.

A	B	C	L ₁	L ₃	J	K	H	L ₂	D	E	F	G	I
155	155	155	155	155	155	155	155	155	155	155	155	155	155
			118	118				118					
116	116	116	116	116	116	116	116	116	116	116	116	116	116
73	73	73	73	73	73	73	73	73	73	73	73	73	73
70	70	70	70	70	70	70	70	70	70	70	70	70	70
61	61	61			61	61	61	61	61	61	61	61	61
60	60	60			60	60	60	60	60	60	60	60	60
56.5	56.5	56.5	56.5	56.5	56.5	56.5	56.5	56.5	56.5	56.5	56.5	56.5	56.5
45	45	45	45	45				45	45	45	45	45	45
43	43	43						43	43	43	43	43	43
			42*	42*				42	42	42	42	42	42
					40.5*	40.5*	40.5*						
39.5*	39.5*	39.5*			39.5	39.5	39.5						
								38*	38*	38*	38*	38*	38*
34	34	34	34	34	34	34	34	34	34	34	34	34	34
31	31	31	31	31	31	31	31	31	31	31	31	31	31
29	29	29	29	29	29	29	29	29	29	29	29	29	29

Fig. 5. Surface proteins of *C. trachomatis* strain UW-4. Chlamydiae were grown in HeLa 229 cells, harvested and the mixture of chlamydiae and host cell materials was surface-labelled with ^{125}I by lactoperoxidase-catalysed oxidation. The labelled chlamydiae were separated from labelled host cell components by Triosil density gradient centrifugation and the purified organisms were denatured at 100 °C for 5 min in 2% (w/v) SDS plus 5% (v/v) 2-mercaptoethanol. Chlamydial polypeptides were separated by SDS-PAGE on a linear gradient of 10 to 25% (w/v) polyacrylamide, individual polypeptide bands were excised from the gel and their radioactivity determined. The graph shows the radioactivity of individual polypeptides versus their molecular weight. Three polypeptides of 155, 40 and 29 kilodaltons (K) became radiolabelled and were thus exposed at the chlamydial surface.

Polypeptide composition

To permit the accurate determination of molecular weights, SDS-treated HeLa cells and chlamydiae were electrophoresed using a reduced load of 25 µg protein per sample and the separated polypeptides were stained with Coomassie blue (Fig. 4). The mean molecular weights of some prominent polypeptides derived from observations of several different

polyacrylamide gels of the same composition are summarized in Table 2. The major polypeptide varied between 38 000 and 42 000 daltons, depending on the individual strain. LGV isolates of *C. trachomatis* (serotypes L₁, L₂ and L₃) possessed a further important polypeptide of 118 000 daltons which was readily detected by fluorography (Fig. 3) but was much less prominent in Coomassie blue-stained preparations. The reason for this is not clear but may reflect a requirement for extensive *de novo* synthesis of this protein during the chlamydial growth cycle resulting in its labelling to very high specific activity with the ³H-labelled amino acid mixture.

Chlamydial surface proteins

Preliminary surface-labelling experiments with ¹²⁵I were performed on six different serotypes of *C. trachomatis* and a typical result (strain UW-4) is shown in Fig. 5. After separation of the chlamydial polypeptides by SDS-PAGE and determination of their radioactivity, three major peaks of radioactivity were observed for all strains, demonstrating that the major polypeptide (40 000 daltons for UW-4 in Fig. 5) and the polypeptides of 155 000 and 29 000 daltons were located at the surface of the chlamydial elementary body.

DISCUSSION

The data presented in this report show the feasibility of the large-scale growth and purification of TRIC agents once chlamydial yields are maximized by the use of optimal concentrations of DEAE-dextran. By this means, 14 of the 15 internationally recognized serotypes of *C. trachomatis* were grown in milligram amounts, the only serotype not studied being Ba which has largely been restricted to trachoma in Red Indian populations of North America. The use of tissue culture cells rather than the embryonated chicken yolk sac as the starting point for chlamydial growth facilitated chlamydial purification by a simple procedure involving centrifugation on a discontinuous Triosil density gradient. The suitability of the technique for chlamydial purification was demonstrated by its ability to separate radiolabelled host cell material from non-labelled chlamydial elementary bodies and by the finding that if a mixture of non-labelled host cells and radiolabelled chlamydiae was applied to the gradient, more than 90% of the radioactivity was recovered with the band of purified chlamydiae. Emetine played a crucial role in studies of chlamydial purity enabling the selective radiolabelling of chlamydial polypeptides with ³H-labelled amino acids. The different polypeptide composition on SDS-PAGE of purified chlamydiae compared with host cells, the absence of marker HeLa cell polypeptides from the chlamydial preparations, the finding that the observed polypeptides were all radioactive if the chlamydiae had first been selectively radiolabelled with ³H-labelled amino acids in the presence of emetine, all confirmed that preparations of each chlamydial strain were sufficiently pure for detailed analysis. Electron microscopic observations showed that the purified chlamydiae studied consisted almost entirely of elementary bodies.

The genome of *C. trachomatis* at 11×10^5 nucleotide pairs (Sarov & Becker, 1969) has the capacity to produce large numbers of polypeptides, making detailed comparisons between serotypes difficult. Resolution of the complex chlamydial polypeptides was greatly enhanced by the use of pore-limiting gel electrophoresis with its improved zone sharpening effect compared with non-gradient electrophoretic techniques. Comparison of the polypeptide compositions of each strain revealed a major polypeptide of between 38 000 and 42 000 daltons depending on the serotype. Interestingly, the distribution of this major polypeptide according to molecular weight correlated closely with the grouping of chlamydiae on the basis of the type of human infection produced. Thus, serotypes A B C with a major polypeptide of 39 500 daltons are responsible for severe ocular infection in third world countries (hyper-endemic trachoma); serotypes D E F G I with a major polypeptide of 38 000 daltons cause most of the cases of genital tract or mild ocular (para trachoma)

infection in the developed world with serotypes H J K a less common cause of similar infections. Chlamydiae of serotypes L₁, L₂ and L₃ all cause lymphogranuloma venereum, a more invasive form of chlamydial genital tract infection characterized by marked lymphoid pathology. Serotypes L₁ and L₃ were closely related to each other by their unique polypeptide composition and a common major polypeptide of 42 000 daltons. L₂ was related to L₁ and L₃ by the joint possession of a unique polypeptide of 118 000 daltons, prominent on fluorography and not found in other serotypes. However, the major polypeptide of L₂ at 38 000 daltons has the same molecular weight as that of the para trachoma D E F G I group. On this basis L₂ organisms might be intermediates between the TRIC and LGV agents. Ideally, if differences in polypeptide composition are to be ascribed to individual serotypes it is desirable to test more than one strain per serotype. However, the fact that the strains selected were recognized 'type' strains, together with the close similarities in the polypeptide composition of related serotypes, suggests there will be no significant differences in the polypeptide composition of strains within the same serotype.

The surface of the chlamydial elementary body must play a crucial role in chlamydial attachment to the host cell and provides a major target for attack by host immune defences. An attempt was therefore made to identify the chlamydial surface polypeptides by radio-labelling with ¹²⁵I using lactoperoxidase-catalysed oxidation. A major consideration was to prevent chlamydial lysis during processing which would have resulted in the labelling of internal, cytoplasmic proteins. To minimize lysis, the chlamydiae were radiolabelled as rapidly as possible after their release from the host cell and prior to density gradient purification. The six strains of *C. trachomatis* for surface labelling were selected as being representative of each of the major groups of polypeptide composition revealed by SDS-PAGE. In all these strains the major polypeptide and the polypeptides of 155 000 and 29 000 daltons were definitely identified as surface polypeptides. Unfortunately, as the proportion of chlamydiae present in the crude mixture being radiolabelled was unknown and the degree of radiolabelling was low, the specific activity of each polypeptide could not be determined accurately. Nevertheless, it was clear that both the 155 000 and 29 000 dalton polypeptides were labelled to higher specific activity than the major polypeptide. This could reflect either a greater surface accessibility of these polypeptides or a greater abundance of tyrosine in their amino acid composition. In the LGV strains some polypeptides in the 118 000 dalton region were also radiolabelled. Further studies with more intense labelling are necessary to establish the reproducibility of surface labelling in regions other than the three major regions described.

There are no reports in the literature on the detailed polypeptide composition of the different serotypes of *C. trachomatis*. A species-specific antigen of 155 000 daltons was identified by Caldwell *et al.* (1975) in a Triton X-100 extract of the same LGV agent of serotype L₂ as was used in this study. This antigen is probably identical to the 155 000 dalton surface polypeptide common to all serotypes described here. The 118 000 dalton LGV-specific polypeptide and the major polypeptide of between 38 000 and 42 000 daltons have not been described before. In view of the abundance and surface location of the major polypeptide it is suggested that this may be the principal outer membrane protein of the elementary body; the similarity but not identity of its molecular weights in different serotypes might be attributed to minor modifications of the same protein. The role of the major and LGV-specific polypeptides in the pathogenesis of chlamydial infections and in the hypersensitivity reactions to trachoma vaccines should be determined. The 29 000 dalton surface polypeptide is probably identical to the type-specific polypeptide of 30 000 daltons purified by Hourihan *et al.* (1980) in nanogram amounts from alkali/Triton X-100 extracted lysates of BHK21 cells infected with trachoma agents of serotypes A, B or C. It is to be hoped that more detailed knowledge of the immunochemistry of chlamydial surface antigens will provide a better understanding of both the pathogenesis of chlamydial infections and the prospects for prophylactic immunization.

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