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Expression of *Chlamydia trachomatis* Inclusion Membrane Protein Genes *IncB* and *IncC* in *Escherichia coli*

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Abstract—In this study, we have cloned the *Chlamydia trachomatis* genes *incB* and *incC* into the expression plasmid vectors from pET series for the subsequent isolation of recombinant proteins. As a result, we have obtained the first full-length recombinant *C. trachomatis* proteins IncB and IncC, which can be used for following antibody production and for study of their protein–protein interaction.

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Chlamydia trachomatis is an obligate intracellular parasite causing human diseases, such as trachoma, lymphogranuloma inguinale, and urogenital chlamydiasis (chlamydia). *Chlamydia trachomatis* is now recognized as the most widespread sexually transmitted bacterial agent [1]. Chlamydiae are especially dangerous for the reproductive health of women, because they are involved in development of chronic inflammatory diseases of the small pelvis, which can lead to tubal occlusion, ectopic pregnancy, and infertility [2].

Chlamydiae are perfectly adapted to living conditions inside the eukaryotic cell due to their unique biphasic life cycle. It is an interchange of two metabolic states, infective and vegetative, represented by small spore-like *elementary bodies* and large reproducing *reticulate bodies*, respectively [3]. Chlamydiae are characterized by a specific type of relationship with the host cell, which is considered as absolute energy-dependent parasitism [4]. The bacteria actively use ATP and other high-energy compounds of the eukaryotic cell and compete with it for nutrients, vitamins, and cofactors. Doing so, chlamydiae inhibit, but maintain a certain level of host vitality until completion of their life cycle. One of mechanisms allowing them to "slip away" from the control of eukaryotic cell is the ability of chlamydiae to inhibit apoptotic processes [5, 6].

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The intracellular stage of chlamydial development occurs inside a specific membranous vacuole called an inclusion. It is supposed that the interaction of chlamydiae with the host cell might involve their own unique proteins localized in the inclusion membrane and called Inc-proteins (inclusion proteins) [7]. Inc-proteins are found in virtually all chlamydial species, and their homologs are absent in all other studied organisms. These proteins differ from each other in primary amino acid sequence, but all of them contain a bi-lobed hydrophobic domain composed of about 50-80 amino acid residues, which likely determines localization of these proteins in the inclusion membrane [8]. Moreover, a significant number of open reading frames are found in genomes of chlamydiae that encode proteins with analogous hydrophobic profiles and are absent in all other known genomes [9]. At present, some biological properties are known only for two of them: IncA participates in formation of sole inclusion in multiple chlamydial infections [10] and IncG interacts with the eukaryotic protein 14-3-3β [11]. Functions of other Inc-proteins are still unclear. However, the fact that their hydrophilic domains are localized on the outer (plasmatic) surface of the inclusion membrane and are phosphorylated by host kinases and that expression of genes encoding some Inc-proteins begins within the first half hour after infection of cell culture suggests that they might play a role of mediators in interaction of chlamydia with the eukaryotic cell [7].

Data on the association of Inc-proteins of *C. trachomatis* with proteins and organelles of the infected cell are now of greatest interest. The absence of systems for genetic transformation of chlamydiae considerably impedes studies of the structure and functions of inclusion membrane proteins. Moreover, expression of their genes in heterologous systems is complicated by insolubility of Inc-proteins in cytoplasm and their toxicity for the host cell. Therefore, only hydrophilic recombinant fragments of these proteins have been used so far for genesis of antibodies [12, 13] and analysis of possible interconnection with the secretion system type III [14]. Construction of full-length recombinant Inc-proteins might allow discovery of their partners and lead to better understanding of pathogenesis of chlamydial infection.

We choose two members of the inclusion membrane protein family, IncB and IncC, as objects for this study. Homologs of these proteins are found in other chlamydiae, such as *C. pneumoniae* [15], *C. psittaci* [12], *C. muridarum* [16], and *C. abortus* [17]. The genes *incB* and *incC* are localized in the locus of replication origin both in *C. trachomatis* and *C. pneumoniae* [8], which is a distinctive feature of proteins involved in the most important metabolic processes of the cell, such as replication, transcription, and translation. IncB and IncC are the proteins of the early phase of infection, whose gene expression begins within a half hour after infection of the host cell [18, 19] and is simultaneous with important processes such as formation of an inclusion, its transportation into the perinuclear space, and avoidance of fusion with early lysosomes [7]. These features allow us to suggest that IncB and IncC might play a significant role at early stages of chlamydial infection development and be necessary elements in processes of inclusion formation.

MATERIALS AND METHODS

Bacterial strains and plasmid vectors. The following strains were used: *Escherichia coli* DH5α (Life Technologies, UK) and B834 (DE3) and BL21 TrxB (DE3) [pLysS] (Novagen, USA). The reference strain *C. trachomatis* D/UW-3/Cx (ATCC VR-885) was kindly provided by Dr. Eva Hjelm (Uppsala Universitet, Sweden). Clinical isolates of *C. trachomatis* were obtained from cervical mucosa scrapings of patients from the Ott Institute of Obstetrics and Gynecology, Russian Academy of Medical Sciences (St. Petersburg, Russia) in 1998-1999.

The plasmid vectors pGEM-T/easy (Promega, USA), pET-15b and pET-32a(+) (Novagen) were used for cloning and expression.

Evaluation of genetic polymorphism of genes *incB* and *incC*. Genes *incB* and *incC* were amplified by PCR on an Abbott LCX Probe System programmed thermal cycler (UK) using specific primers and Pfu-polymerase (MBI Fermentas, Lithuania) according to the manufacturer's protocol.

Nucleotide sequences of genes *incB* and *incC* were determined using the Big DyeTM Terminator v.3.0 cycle sequencing kit (Applied Biosystems, USA). The reaction mixture provided by the manufacturer (Terminator Ready Reaction Mix) contained 10 ng of plasmid or genomic DNA and 3.2 pM of specific primer in the total volume of 20 μl.

Reaction products were analyzed on an ABI Prism 3100 Genetic Analyzer automated sequencer (Applied Biosystems, USA; Hitachi, Japan).

Nucleotide sequences of genes *incB* and *incC* were compared using the Vector NTI software (InforMax Inc, USA).

Cloning of full-length genes *incB* and *incC*. The full-length genes *incB* and *incC* were amplified using specific primers (table) as described above. Amplification products were purified using the Wizard PCR Preps DNA Purification System kit (Promega, USA) and cloned in a plasmid pGEM-T/easy (Promega) according to the manufacturer's protocol. Subsequently, the Inc-protein genes were sub-cloned in the plasmid vectors pET-15b and pET-32a according the standard protocols [20] using restriction endonucleases (table) and T4 DNA-ligase (MBI Fermentas).

***Escherichia coli* cell transformation with plasmid DNA.** Competent cells from the *E. coli* strains DH5α, B834 (DE3), and BL21 TrxB (DE3) [pLysS] were prepared and transformed with recombinant plasmid DNA by the method of Hanahan [21]. Recombinant clones were selected by screening using isopropyl-β-D-thiogalactoside (IPTG) and X-gal and by contra-selection on media with antibiotics (either 50 μg/ml of ampicillin or 50 μg/ml of ampicillin, 34 μg/ml of chloramphenicol, and 15 μg/ml of kanamycin for strains B834 and BL21 TrxB (DE3) [pLysS], respectively).

Induction of expression of genes *incB* and *incC* in *E. coli* cells. The culture of *E. coli* B834 (DE3) or BL21 (DE3) [pLysS] TrxB was transformed by the plasmid constructs pET-15b/IncB, pET-15b/IncC, pET-32a/IncB, or pET-32a/IncC (Fig. 1). Several recombinant colonies were inoculated into 100 ml of the TB medium (1.2% bactotryptone, 2.4% yeast extract, 4% glycerol, 17 mM KH_2PO_4, and 72 mM K_2HPO_4) containing either 50 μg/ml of ampicillin or 50 μg/ml of ampicillin, 34 μg/ml of chloramphenicol, and 15 μg/ml of kanamycin for strains B834 [pLysS] and BL21 TrxB (DE3) [pLysS], respectively, and grown at 37°C with continuous agitation until culture $OD_{600} = 1.2$. Expression of the target protein gene was induced by isopropyl-β-D-thiogalactoside (final concentration 0.1 mM). The culture was incubated overnight at 30°C with continuous agitation, centrifuged at 6000*g* for 30 min, and the pellets were frozen at −75°C. The presence of the target protein was examined by denaturing electrophoresis in 12-15%

Primers for cloning of full-length genes *incB* and *incC* in plasmid vectors pET-15b and pET-32a

Gene	Primer type	Sequence (5'-3')	Restriction site
		pET-15b	
incB	forward	**catatg**gttcattctgtatacaattc	NdeI
	reverse	**ggatcc**cctattcttgaggttttgtttg	BamHI
incC	forward	**catatg**acgtactctatatccgat	NdeI
	reverse	**ctcgag**ttagcttacatataaagtttg	XhoI
		pET-32a	
incB	forward	**ccatgg**ctatggttcattctgtatacaattc	NcoI
	reverse	**ggatcc**ttattcttgaggttttttgttg	BamHI
incC	forward	**ccatgg**ctatgacgtactctatatccgat	NcoI
	reverse	**gaattc**ttagcttacatataaagtttgag	EcoRI

Note: Restriction sites are indicated in bold.

polyacrylamide gel according to the standard protocol [20].

Mass-spectrometry of recombinant proteins TrxA-IncB and TrxA-IncC. To prepare the protein samples for mass-spectrometric identification, the bands of interest were cut out of the gel and disintegrated to fragments about 1 × 1 mm in size. The fragments were placed into tubes (one or two fragments in each) and incubated for 15 min at room temperature in 150 µl of the mixture of 40% methanol and 5% acetic acid. The supernatant was removed, and 150 µl H_2O was added to the pellet with following incubation for 5 min at room temperature. After removal of the supernatant, 150 µl of 50% acetonitrile in 50 mM NH_4HCO_3 was added to the pellet and incubated for 20 min at 56°C for removal of dye. If necessary, this step was repeated. Then 150 µl of acetonitrile was added to the pellet and incubated for 10 min at room temperature followed by desiccation for 30 min in vacuum desiccator. Dry gel fragments were trypsinized in the following solution: 10 ng/µl trypsin, 20 mM NH_4HCO_3, and 50 µM dithiothreitol, 1-1.5 µl per gel fragment. After incubation for 10 min at 5°C, the same volume (1-1.5 µl per gel fragment) of H_2O was added to the mixture and incubated for 15 h at 37°C. The supernatant was sampled, dried for 30 min at 56°C, resuspended in 0.1% trifluoroacetic acid, and incubated for 15 min at room temperature. Equal volumes (0.5 µl) of the suspended supernatant and 2,5-dihydroxybenzoate (20 mg/ml) solution in acetonitrile (20%) with trifluoroacetate (0.1%) were mixed on the target and air-dried. Mass-spectrometry was accomplished using a MALDI-TOF Reflex IV apparatus (Bruker Daltonics, Germany), UV-laser, 336 nm, in the mode of positive ions, with the reflector.

Isolation of recombinant proteins IncB, IncC, TrxA-IncB, and TrxA-IncC from *E. coli* cells. Pellets of *E. coli* cultures were thawed at 0°C and suspended in 10 ml of specific buffer (20 mM Na_3PO_4, 20% glycerol, 0.5 M NaCl, and 10 mM imidazole). The mixture was ultrasonicated (22 kHz, 15 × 10 sec) to homogeneity. Then 0.5% Emulgen 913 was added, and the mixture was incubated for 30 min on ice with agitation and centrifuged for 1.5 h at 45,000*g*. The recombinant protein was determined in the pellet and supernatant by denaturing electrophoresis in 12-15% polyacrylamide gel according to the standard protocol [20].

To isolate the recombinant protein from the soluble fraction of cell lysate, the pellets of *E. coli* cultures were suspended in 10 ml of the buffer (20 mM Na_3PO_4, 20% glycerol, 0.5 M NaCl, and 10 mM imidazole) and centrifuged for 1.5 h at 45,000*g*.

To isolate the recombinant protein from the insoluble fraction of cell lysate, the pellets of *E. coli* cultures were suspended in 10 ml of the buffer (20 mM Na_3PO_4, 0.5 M NaCl, 10 mM imidazole, and 8 M urea) and centrifuged for 1.5 h at 45,000*g*.

The protein was isolated by metal chelate affinity chromatography on a HisTrap™ Kit column (Amersham Biosciences AB, Sweden) according to the manufacturer's protocol.

Fission of fusion proteins TrxA-IncB and TrxA-IncC. Hydrolysis of the fusion proteins was conducted at 4°C for 22 h. The reaction mixture contained 20 mM sodium phosphate buffer, 0.5 M NaCl, 20% glycerol, 1 mM $CaCl_2$, 2 mg fusion protein, and 2 U of human recombinant enteropeptidase light chain (Invitrogen, USA). Peptides produced were separated on a HisTrap™ Kit column (Amersham Biosciences AB) according to the manufacturer's protocol.

RESULTS

Detection of genetic polymorphism of genes *incB* and *incC*. To reveal genetic polymorphism, we determined

nucleotide sequences of genes *incB* and *incC* in eight clinical isolates of *C. trachomatis*. Two of them belonged to serovar B, three to serovar E, and two to serovar G; the serovar of the eighth strain was unknown. A comparison of the determined sequences demonstrated a conservativeness of *C. trachomatis* proteins IncB and IncC among different clinical isolates and absence of amino acid substitutions in comparison with the same proteins from the laboratory strain D/UW-S/Cx and sequences published in GeneBank (AE001296). Three insignificant nucleotide substitutions 366A→G, 390C→T, and 477T→C, which were not associated with serovar and sensitivity to antibiotics, were found in one of the clinical strains (serovar E).

Cloning of genes *incB* and *incC* in plasmid vector pET-15b and expression in *E. coli* cells. The full-length genes *incB* and *incC* were cloned in the plasmid vector pET-15b for synthesis of fusion proteins with a sequence of six histidine residues. Thus prepared constructs called pET-15b/IncB and pET-15b/IncC (Fig. 1) and the initial vector were used for transformation of *E. coli* strain B834 (DE3). Denaturing PAGE of lysates from the cells carrying the recombinant plasmids demonstrated the presence of protein fractions with molecular masses corresponding to that predicted for the recombinant His-IncB and His-IncC (15.1 and 22.1 kD, respectively). Similar fractions were not found in lysates of the cells carrying the initial plasmid vector. These fractions appeared in lysates of cells transformed with the construct pET-15b/IncC after addition of IPTG, achieved maximum concentration 2 h after induction, and virtually disappeared in overnight culture. The maximum level of His-IncB in lysates of the cells transformed with the construct pET-15b/IncB was achieved for overnight culture.

Cloning of genes *incB* and *incC* in plasmid vector pET-32a(+) and expression in *E. coli* cells. The full-length genes *incB* and *incC* were cloned in the plasmid vector pET-32a(+) for synthesis of fusion proteins with thioredoxin A (TrxA). These constructs, called pET-32a/IncB and pET-32a/IncC (Fig. 1), and the initial vector were used for transformation of *E. coli* strain BL21 TrxB (DE3) [pLysS]. After induction of expression under the above conditions the denaturing PAGE of lysates from the cells carrying the recombinant plasmids demonstrated the

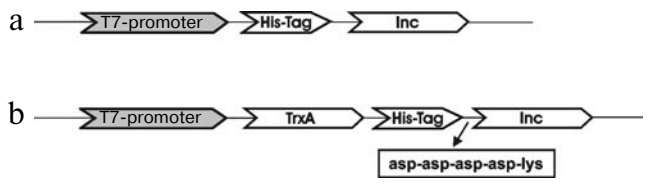


Fig. 1. Schemes of recombinant constructs for expression of genes *incB* and *incC* based on vectors pET-15b (a) and pET-32a(+) (b). The arrow indicates the hydrolysis site for human enteropeptidase.

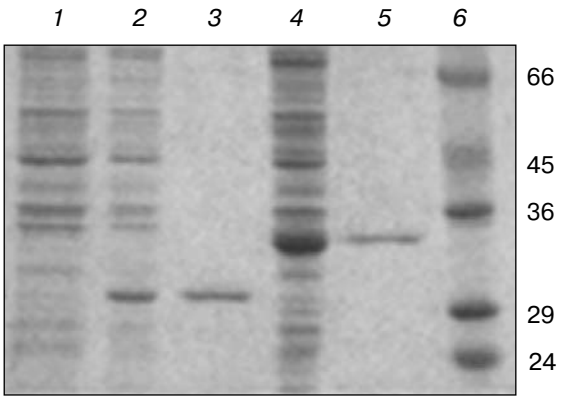


Fig. 2. Electrophoretic monitoring of purification of the fusion proteins TrxA-IncB and TrxA-IncC: *1*) lysate of *E. coli* cells transformed with the recombinant vector pET-32a(+); *2*) lysate of *E. coli* cells transformed with the vector pET32-IncB, induction with 0.1 mM IPTG; *3*) recombinant protein TrxA-IncB purified by affinity chromatography on Ni^{2+}-diacetate agarose; *4*) lysate of *E. coli* cells transformed with the vector pET32-IncC, induction with 0.1 mM IPTG; *5*) recombinant protein TrxA-IncC purified by affinity chromatography on Ni^{2+}-diacetate agarose; *6*) molecular mass marker, kD.

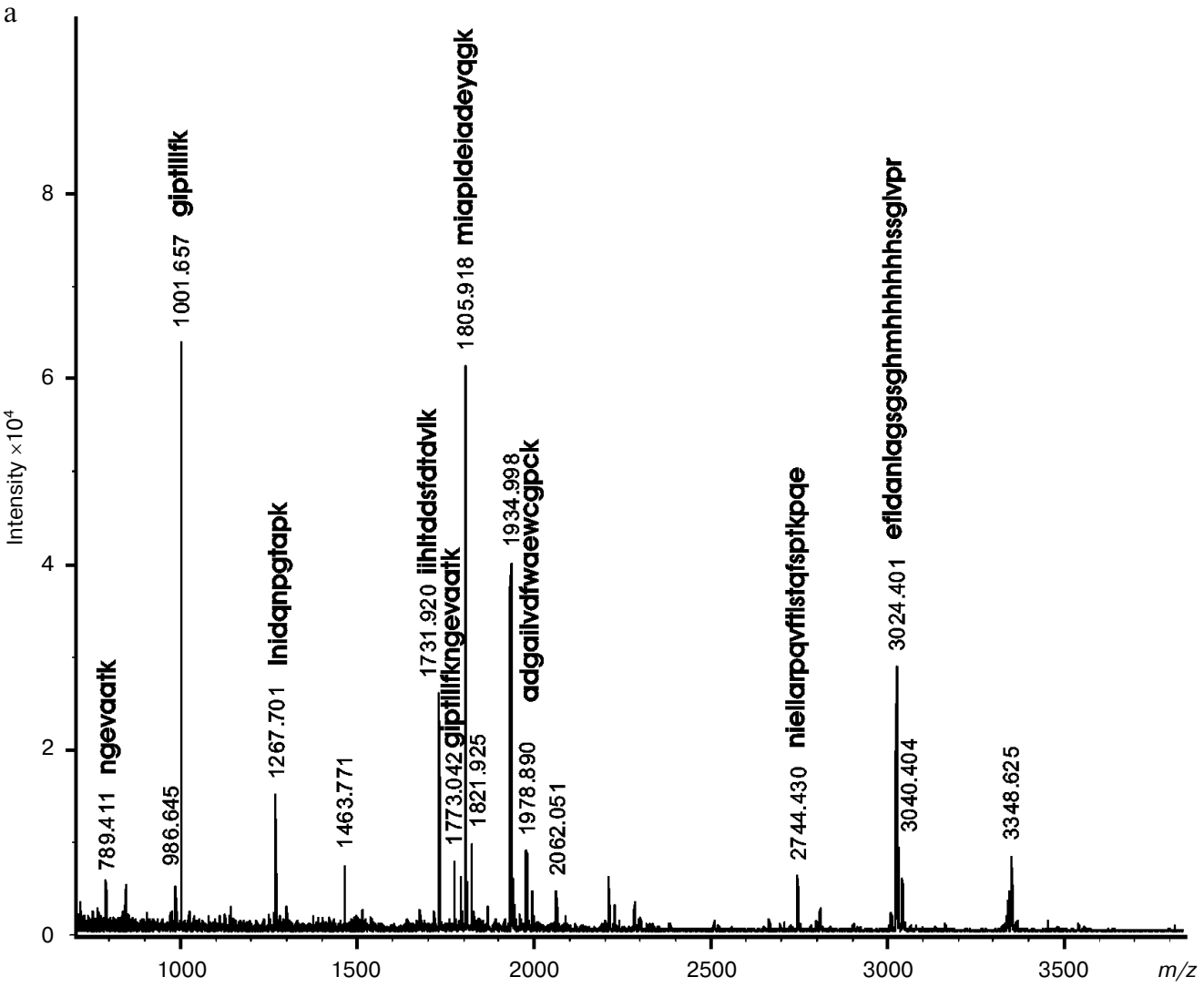
presence of protein fractions with molecular masses corresponding to those predicted for the fusion proteins TrxA-IncB and TrxA-IncC (30.6 and 37.1 kD, respectively); these fractions were not found in lysates of the cells carrying the initial plasmid vector (Fig. 2). The maximum level of the recombinant Inc-proteins was observed in lysates of overnight *E. coli* cell culture. The conformity between these fractions in polyacrylamide gel and the target fusion proteins was confirmed by mass-spectrometric analysis after pretreatment with trypsin, which demonstrated the presence of protein fragments with polypeptide chains identical to amino acid sequence fragments of TrxA-IncB and TrxA-IncC (Fig. 3).

Isolation of recombinant proteins IncB, IncC, TrxA-IncB, and TrxA-IncC from *E. coli* cells. Isolation and purification were performed on HisTrap™ Kit columns (Amersham Biosciences AB) with preliminary testing of solubility of recombinant proteins in the application buffer recommended by the manufacturer. According to the results of fractioning, the recombinant proteins were isolated from 2.5-h cultures of *E. coli* containing TrxA-IncB and TrxA-IncC in the buffer without 8 M urea, whereas from 2.5-h culture of *E. coli* containing His-IncC and from the overnight culture of *E. coli* containing His-IncB were isolated in buffer with 8 M urea.

Purification yielded electrophoretically pure fusion proteins TrxA-IncB and TrxA-IncC (Fig. 2); the yield of the recombinant proteins in 2.5-h *E. coli* cultures was 6 and 3 mg/liter for TrxA-IncB and TrxA-IncC, respectively.

Separation of fusion proteins TrxA-IncB and TrxA-IncC. The purified fusion proteins were hydrolyzed with

a



b

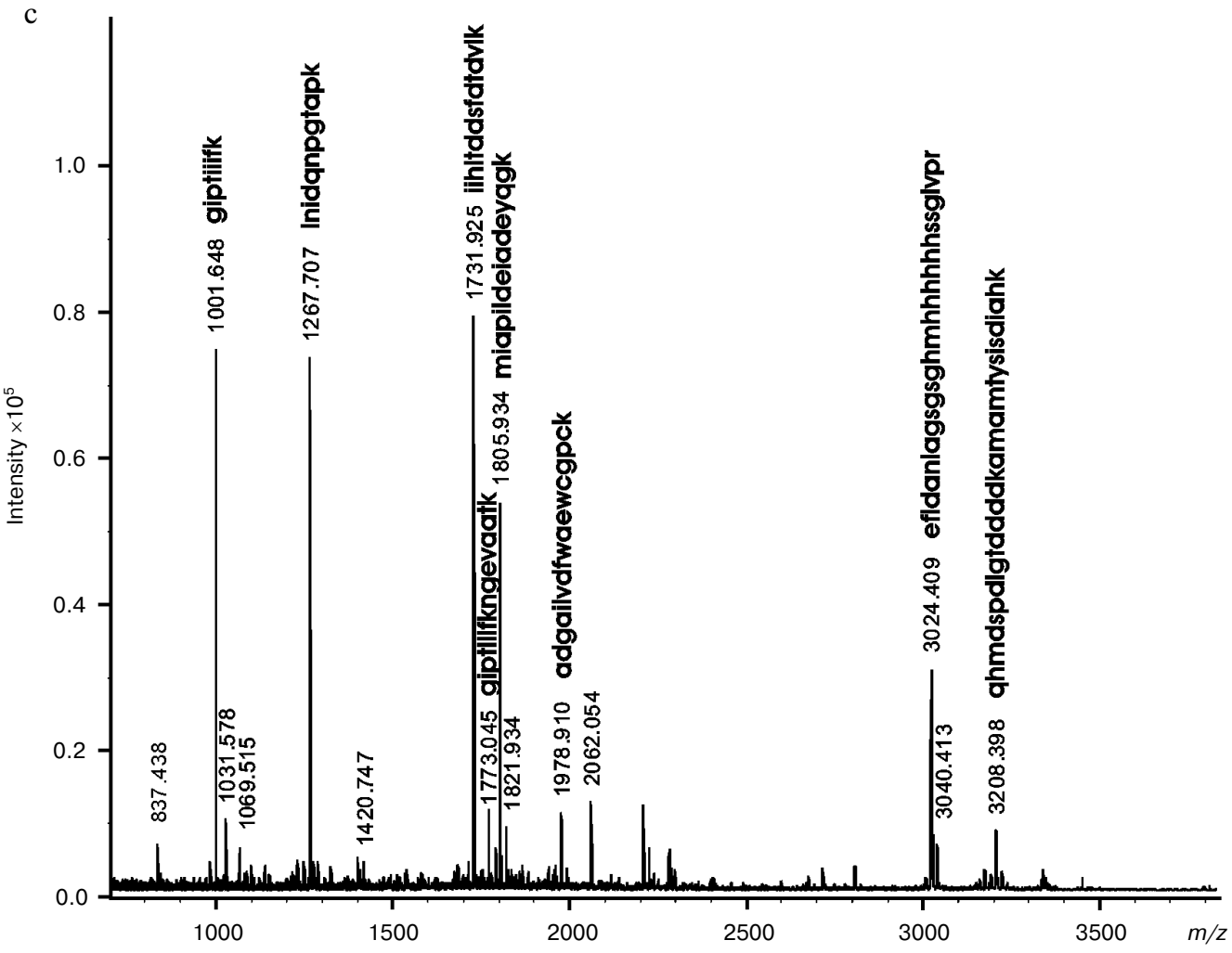
MSDK**IIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGK**LTVAK**LNIDQNPGTAPK**YGIR**GIPTLL**
LFKNGEVAATKVGALSKGQLK**EFLDANLAGSGSGHMHHHHHHSSGLVPR**GSGMKETAAAKFERQHMDSPDLGTDDDDKAM
AMVHSVYNSLAPEGFSQVSIQPSQIPTSKKVMIAIMTLFALTAIAAIVLSIVTVCGGFPFLLAALNTVTIGACVSLPVFT
CIATTLLLLCLR**NIELLARPQVFTLSTQFSPTKPQE***

Fig. 3. Mass-spectrometric analysis of trypsinolytic fragments of the recombinant proteins TrxA-IncB (a, b) and TrxA-IncC (c, d). a, c) Mass-spectra of trypsinolytic fragments; b, d) amino acid sequences of the proteins TrxA-IncB and TrxA-IncC, respectively; the marked fragments are those identified by mass-spectrometry.

human recombinant enteropeptidase light chain (Fig. 4) to prepare the mixtures of Inc-protein (IncB, 12.88 kD or IncC, 19.87 kD) and thioredoxin A (17.43 kD) linked with a sequence of six histidines (Fig. 1). The final purification of recombinant Inc-proteins was performed by the method of metal chelate affinity chromatography: thioredoxin A bound with the resin, whereas Inc-protein was eluted with the application buffer.

DISCUSSION

In the first step of this work, we studied Inc-protein polymorphism among the clinical isolates of *C. trachomatis*. No nucleotide substitutions leading to amino acid substitutions were found for both IncB and IncC. A comparison of IncB and IncC sequences from the *C. pneumoniae* strains AR39, CWL029, J138, and TW-183 pub-



d

MSDK**IIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGK**LTVAK**LNIDQNPGTAPK**YGIR**GIPTLL**
LFKNGEVAATKVGALSKGQLK**EFLDANLAGSGSGHMHHHHHHSSGLVPR**GSGMKETAAAKFER**QHMDSPDLGTDDDDKAM**
AMTYSISDIAHKSDISNPTSPAPSRKRGSFPPQSPSAVGSLEGANFSTWGPGPFFTVPVYPQQLAAMQNNLFTLQTEVSA
LKKKLVQSSQTRGSLGLGPQFLAACLVAATILAVAVIVLASLGLGGVLPFVLVCLAGSTNAIWAIVSASITTLICCVSIA
CIFLAKCDKGSDPQTLYVS*

Fig. 3 (Contd.).

lished in GeneBank (AAF38304, AAD18440, BAA9850, AAP98233, NP 876577, BAA502, and H7 2095) also demonstrated a conservativeness of these proteins within the given species. At the same time, we found a significant number of amino acid substitutions in other inclusion membrane proteins, such as IncA, IncD, IncE, IncF, and IncG (data not shown). According to the data from the literature, some mutations in the *incA* gene lead to formation of multiple inclusions in *C. trachomatis*, but this mechanism is not unique [22]. The data might suggest great significance of IncB and IncC for physiology of chlamydiae, because conservativeness is particularly characteristic of proteins involved in crucial cellular processes.

Down to recent times, all work on production of recombinant Inc-proteins were restricted to preparation of either their hydrophilic domains or composite proteins, because full-length Inc-proteins were insoluble or toxic for the host cell when expressed endogenously. In particular, the recombinant peptide composed of 101 amino acid residues was used for raising antibodies against *C. psittaci* IncB [12]. To raise antibodies to *C. trachomatis* IncA and IncG the carboxy-terminal fragments of these proteins were used, to raise antibodies to IncD its amino-terminal

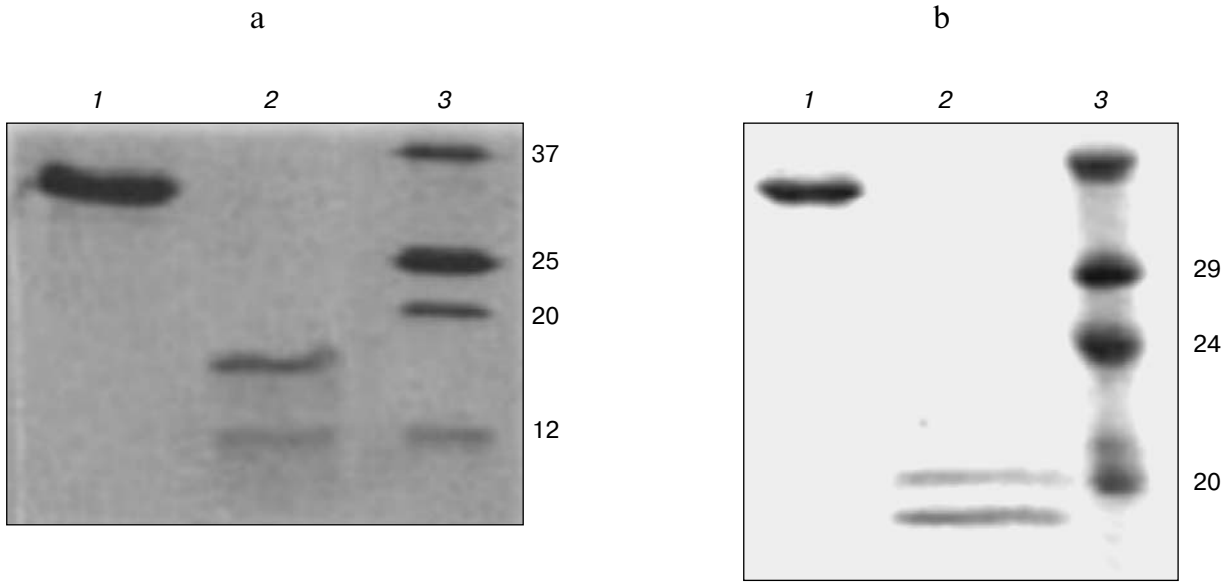


Fig. 4. Electrophoretic analysis of fusion proteins TrxA-IncB and TrxA-IncC hydrolyzed with human recombinant enteropeptidase light chain (a and b, respectively). *1*) Fusion protein; *2*) hydrolytic products; *3*) molecular mass marker, kD.

fragment was used, and synthetic fragments of IncE and IncF were used; none of these peptides contained the hydrophobic fragment characteristic of Inc-proteins [13]. To raise antibodies for localization of predicted Inc-like proteins of *C. trachomatis* and *C. pneumoniae*, the fusions with maltose-binding protein gene were used, and, in several cases, a fragment of Inc-protein gene was built in these fusions, because the constructs containing the full-length gene were toxic [8]. The hydrophilic amino-terminal domains of Inc-proteins were also used to test the possibility of secretion of *C. trachomatis* inclusion membrane proteins by the *Shigella flexneri* type III system [14].

In the study presented here, we aimed to prepare the full-length recombinant proteins IncB and IncC. Initially, we chose the plasmid vector pET-15b to prepare a construct expressing the *C. trachomatis* genes encoding the inclusion membrane proteins IncB and IncC in *E. coli* (Fig. 1). This plasmid allows cloning of the target gene under the control of the inducible promoter of phage T7 for production of fusion protein with amino-terminal sequence contains six histidine residues. The presence of this peptide in the hybrid construct allows fast and effective purification of the recombinant protein by metal chelate affinity chromatography because of high affinity of the His-Tag to bivalent cations.

The protein fraction corresponding to the recombinant His-IncC in lysates of cells transformed with the construct pET-15b/IncC achieved maximum level 2 h after expression was induced and virtually completely disappeared in overnight culture. These data are consistent with the literature data on insolubility of Inc-proteins and their toxicity for cells on expression of their full-length genes in heterologous systems [8, 12, 14]. The construct pET-15b/IncB was less toxic, and the level of IncB was maximal in overnight culture. However, purification of the recombinant IncB and IncC from *E. coli* cells by metal ion affinity chromatography was not successful enough to prepare proteins of the purity required for further studies. The level of admixture proteins remained unchanged upon stiffening of purification conditions, possibly because of high ability of the proteins IncB and IncC to form supramolecular protein complexes.

Since attempts to optimize purification conditions for IncB and IncC did not result in enhancement of purity of prepared specimens, we constructed recombinant vectors on the basis of the plasmid pET-32a(+). These constructs, named pET-32a/IncB and pET-32a/IncC, allowed expression of full-length *incB* and *incC* genes to produce fusion proteins with TrxA (thioredoxin A) (Fig. 1). Incorporation of TrxA in chimeric protein significantly elevates its solubility in the cytoplasm of *E. coli*. This effect is particularly expressive when *E. coli* strains deficient in TrxB (thioredoxin reductase B) are used, because in these strains TrxA not only elevates solubility of the protein complex, but also provides the disulfide bond formation in the proteins [23]. The presence of $(His)_6$-peptide in the recombinant proteins TrxA-IncB and TrxA-IncC allowed their isolation and purification by metal chelate affinity chromatography to prepare electrophoretically pure fusion proteins. The purified fusion proteins were hydrolyzed with human recombinant enteropeptidase light chain, and then full-length recombinant Inc-proteins were purified by metal chelate affinity chromatography.

Thus, we are the first to prepare the full-length *C. trachomatis* inclusion membrane proteins IncB and IncC; these will be used both for antibody production and for detection of partner proteins in protein–protein interactions. We suppose that the discovery of partner proteins might bring significant changes in understanding of early processes of chlamydial infection, which remain to date one of the most intriguing enigmas of modern intracellular parasitology.

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