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Lysine acetylation of major *Chlamydia trachomatis* antigens

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ABSTRACT

Chlamydia trachomatis (Ct) is a human pathogen causing trachoma and infertility. We investigated acetylation at lysine residues of chlamydial antigenic proteins: major outer membrane protein (MOMP), 60 kDa chaperonin (chlamydial Hsp60), elongation factor G (EF-G), enolase and the polymorphic membrane proteins PmpB, PmpE and PmpF. 60 kDa chaperonin, EF-G and PmpB showed the highest degree of acetylation.

Our data show that important Ct antigens could be post-translationally modified by acetylation of lysine residues at multiple sites. Further studies are needed to investigate total acetylome of Ct and the impact PTMs might have on Ct biology and pathogenicity.

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1. Introduction

Chlamydia trachomatis (Ct) is an obligate intracellular pathogen causing socioeconomically significant morbidities as blindness (trachoma) and female infertility [1]. Ct species is divided into three biovars and various serovars that differ in their major outer membrane proteins and are associated with a number of diseases. Serovars A, B, Ba and C are considered ocular strains, serovars D–K cause sexually transmitted diseases worldwide, and serovars L1–L3 inflict severe systematic infection lymphogranuloma venereum [2]. Factors that contribute to chlamydial pathogenicity and persistence in tissues are still not fully understood. Proteomic approaches could help elucidate involved mechanisms, since post-translational modifications (PTMs) and level of protein expression are taken into account – categories otherwise frequently under-estimated or overlooked in transcriptomics and genomics studies.

In order for an organism to survive and thrive in ever changing and hostile environments it needs flexible survival mechanisms. PTM of proteins is a strategy that organisms employ to control their biological processes and to adapt to rapid environmental changes. Some of the most studied PTMs include phosphorylation (Ser, Thr, Tyr), ubiquitination and sumoylation (Lys), methylation (Arg, Lys) and acetylation (Lys) [3].

Acetylation of lysine is an important, functional PTM that in eukaryotes, among many significant regulatory mechanisms, also affects cell cycle and tumour suppression through activation of p53 by acetylation of its distinct lysine residues. It occurs on the ε-amino group of lysine, and it is one of the easiest PTM sites to identify, due to the 42 Da mass shift (resulting from the replacement of one of the hydrogens from the amine group with $COCH_3$) on the modified amino acid. It is a dynamic process performed by acetyl transferases, while the reverse is performed by deacetylases. Until recently it was thought to occur only in eukaryotes, but emerging data suggest that it occurs in prokaryotes as well [4]. Moreover, this reversible process enables some bacterial species to regulate cellular processes, infect, survive and even thrive in hostile environments [5]. Whether there are post-translationally acetylated lysine Ct antigenic proteins is unknown, as well as their impact and role within pathobiology of Ct.

Lysine acetylation could affect Ct infectious potential and pathogenicity and also alter the immunogenicity of Ct proteins. "If" and "how" need to be answered in order to help reveal the role of specific antigens in immunopathology and protection. Many immunological studies on Ct, mainly on genital and lesser on ocular serovars, have identified several major antigens eliciting

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strong humoral antibody response with a protective role. These antigens include a serotyping agent like the major outer membrane protein (MOMP) [6], polymorphic membrane proteins (Pmps) and chlamydial Hsp60 (cHsp60; known as GroEL and 60 kDa chaperonin) associated with Ct pathology and other dominant antigens from cytosol with an unknown role, as highly conserved metabolic/modifying and general secretion pathway enzymes [7]. Within this complex story of Ct pathologies, we have recently undertaken a quantitative immunoproteomic and chemometrics study of ocular Ct serovar B (CtB) and its severe trachoma relevant antigen characterization raising IgG response within 2 endemic African country populations at the stage of trachoma-caused blindness (data unpublished). There are no proteomic studies on ocular serovar B and consequently no immunoproteomic research with human trachoma patients and CtB immunodominant antigens, at this point.

Based on this immunoproteomic study, we have selected for our current preliminary research on the existence of Ct proteins' lysine acetylation, 5 most impactful major antigens, that have the highest influence on endemic trachoma patients and healthy control differentiation with significantly higher or exclusive IgG response in trachoma patients elicited by: MOMP, cHSP60), elongation factor G (EF-G), enolase and PmpF. Additionally, lysine acetylation of PmpB as pelvic infection disease dominant antigen [8] and PmpE as promising vaccine candidate for genital disease model [9], were also selected for examination. Out of the seven selected proteins, MOMP, 60 kDa chaperonin and Pmps have been characterized as major (dominant) Ct antigens in all serovars (ocular, genital and LGV) [1].

In this study we assessed three biological samples obtained at different time points of CtB with the gel-aided shotgun proteomics as a model to investigate the acetylation pattern of seven important CtB antigens: MOMP, 60 kDa chaperonin, EF-G, enolase, PmpB, PmpE and PmpF, that has been supported by Western blot of CtB proteome probed with anti-acetylated lysine specific antibody.

2. Materials and methods

2.1. Ct growth conditions and EBs purification

CtB (ATCC® VR-573™) were propagated in McCoy cells (ATCC® CRL-1696™, passages varying from P3 to P26) according to standard procedures [10]. Harvested stocks were centrifuged at 200 × g to pellet cellular debris. To purify EBs supernatants were layered over discontinuous Renografin gradients as described by Caldwell et al. [11]. The resulting EB fraction was washed twice in 0.01 M sodium phosphate (pH 7.2) containing 0.25 M sucrose and 5 mM L-glutamic acid (SPG). Pellets were resuspended in SPG and stored at −80 °C until analysis. For heat-inactivation samples were incubated at 56 °C for 30 min.

2.2. Electrophoresis

SDS-PAGE was carried out according to Laemmli [12] using a Hoefer scientific instrumentation apparatus (Amersham Biosciences, USA) with a discontinuous buffer system. Protein components were resolved on 12% polyacrylamide gels (PAA), which were stained using Coomassie Brilliant Blue R-250 (Sigma–Aldrich, Germany).

2.3. Western blot

CtB lysate (20 μg/lane) was resolved on 12% PAA and subsequently transferred to a nitrocellulose membrane, 0.2 μm (Bio-Rad, Germany) by blotting according to Towbin et al. [13] using BlueFlash™ Semi-Dry Blotter (Serva electrophoresis, Germany). The membrane was blocked with 1% bovine serum albumin (BSA) and probed with commercial anti-acetyl lysine IgG developed in rabbit (Abcam, ab80178) in 1:1000 dilution. BSA solution (0.5%) was used instead of primary antibody as a control. Western blot was developed using alkaline phosphatase conjugated goat anti rabbit IgG (Jackson ImmunoResearch, 111-055-045) in 1:1000 dilution, as a secondary antibody. The phosphatase activity was visualized using 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium (NBT). Imaging and analyses of the immunoblot bands were performed by laser Typhoon 7000 series scanner and Image Quant TL 7.0 software (GE Healthcare, USA). The procedure is explained in Supplementary data.

2.4. Shotgun proteomics of CTB antigens

2.4.1. Bands excision and preparation for MS

CtB preparations (4 μg, 8 μg and 14 μg) corresponding to 3 biological batches of CtB were electrophoretically resolved on a 12% polyacrylamide gel. After colloidal CBB staining bands from all resolved preparations were excised and in gel digested according to Shevchenko et al. [14]. Briefly, in-gel digestion procedure is compatible with downstream nano-LC–MS/MS characterization of digests derived from protein bands. When complex protein mixtures are gel-separated and analyzed by LC–MS/MS, the in-gel digestion procedure enables the analysis of entire proteomes of organelles and the majority of proteins in cell lysates. First step is band excision from the gel with a surgical scalpel. After washing with 25 mM ammonium bicarbonate buffer (ABC) and solution of 50% acetonitrile with 25 mM ABC in order to eliminate contaminants related to gel dying, the protein bands were treated with 10 mM DTT to reduce disulphide bridges. Afterwards, bands were alkylated with 55 mM iodoacetamide to block cystein residues and prevent re-formation of disulphide bonds. Proteins in gel bands were digested overnight at 37 °C with 10–20 μL of 15 ng/μL trypsin (proteomics grade, Sigma, Germany) applied per band depending on its size (the square area range of the band treated was 5–10 mm^2). Peptide mixtures were filtered by zip tips prior to MS analysis.

2.4.2. Nano-LC–MS/MS

All excised and digested bands were analyzed by LC–MS/MS. Peptides were chromatographically separated using the EASY-nLC II system (Thermo, Germany) with a 2 column set up: trap column C18-A1, 2 cm (Thermo, Germany) and analytical column PepMap C18, 15 cm × 75 μm, 3 μm particles, 100 Å pore size (Thermo, Germany). Mobile phases used were A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile. All solvents used were MS grade (Sigma, Germany). Total of 2 μL of each sample was loaded and separated by a gradient over the course of 80 min with a flow rate of 300 nL/min. The flow gradient was (i) 0–5 min at 5% B, (ii) 5–55 min, 5–70% B, (iii) 55–60 min 70–95% B, (iv) 60–70 min 95% B, (v) 70–75 min 95–5% B, (vi) 75–80 min 5% B.

Peptides were analyzed by LTQ Orbitrap XL mass spectrometer in data dependent mode with nano-ESI spray voltage of 1.9 kV, capillary temperature of 275 °C and tube lens value set at 110 V. All spectra were acquired in positive mode with high-resolution full scan in the mass range m/z 300–2000 and Orbitrap resolution of 30,000. The 5 most intense precursors were subjected to collision induced dissociation (CID) with normalized collision energy of 35 and activation time of 30 ms. Dynamic exclusion with 1 repeat count over 10 s and exclusion for 10 s was applied.

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