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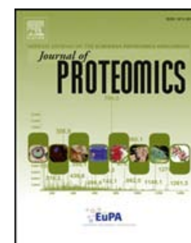

Proteomic characterization of the subpellicular cytoskeleton of *Toxoplasma gondii* tachyzoites☆

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ABSTRACT

Toxoplasma, the causative agent of toxoplasmosis in animals and humans, has a subpellicular cytoskeleton that is involved in motility, cell shape and invasion. Knowledge of components of the cytoskeleton is necessary to understand the invasion mechanisms as well as for the identification of possible therapeutic targets. To date, most cytoskeletal components of *Toxoplasma* remain unidentified due mainly to the lack of reproducible methods for their isolation. Based on the successful isolation of the cytoskeleton, it was possible to report for the first time, the proteomic characterization of the subpellicular cytoskeleton of *Toxoplasma* formed by 95 cytoskeletal proteins through proteomic analysis by tandem mass spectrometry of one dimension SDS PAGE. By bioinformatic analysis of the data, proteins were classified as: 18 conventional cytoskeletal proteins; 10 inner membrane complex proteins, including 7 with alveolin repeats; 5 new proteins with alveolin like repeats; 37 proteins associated with other organelles and 25 novel proteins of unknown function. One of the alveolin like proteins not previously described in *Toxoplasma* named TgArticulin was partially characterized with a specific monoclonal antibody. Presence of TgArticulin was exclusively associated with the cytoskeleton fraction with a cortical distribution. Functions for the several molecules identified are proposed.

Biological significance

This manuscript describes, for the first time, the proteome of the subpellicular cytoskeleton of Toxoplasma gondii. *The importance of this study is related to the role of the cytoskeleton in the highly invasive capability of a parasite that causes abortion, blindness, and death by encephalitis in immunocompromised patients.*

Proteomic characterization of the cytoskeleton of *T. gondii* tachyzoites was possible by the development of a successful procedure for the isolation of the subpellicular cytoskeleton. Knowledge of the composition of the cytoskeleton of *Toxoplasma* is fundamental for the understanding of the motility and host cell invasion mechanisms, and for the future design

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and development of toxoplasmicidal drugs with effects against specific components of the cytoskeleton of this parasite that are absent in mammal host cells.
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1. Introduction

Toxoplasma gondii is an obligate intracellular parasite worldwide distributed belonging to the phylum Apicomplexa. It infects warm-blooded animals including humans, causes abortion and in immunocompromised individuals, encephalitis and death [1].

The most invasive form of *Toxoplasma* is the tachyzoite, which invades host cells through an active process based on its motility and in the secretion of different organelles [2,3]. During invasion the parasite adheres to the target cell by proteins secreted from the micronemes, followed by the induction of gliding motility and conoid extrusion, both upon the host cell membrane. Gliding motility and conoid extrusion are two dynamic events that are based on parasite cytoskeleton [3–5]. As a retractile organelle, the conoid exerts pressure on the target cell membrane, through a process triggered by the increase of cytosolic calcium and with the participation of actin filaments and myosin molecules, thus facilitating the interaction and fusion between the apical membrane of the parasite and the host cell plasma membrane [6,7]. Afterwards, through the secretion of the rhoptries, the parasite penetrates the host cell by twirling gliding movements and then locates into a parasitophorous vacuole (PV) which is modified by the secretion of components of the dense granules, followed by the proliferation inside the PV through the asexual process called endodiogeny. Since *Toxoplasma* has no motile organelles, motility only occurs by gliding over the cell membrane through a molecular complex called glideosome, whose function is related to its invasive capability and tissue dissemination [8,9].

The tachyzoite is surrounded by a trimembrane pellicle, formed by the plasma membrane and 11 longitudinal flattened vesicles located beneath the membrane that are distributed along the axis of the parasite and are fused together providing a bimembranal structure called inner membrane complex (IMC), which is interrupted at apical and posterior ends. Associated with the pellicle there is a subpellicular cytoskeleton that includes different structures, such as conoid, preconoidal rings, apical polar ring, 22 subpellicular microtubules, a subpellicular network of filaments and polar posterior ring. The conoid is an apical retractable hollow truncated cone made up of 14 helical structures rich in α-tubulin. In its interior there are 2 intraconoidal paired microtubules that apparently participate in transporting cargo proteins towards the anterior end of the parasite, as well as in the correct location of the neck rhoptries inside the conoid [10]. Anchored to the conoid there is a polar apical ring that acts as an organizing center for the 22 subpellicular microtubules. The subpellicular microtubules extend helically from the apical polar ring along three quarters of the length of the parasite and are anchored to the cytosolic side of the IMC [11]. The presence of intra membrane particles organized in simple and double lines, have been described at the cytosolic face of the IMC [11,12]. The double particle lines apparently function as an anchorage system for the subpellicular microtubules [11–13]. In contrast, the function of the simple alignment particles remains unknown [14]. Associated with the IMC, there is a subpellicular network resistant to the extraction with the non-ionic detergent triton X-100. This network that is constituted by numerous cross-linked longitudinal filaments of about 8–10 nm, is anchored at one extreme to the posterior polar ring and at the other to the apical polar ring. Although it surrounds the whole parasite, most authors describe its localization between the IMC and the subpellicular microtubules [15–19]. Its precise functions have not yet been determined, however it has been suggested to have a structural role for the maintaining of the cell shape and to provide mechanical strength in mature tachyzoites [15–19]. The machinery for the parasite motility is associated with the pellicle as a multiprotein complex called glideosome [20]. It consists of a hexameric transmembrane complex formed by MIC2 and MIC2 associated protein (MIC2AP), which at the cytosolic side is bound to the bridge protein aldolase that links actin filaments [21]. Actin filaments bind to the molecular motor, myosin A (TgMyoA)-myosin light chain (TgMLC), forming the dynamic system for gliding motility, which is additionally anchored to the IMC integral proteins TgGAP45 and TgGAP50 [20,22].

Complete annotation of the genome of the three genotypes of *Toxoplasma* has facilitated the molecular characterization of different components, some of them previously isolated through subcellular fractionation [23]. Nevertheless the importance of the subpellicular cytoskeleton to parasite shape and motility, its proteomic composition and structural organization have been poorly described [15,18,24–27]. In an earlier study, we reported a successful method for isolation of the subpellicular cytoskeleton by treatment of tachyzoites with the non-ionic detergent triton X-100 in an intracellular medium with a mixture of protease inhibitors [18]. By using this methodology, we report for the first time in the present work, the proteome of the subpellicular cytoskeleton of *T. gondii* tachyzoites using tandem mass spectrometry (LC MS/MS), including the identification of new alveolin related proteins and a group of new undescribed proteins.

2. Materials and methods

2.1. Animals

Female Balb/c mice used for parasitic infections were maintained in an animal facility with regulated environmental conditions of temperature, humidity and filtered air. Handling was performed according to the Mexican Official Norm (NOM-062-ZOO-1999) for the production, care and use of laboratory animals.

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