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ORIGINAL ARTICLE

Phosphoproteome profiling of the sexually transmitted pathogen *Trichomonas vaginalis*

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Background/Purpose(s): Trichomoniasis caused by *Trichomonas vaginalis* is the most common non-viral sexually transmitted infection. Morphological transformation from the trophozoite stage to the amoeboid or pseudocyst stage is crucial for *T. vaginalis* infection and survival. Protein phosphorylation is a key post-translational modification involved in the regulation of several biological processes in various prokaryotes and eukaryotes. More than 880 protein kinases have been identified in the *T. vaginalis* genome. However, little is known about the phosphorylation of specific proteins and the distribution of phosphorylated proteins in different stages of the morphological transformation of *T. vaginalis*.
Methods: To obtain a more comprehensive understanding of the *T. vaginalis* phosphoproteome, we analyzed phosphorylated proteins in the three morphological stages using titanium dioxide combined with LC-MS/MS.
Results: A total of 93 phosphopeptides originating from 82 unique proteins were identified. Among these proteins, 21 were detected in all stages, 29 were identified in two different stages, and 32 were stage specific.

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Conclusion: Identification of stage-specific phosphorylated proteins indicates that phosphorylation of these proteins may play a key role in the morphological transformation of *T. vaginalis*.
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Introduction

Trichomonas vaginalis is the causative agent of one of the most common sexually transmitted diseases, trichomoniasis.[1] Approximately 5 million cases of trichomonal vaginitis are reported annually in North America. Worldwide, over 170 million cases of trichomoniasis are reported each year. Infection is acquired primarily through transmission of trophozoites by direct sexual contact, although neonatal infection has also been reported.[2] Trichomoniasis is associated with adverse pregnancy outcomes, enhanced predisposition to HIV infection and risk of cervical neoplasia.[3,4]

A unique feature of the *T. vaginalis* life cycle is the reversible morphological transformation from the trophozoite stage to the amoeboid or pseudocyst stage. The parasite multiplies in the vagina or prostate of infected hosts in the trophozoite form. When it attaches to the surface of vagina epithelial cells, the trophozoite transforms to an amoeboid stage and induces acute inflammation of the vagina, resulting in a yellowish-green foul-smelling discharge in some females. Transformation to the amoeboid stage is crucial for establishment and maintenance of *T. vaginalis* infection in the vagina. Several proteins that mediate adherence, such as surface proteins (AP120, AP65, AP51, AP33, AP23),[5,6] cysteine proteinases,[7] glyceraldehyde 3-phosphate dehydrogenase,[8] and lipophosphoglycan,[9] have been identified. Increasing evidence demonstrates that *T. vaginalis* can survive in the form of a pseudocyst under unfavorable environmental conditions, such as nutrient deprivation, the presence of drugs or dramatic changes in temperature.[10] In this form, the parasite becomes non-motile and the flagellae are internalized, but a true cyst wall is not formed. Recent studies indicate that the pseudocyst stage is reversible and represents a defense mechanism against conditions detrimental to trichomonad survival.[11,12] This pseudocystic stage is probably responsible for transmission of trichomoniasis via non-sexual contact. At present, how transformation between the trophozoite, amoeboid and pseudocyst stages is controlled and regulated in *T. vaginalis* remains largely unknown.

The modulation of protein phosphorylation through the antagonistic effects of protein kinases and protein phosphatases is a major regulatory mechanism for most cellular processes. Protein kinases are one of the major classes of enzymes involved in signal transduction and signaling networks. Many growth factors, cytokines and antigens initiate signaling events through protein phosphorylation, leading to cellular responses such as gene expression, changes in cell shape and migration, entry into the cell cycle, and proliferation. Protein phosphorylation is a major mechanism by which external stimuli are transformed into intracellular signals to which cells respond. It has been estimated that 30–50% of intracellular proteins are phosphorylated at some point in their lifetime.[13] The *T. vaginalis* kinome comprises more than 880 eukaryotic protein kinases and 40 atypical protein kinases, making it one of the largest kinomes identified to date.[14] Large-scale gene expression analysis using expressed sequence tags showed that 451 kinase genes (50% of the kinome) are expressed. At present, it is not known if any of these kinases participate in the transformation of trophozoites into amoeboids or pseudocysts. An alternative approach to explore the function of these kinases in the transformation process is to investigate the phosphoproteome of *T. vaginalis* and to use the resulting data set as a basis for deducing the kinase–substrate interaction network. In the present study, we elucidated the expression profile of phosphoproteins in the trophozoite, pseudocyst and amoeboid stages of T. *vaginalis* using a reverse-phase LC/MS/MS system.

Methods

Cell culture

T. vaginalis isolate ATCC30236 (JH 31A#4) was maintained in YIS medium, pH 5.8, containing 10% heat-inactivated fetal calf serum at 37 °C.[15] The number of viable cells was determined based on hemocytometer counts using Trypan blue exclusion. Trophozoites grown to the late logarithmic phase with more than 90% viable cells were harvested for further study. The pseudocyst stage was induced by incubating trophozoites in late logarithmic phase on ice for 4 hours. The amoeboid stage was induced by growing trophozoites in late logarithmic phase in fibronectin-coated T-75 flasks for 3 hours at 37 °C.

Preparation of cell extracts, digestion of protein mixtures and enrichment of phosphopeptides

T. vaginalis cells from trophozoites, pseudocysts and amoeboids were harvested by centrifugation at 3000 rpm for 15 minutes and were washed there times in normal saline. Cell pellets were resuspended in lysis buffer (8 M urea, 4% CHAPS) containing protease inhibitors (Roche Diagnostics, Basel, Switzerland) at a cell density of approximately 1.2×10^8 cells/mL. Cells were disrupted by ultrasonication (XL2000, Misonix Inc., Farmingdale, NY, USA) in an ice bath for eight cycles, each consisting of 10 seconds of sonication followed by a 10-second break. After centrifugation at 4 °C and 13,000 rpm for 15 minutes, impurities in the supernatant were removed using a 2D cleanup kit (GE Healthcare, Taipei, Taiwan). For tryptic digestion, the protein mixtures were diluted in 50 mM ammonium bicarbonate (Sigma, St. Louis, MO, USA) and digested with sequencing-grade trypsin (1:50 w/w; Promega, Madison, WI, USA) at 56°C for 1 hour. The digested lysate was reduced with 25 mM NH_4HCO_3 (Sigma) containing 10 mM dithiothreitol (DTT; Sigma) at 37°C for 30 minutes and then alkylated with 55 mM iodoacetamide (Sigma) at room temperature for 30 minutes. After further reduction with

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