Molecular & Biochemical Parasitology 207 (2016) 10–18

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Trichomonas vaginalis NTPDase and ecto-5′-nucleotidase hydrolyze guanine nucleotides and increase extracellular guanosine levels under serum restriction

Camila Braz Menezes[a], Juliano Durgante[b], Rafael Rodrigues de Oliveira[c], Victor Hugo Jacks Mendes dos Santos[c], Luiz Frederico Rodrigues[c], Solange Cristina Garcia[b], Odelta dos Santos[a], Tiana Tasca[a,*]

[a] *Laboratório de Pesquisa em Parasitologia, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, 90610-000 Porto Alegre, RS, Brazil*
[b] *Laboratório de Toxicologia, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, 90610-000 Porto Alegre, RS, Brazil*
[c] *Instituto do Petróleo e dos Recursos Naturais, Pontifícia Universidade Católica do Rio Grande do Sul, Av. Ipiranga 6681, 90619-900 Porto Alegre, RS, Brazil*

ARTICLE INFO

Article history:
Received 26 November 2015
Received in revised form 14 April 2016
Accepted 29 April 2016
Available online 2 May 2016

Keywords:
Trichomonas vaginalis
Ectonucleoside triphosphate diphosphohydrolase
Ecto-5′-nucleotidase
Serum limitation
Guanosine

ABSTRACT

Trichomonas vaginalis is the aethiologic agent of trichomoniasis, the most common non-viral sexually transmitted disease in the world. The purinergic signaling pathway is mediated by extracellular nucleotides and nucleosides that are involved in many biological effects as neurotransmission, immunomodulation and inflammation. Extracellular nucleotides can be hydrolyzed by a family of enzymes known as ectonucleotidases including the ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases) family which hydrolyses nucleosides triphosphate and diphosphate as preferential substrates and ecto-5′-nucleotidase which catalyzes the conversion of monophosphates into nucleosides. In *T. vaginalis* the *E*-NTPDase and ecto-5′-nucleotidase activities upon adenine nucleotides have already been characterized in intact trophozoites but little is known concerning guanine nucleotides and nucleoside. These enzymes may exert a crucial role on nucleoside generation, providing the purine sources for the synthesis *de novo* of these essential nutrients, sustaining parasite growth and survival. In this study, we investigated the hydrolysis profile of guanine-related nucleotides and nucleoside in intact trophozoites from long-term-grown and fresh clinical isolates of *T. vaginalis.* Knowing that guanine nucleotides are also substrates for *T. vaginalis* ectoenzymes, we evaluated the profile of nucleotides consumption and guanosine uptake in trophozoites submitted to a serum limitation condition. Results show that guanine nucleotides (GTP, GDP, GMP) were substrates for *T. vaginalis* ectonucleotidases, with expected kinetic parameters for this enzyme family. Different *T. vaginalis* isolates (two from the ATCC and nine fresh clinical isolates) presented a heterogeneous hydrolysis profile. The serum culture condition increased E-NTPDase and ecto-5′-nucleotidase activities with high consumption of extracellular GTP generating enhanced GDP, GMP and guanosine levels as demonstrated by HPLC, with final accumulation of the nucleoside. The transcript levels of the five *TvNTPDases* gene sequences were analyzed by qRT-PCR and the highest gene expressions were found for *TvNTPDase 2* and *4*. The extracellular guanosine uptake was observed as ^{13C}GTP nucleotide into parasite DNA and it was lower than that observed for adenosine, labeled as ^{13C}ATP. These findings indicate the *T. vaginalis* preference for adenosine uptake and the accumulation of guanosine in the extracellular milieu, corroborating with HPLC data. Our data demonstrate, for the first time, the cascade of guanine nucleotides in *T. vaginalis* and open possibilities on the study of guanine-related purines other than the classical intracellular activity of G proteins for signal transduction.

1. Introduction

The protozoan *Trichomonas vaginalis* is the ethiologic agent of trichomoniasis, the most common non-viral sexually transmitted disease, infecting approximately 276 million people worldwide

* Corresponding author.
E-mail address: tiana.tasca@ufrgs.br (T. Tasca).

http://dx.doi.org/10.1016/j.molbiopara.2016.04.003
0166-6851/

annually [1]. Symptoms of trichomoniasis range from mild to severe inflammation and genital pruritis and may also include cervicovaginal or urethral discharge [2]. It is important to emphasize that the infection may be asymptomatic in 70–85% of infected women, thus facilitating carrying of the parasite and increasing the number of new infected people [3,4]. The impact of trichomoniasis on human health is significant since the infection has been associated with infertility, increased risk of cervical and prostate cancer and even more relevant is trichomoniasis as a co-factor of HIV acquisition and transmission [5,6]

Since the introduction of the concept of the purinergic signaling, clear signaling roles for extracellular ATP and other nucleotides have been established, such as regulation of epithelial cell responses and immune cascades [7,8]. Despite been usually related to adenine-based purines (mainly ATP and adenosine), guanine-based purines, such the nucleotides GTP, GDP, GMP and the nucleoside guanosine are also part of this signaling cascade [9]. The role for guanine-based purines has been traditionally studied concerning the intracellular activity of G proteins for signal transduction. Nevertheless, guanine-based purines have been shown to exert extracellular functions not related to their intracellular modulation of G proteins, including *in vitro* and *in vivo* activities [10,11].

The enzymes involved in extracellular nucleotide hydrolysis consist in a group of ectoenzymes known as ectonucleotidases, including the ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) family that hydrolyses nucleoside tri and diphosphates, as well as the ecto-5′-nucleotidase that hydrolyses nucleoside monophosphates [12]. In the human immune system, these enzymes have already been characterized and may influence the host response to infection, as extracellular ATP and other nucleotides constitute potent "danger signals" for the host immune and inflammatory responses [13]. Besides this crucial involvement in inflammation, ectonucleotidases may play an important role in purine scavenging, since most of parasites, including *T. vaginalis*, lack *de novo* purine [14] and pyrimidine [15] nucleotides synthesis. The *T. vaginalis* unique purine salvage pathway comprises a bacterial type purine nucleoside phosphorylase purine nucleoside phosphorylase (PNP), which catalyzes inter-conversion between purine bases and purine nucleosides and the PNK converting the nucleosides to nucleotides. It relies primarily on the sequential actions of the two enzymes to incorporate exogenous adenine and guanine into its purine nucleotide pool [16]. Considering nucleoside transporters it was already demonstrated that nucleoside uptake is mediated by two separate carries: one that transports all nucleosides and a second specific for adenosine, guanosine and uridine [17].

Besides the modulation on host-defense interactions at the site infection, the nucleotides hydrolysis promoted by the ectonucleotidase cascade in *T. vaginalis* provides the primary precursors of purine and pyrimidine nucleotides for the parasite growth and survivor [14,18]. The hydrolysis of non-adenine nucleotides by the NTPDases has already been shown in many organisms. In *Legionella pneumophila* for example, E-NTPDases hydrolyze purine and pyrimidine bases such as GTP, UTP, and CTP with efficiencies similar to those for ATP and ADP [19]. In parasites, E-NTPDases that do efficiently hydrolyze non-adenine nucleotides include those found in the *Toxoplasma gondii* [20], *Tritrichomonas foetus* [21], trypanosomes [22,23] and *Schistosoma mansoni* [24]. Previous studies from our group have characterized the E-NTPDase [25] and ecto-5′-nucleotidase [26,27] activities in adenine and non-adenine nucleotides in *T. vaginalis* trophozoites, although little is known concerning guanine nucleotides hydrolysis profile and the pattern of the nucleoside incorporation to the intracellular nucleotide pool in *T vaginalis* trophozoites.

Mammalian serum is considered essential for the growth of trophozoites of many parasites such as *Giardia duodenalis* and other protozoa, like *Entamoeba histolytica* and *T. vaginalis* [28], as an important source of nutrients. *In vitro, T. vaginalis* culture is traditionally supplemented with bovine, horse or human serum as an important source of purines and pirimidines for growth. Some studies have already shown the effect of serum limitation on cell growth and metabolism. Rapaport and Zamecnik showed that in mammalian cell lines, serum limitation altered cellular growth and promoted an increase in the incorporation of adenosine into adenine nucleotides [29]. In parasites, biochemical adaptation to nucleoside starvation leads to upregulation of purine and pyrimidine salvage pathways which includes the ectonucleotidases and nucleoside transporters. It has already been shown that under conditions of insufficient purines (purine stress) *Crithidia luciliae* increased the rates of transport of nucleosides and bases from the environment by enhancing the activity of the ectoenzyme 3′-nucleotidase (3′NTase) [30] The regulation of the activity of purine transporters in two protozoan species, *Crithidia fasciculata* and *Trypanosoma brucei*, was already investigated and the purine transport activity was also stimulated 5–15-fold following growth in purine-depleted medium[31]. In *Leishmania donovani* purine starvation parasites for leads to a rapid amplification in purine nucleobase and nucleoside transport and parasites remodel their purine metabolic pathway to maximize salvage [32]. A recent study from our group demonstrated that serum-limited *T. vaginalis* strongly increased the E-NTPDase and ecto-5′-nucleotidase hydrolysis profiles as well as the E-NTPDase transcripts levels, causing and arrest in the parasite cell cycle suggesting adenosine uptake [33].

Considering that (i) guanine nucleotides and nucleoside are involved in the purinergic signaling and (ii) the hydrolysis profile of these nucleotides in *T. vaginalis* trophozoites is unkown and (iii) that serum limitation modulates the ectoenzymes hydrolysis profile, the aims of this study were to investigate the hydrolysis profile and the metabolism of guanine nucleotides in *T. vaginalis* under a limited serum condition. The results show that all different *T. vaginalis* isolates hydrolyzed GTP, GDP, and GMP with accumulation of extracellular guanosine as demonstrated by HPLC and uptake assays.

2. Material and methods

2.1. Chemicals

Nucleotides (GTP, GDP, GMP, ^{13}CATP, ^{13}CGTP), nucleosides (guanosine, adenosine), enzyme inhibitors (ouabaine, orthovanadate, tretramisole, suramin, and ammonium molybdate) and dilazep were purchased from Sigma Chemical Co (USA).

2.2. T. vaginalis *culture*

T. vaginalis isolates 30236 and 30238 (from the American Type Culture Collection); TV-LACM1, TV-LACM2, TV-LACM6,TV-LACM15 and TV-LACM16 (fresh clinical isolates from female patients); TV-LACH1, TV-LACH2, TV-LACH3, TV-LACH4 (fresh clinical isolates from male patients) all from Laboratório de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, UFRGS, Brazil (project approved by UFRGS Ethical Committee, number 18923) were used in this study. Trichomonads were cultured axenically *in vitro* on trypticase yeast-extract maltose (TYM) medium (pH 6.0) supplemented with 10% (v/v) heat-inactivated bovine serum (HIBS) and incubated at 37 °C (±0.5) [34]. After 24 h, trophozoites exhibiting motility and normal morphology were harvested and washed for the experiments described below.