Available online at www.sciencedirect.com
ScienceDirect
European Journal of Protistology 44 (2008) 103–113
European Journal of PROTISTOLOGY
www.elsevier.de/ejop

Heterogeneous production of metallo-type peptidases in parasites belonging to the family Trypanosomatidae

André Luis Souza dos Santos[a,*], Rosangela Maria de Araújo Soares[a], Celuta Sales Alviano[a], Lucimar Ferreira Kneipp[b]

[a]*Departamento de Microbiologia Geral, Instituto de Microbiologia Prof. Paulo de Góes, Universidade Federal do Rio de Janeiro, Cidade Universitária, Ilha do Fundao, 21941-902 Rio de Janeiro, RJ, Brazil*
[b]*Departamento de Micologia, Instituto Oswaldo Cruz–Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil*

Received 24 May 2007; received in revised form 25 July 2007; accepted 23 August 2007

Abstract

Proteolytic enzymes play a central role in the physiology of all living organisms, participating in several metabolic pathways and in different phases of parasite–host interactions. We have identified cell-associated peptidase activities in 33 distinct flagellates, including representatives of almost all known trypanosomatid genera parasitizing insects (*Herpetomonas*, *Crithidia*, *Leishmania*, *Trypanosoma*, *Leptomonas*, *Phytomonas*, *Blastocrithidia* and *Endotrypanum*) as well as the biflagellate kinetoplastid *Bodo*, by using SDS–PAGE containing gelatin as co-polymerized substrate and proteolytic inhibitors. Under the alkaline pH (9.0) conditions employed, all the flagellates presented at least one peptidase, with the exception of *Crithidia acanthocephali* and *Phytomonas serpens,* which did not display any detectable proteolytic enzyme activity. All the proteolytic activities were completely inhibited by 1,10-phenanthroline, a zinc-chelating agent, putatively identifying these activities as metallo-type peptidases. EDTA and EGTA, two other metallopeptidase inhibitors, E-64 (a cysteine peptidase inhibitor), pepstatin A (an aspartyl peptidase inhibitor) and PMSF (a serine peptidase inhibitor) did not interfere with the metallopeptidase activities detected in the studied trypanosomatids. Conversely, *Bodo*-derived peptidases were resistant to 1,10-phenanthroline and only partially inhibited by EDTA, showing a distinct inhibition profile. Together, our data demonstrated great heterogeneity of expression of metallopeptidases in a wide range of parasites belonging to the family Trypanosomatidae.

Keywords: Cellular proteolytic enzymes; Metallopeptidases; Trypanosomatidae

Introduction

The family Trypanosomatidae (order Kinetoplastida) is composed exclusively of parasitic organisms with a single flagellum and a kinetoplast (Vickerman 1994). Trypanosomatids parasitize all classes of vertebrates as well as some invertebrates, preferentially insects from the orders Diptera and Hemiptera, and also plants. Invertebrates act as hosts of monogenetic parasites, such

*Corresponding author. Laboratório de Estudos Integrados em Bioquímica Microbiana, Departamento de Microbiologia Geral, Instituto de Microbiologia Prof. Paulo de Góes (IMPPG), Bloco I-subsolo, Centro de Ciências da Saúde (CCS), Universidade Federal do Rio de Janeiro (UFRJ), Avenida Carlos Chagas Filho, 373, Cidade Universitária, Rio de Janeiro, RJ 21941-902, Brazil.
Tel.: +5521 2562 6740; fax: +5521 2560 8344.
E-mail address: andre@micro.ufrj.br (A.L.S. Santos).

0932-4739/$ - see front matter
doi:10.1016/j.ejop.2007.08.006

as *Blastocrithidia*, *Crithidia*, *Herpetomonas* and *Leptomonas*, or serve as vectors of digenetic genera *Trypanosoma*, *Endotrypanum*, *Leishmania* and bug-transmitted parasites of the genus *Phytomonas*, found in plants. In humans and domestic animals *Trypanosoma* and *Leishmania* cause severe diseases, including sleeping sickness, Chagas' disease and kala-azar (Vickerman 1994), while *Phytomonas* are pathogens of different families of plants including coconut, oil palm, tomato and corn (Camargo 1999). Conversely, insect trypanosomatids are used routinely as safe models for initial biochemical and molecular studies on conserved features of the family Trypanosomatidae because they are easily cultured under axenic conditions and are traditionally nonpathogenic to humans (Vickerman 1994). However, some possible mammalian infections due to insect flagellates have been described (reviewed by Chicharro and Alvar 2003). Interestingly, *Phytomonas serpens* expresses immunological similarities with the human trypanosomatid pathogens (reviewed by Santos et al. 2007). These works emphasize the real importance of proper biochemical and molecular studies using insect trypanosomatids.

Microbial peptidases have been extensively used in the food, dairy and detergent industries since ancient times. There is a renewed interest in peptidases as targets for developing therapeutic agents against relentlessly spreading fatal diseases such as cancer, AIDS, malaria and trypanosomiases (Barrett et al. 2001; Vermelho et al. 2007). Peptidases are a unique class of enzymes that occupy a pivotal position in the physiology of all eukaryotic cells: examples are the removal of abnormal proteins, degradation of regulatory proteins for metabolic pathways and cell cycle progression, as well as breakdown of proteins for energy production and antigen presentation. Proteolysis also plays a role in cellular differentiation and death (Barrett et al. 2001). Catalysis can be initiated either within a polypeptide chain (endopeptidase) or from amino or carboxyl ends (exopeptidase activity). Proteolytic enzymes have been divided into 6 major groups on the basis of the catalytic mechanism used during the hydrolytic process: serine-, threonine-, glutamic-, aspartic-, metallo- and cysteine-type peptidases (Barrett et al. 2001).

Various microorganisms that are obligate or opportunistic human pathogens produce metallopeptidases with a wide multiplicity of pathological actions, in which zinc is an essential metal ion for the catalytic activity. For instance, in local infections, the metallopeptidases can cause necrotic or hemorrhagic tissue damage through digestion of structural components of the ground substance, and also form edematous lesions through stimulation of inflammatory mediators, while in systemic infections this class of peptidases can act as a synergistic virulence factor through disordered proteolysis of many plasma proteins (Hooper 1994; Miyoshi and Shinoda 2000). Our research group has extensively described metallopeptidase activities in several monogenetic and digenetic trypanosomatids (d'Avila-Levy et al. 2006a, b; Elias et al. 2006; Nogueira de Melo et al. 2006; Santos et al. 1999, 2001, 2002a, 2003, 2005, 2006a, 2007). In the present work, we have extended these studies in an effort to identify metallo-type proteolytic enzymes in 33 distinct flagellates, including representatives of almost all known trypanosomatid genera that parasitize insects (*Herpetomonas*, *Crithidia*, *Leishmania*, *Trypanosoma*, *Leptomonas*, *Phytomonas*, *Blastocrithidia* and *Endotrypanum*) as well as the biflagellate kinetoplastid *Bodo*, by using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) containing gelatin as co-polymerized proteinaceous substrate and metallopeptidase inhibitors.

Materials and methods

Microorganisms and growth conditions

The 33 parasites used in this study are listed in Table 1. For the experiments, trypanosomatids were grown at 26 °C in 250-mL Erlenmeyer flasks containing 100 mL of liver infusion-tryptose (LIT) medium, except for the digenetic trypanosomatids (*Trypanosoma* spp., *Leishmania* spp., *Phytomonas* spp. and *Endotrypanum schaudinni*), which were cultured in LIT medium supplemented with 10% fetal bovine serum (FBS) (Santos et al. 2005). *Bodo* sp. were isolated from the sap of *Cocos nucifera* (Tavares da Silva et al. 1988) and were cultured in LIT medium supplemented with 10% FBS. Bacteria originating in the plant sap that multiply in the medium are actively ingested by this flagellate and seem to be essential for *Bodo* nutrition. Parasite growth was estimated by determining the cell concentration in a Neubauer chamber. Cellular viability was assessed by motility and exclusion of trypan blue dye from cells. The viability of the parasites was not affected by the culture conditions employed in this work.

Parasite extracts

Three-day-old cultured flagellates grown to a density of 1×10^7 cells/mL were harvested by centrifugation (1500*g*/10 min/4 °C), and washed 3 times with cold phosphate-buffered saline (PBS; 150 mM NaCl, 20 mM phosphate buffer, pH 7.2). The flagellates (1×10^8 cells) were resuspended in 50 μL of PBS and lysed by the addition of 150 μL of SDS–PAGE sample buffer (125 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 0.002% bromophenol blue). The cells were broken in a vortex mixer by alternate 1 min shaking and 2 min cooling intervals (5 cycles), followed by centrifugation