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Glycosidases in mucin-dwelling protozoans

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A range of protozoans were tested for the presence of glycosidases using p-nitrophenyl sugars as substrates. Some of the organisms were mucin dwellers whereas others were blood borne parasites. It had been hypothesized that glycosidase production would be significantly higher in the mucin dwellers. The results obtained demonstrated that the urogenital protozoans *Tritrichomonas foetus* and *Trichomonas vaginalis* produced a vast range of glycosidases which included those required for mucin breakdown. The gut dwelling protozoans *Giardia lamblia* and *Entamoeba histolytica* both produced *β-N*-acetylglucosaminidase. *G.lamblia* also had detectable *β N*-acetylgalactosaminidase activity, and small amounts of *β* mannosidase were found in the extracts from *E. histolytica*. In contrast, little or no glycosidase activity was detected under the same experimental conditions in *Leishmania donovani*, *Trypanosoma brucei* or *T. cruzi*. The mucin dwelling protozoans all produce *β-N*-acetylglucosaminidase but only the Trichomonads produced the range of enzymes required for complete breakdown of mucin. This seems to suggest that mucin breakdown is not a characteristic of all mucin dwelling protozoans.

***Keywords*: protozoans, glycosidases, mucins**

Introduction

Parasitic protozoans are numerous and diverse and many are pathogens of man and animals. The protozoa causing disease in humans usually fall into one of the following groups, namely, Sarcodina (the amoebas); Mastigophora (the flagellates); the Ciliates and the Sporozoa (1). The organisms used in this study are shown in Table 1.

Amongst the important protozoan pathogens of man are those causing malaria, amoebic dysentery, Chaga's disease, African sleeping sickness and leishmaniasis [1, 2]. Many others cause serious disease in domestic animals, such as bovine trichomoniasis and Nagana, and so are important economically [2–6]. Parasitic protozoa are complex in nature and have adapted themselves well for existence within the system as well as other host systems. Their nutrition, physiology and biochemistry have been specially adapted to their habitat where they have evolved many ways of invading the host and evading the immune system [3]. It is for this reason that the control of these parasitic diseases has for the most part, remained a difficult and unsuccessful process.

It is not in the best interest of the parasite to cause serious damage to its host. However, a variety of parasites, in particular those mentioned, cause injury to their host as part of their mechanism of dispersal from one host to another. For example, diarrhoea caused by *Entamoeba* or *Giardia* infection of the intestine, probably serves in the spreading of these parasites. The role of nutrition in host parasite interactions may also cause pathogenic effects, even though the mechanism remains unclear. The kinetoplastid flagellates namely, *Leishmania* and *Trypanosoma*, are predominantly haemoflagellates and are transmitted by insect vectors (Table 2). The remaining pathogens mentioned are mainly intestinal and/or urogenital protozoans and share a common feature in that they parasitize an environment that is rich in mucin. They also differ from the kinetoplasts in that they are not transmitted by vectors.

Glycosidases may be implicated in the mechanisms of pathogenicity of *Tritrichomonas foetus* and *Trichomonas vaginalis* which inhabit the mucin of the urogenital tract [7, 8]. *In vitro* studies have shown that both *T. foetus* and *T. vaginalis* are able to utilize a range of carbohydrates via the glycolytic pathway at the site of unique organelles known as hydrogenosomes [9]. These protozoans produce a range of glycosidases capable of hydrolysing the carbohydrate chains of mucin [10, 11]. This poses the question "Is glycosidase production a characteristic feature of mucin-dwelling organisms?"

There are many common features associated with the nature of the diseases caused by the mucin-dwelling pathogens. Mucin penetration and impairment of the mucus lining of the vertebrate tracts are often witnessed during infections of *Entamoeba*, *Giardia* and *Trichomonas* [2, 3, 12–14]. The trypanosomes and leishmania however, all have more complex life-cycles and spend part of their time growing multiplying in tissues and the blood of their main hosts causing disease [1, 15].

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Table 1. Classification of the organisms studied. Adapted from Cox [1]

Organism	*Classification (Phylum: Sarcomastigophora)*	*Main host*	*Target tissue*
Tritrichomonas foetus	Subphyllum: Mastigophora Order: Trichomonadida	Bovine	Urogenital tract
Trichomonas vaginalis	As above	Human	Urogenital tract
Giardia lamblia	Subphyllum: Mastigophora Order: Diplomonadida	Human	Small intestine
Entamoeba histolytica	Subphyllum: Sarcodina Order: Amoebida	Human	Gastric and intestinal tract
Leishmania donovani	Subphyllum: Mastigophora Order: Kinetoplastida	Human	Visceral
Trypanosoma brucei brucei	As above	Equine, Sheep, Goats	Tissues and blood
Trypanosoma cruzi	As above	Human	Blood

Table 2 Mode of transmission and pathology. Adapted from Cox [1]

Organism	*Mode of transmission*	*Disease*
T. foetus	Venereal	Trichomoniasis
T. vaginalis	Venereal	Trichomoniasis
G. lamblia	Cysts	Giardiasis
E. histolytica	Cysts	Amoebic dysentery
L. donovani	Phlebotomous vector	Kala Azar
T. brucei brucei	Tsetse fly	Nagana
T. cruzi	Triatomid bug	Chaga's disease

Proteinases, together with glycosidases, have been shown to play a role in host-parasite interactions, in particular, mucin penetration. The presence of these enzymes has been reported in the trichomonads, *E. histolytica* and *G. lamblia* [16–18]. It has been found that in organisms such as *T. vaginalis* and *G. lamblia* for example, motility alone was not sufficient in allowing these pathogens to migrate through mucin layers [18]. The release of 'cytoactive' factors and soluble parasite products could be involved in attacking the extracellular matrix of host cells/tissue. Since mucin is a glycoprotein composed largely of carbohydrate surrounding a protein 'backbone', glycosidases are implicated in exposing the protein core by hydrolysing the protective carbohydrate chains. This then leaves the backbone exposed to the action of host and/or parasite proteinases.

Aims

(i) To compare glycosidase activities in mucin- and non-mucin dwelling protozoans. (ii) To determine whether production of glycosidases is related to the mucin environment of the organism.

Methods

Confluent cultures of a range of protozoans were obtained from St Pancras Hospital. The trichomonad strains were prepared in modified Diamonds medium [10, 19]. The cells were harvested by centrifugation at 1000 g for 10 min and washed with 0.15 M NaCl. The cell-free culture fluid (supernatant) was retained and stored at $-20\,^{\circ}C$ until use. The cells were resuspended at a density of 2×10^7 cells per ml in sterile distilled water and freeze-thawed to produce a lysate. This was stored at $-20\,^{\circ}C$ until use.

Glycosidases were assayed using para-nitrophenyl sugar substrates. Volumes of 20 µl of either the supernatant or the lysed cells were mixed with 50 µl 0.0067 M para-nitrophenyl glycoside and 10 µl 0.1 M Tris-HCl buffer pH 6.5. The mixture was incubated at $37\,^{\circ}C$ for 2 h. One ml of 0.2 M sodium hydrogen carbonate was added to the mixture which was mixed well. The absorbance was read at 420 nm against a blank containing no added substrate (or no enzyme source). Results were expressed as total enzyme produced, U ml^{-1} where enzyme units are defined as µmoles of sugar liberated per min and where 1 ml of enzyme was derived from 2×10^7 cells

Results

The mucin-dwelling organisms have a much wider range of glycosidases than the non-mucin dwellers (Table 3). Additionally, the glycosidases involved in destruction of the repetitive *N*-acetyllactosamine groups of the mucin sugar backbone are the predominant enzymes detected.

The urogenital pathogens, *T. foetus* and *T. vaginalis* were both found to possess a wide range of intracellular glycosidases. The most active were β-galactosidase, α-*N*-acetylgalactosaminidase and β-*N*-acetylglucosaminidase.

Table 3. Summary of the production of enzymes in protozoans

Enzyme	*Total activity (U ml $^{-1}$)*[a]						
	T. foetus	*T. vaginalis*	*G. lamblia*	*E. histolytica*	*L. donovani*	*T. brucei*	*T. cruzi*
α-Galactosidase	4	4	0.2	–	–	–	–
β-Galactosidase	20	15	–	–	0.2	–	–
α-Glucosidase	2	2	–	–	0.2	0.2	0.5
β-Glucosidase	7	6	–	–	0.2	–	–
β-Glucuronidase	–	1	–	–	–	–	–
α-*N*-Acetyl Galactosaminidase	22	25	–	–	–	–	–
β-*N*-Acetyl Galactosaminidase	3	2	–	2	0.5	–	–
α-*N*-Acetyl Glucosaminidase	–	1	0.5	–	–	–	–
β-*N*-Acetyl Glucosaminidase	30	30	1.5	25	0.5	–	–
α-Mannosidase	1	2	0.5	0.5	–	–	–
β-Mannosidase	–	–	NT	NT	–	NT	1.5
β-Xylosidase	–	–	0.2	–	–	NT	NT

[a]Enzyme activity from 1 ml supernatant plus 1 ml cell lysate
'–' 0.1 U ml^{-1} or below; NT not tested.

E. histolytica produced an active β-*N*-acetylglucosaminidase and *Giardia* also produced this enzyme, although in smaller amounts together with a more varied distribution of other glycosidases. A limited number of glycosidases were observed in *Leishmania* and only β-mannosidase and low amounts of α-glucosidase were found in the trypanosomes *T. brucei* and *T. cruzi*.

Discussion

Glycosidase production, in particular, the production of β-*N*-acetylglucosaminidase, appears to be a distinguishing feature of the mucin-dwelling pathogens used in this study based on the paranitrophenyl assay.

The urogenital and gastrointestinal tracts are protected by thick layers of mucin and so are able to provide a rich source of substrate carbohydrate for the action of both exo- and endoglycosidases for the release of oligosaccharides and monosaccharides [10]. Mucin is a high molecular weight glycoprotein comprising up to 80% carbohydrate. This carbohydrate portion surrounds and protects the protein backbone of the molecule and is composed of chains of repetitive units of *N*-acetyllactosamine [20]. It appears that organisms living in this environment and producing glycosidases have the capacity to hydrolyse this complex molecule. Organisms producing either β-*N*-acetylglucosaminidase, β-galactosidase or α-*N*-acetylgalactosaminidase, such as *T. foetus* and *T. vaginalis* have the ability to destroy the mucin either as part of the pathological process or in order to provide energy.

Degradation of this mucin would result in the impairment of its structure possibly leading to the formation of lesions in the tract linings that are so often associated in diseases such as those caused by *Giardia*, *Entamoeba* and *Trichomonas* spp [12–14]. The *Trichomonads*, *Entamoeba* and *Giardia* all produced β-*N*-acetylglucosaminidase when tested with paranitrophenyl substrates. However, the trichomonads appeared unique in that they produced a greater range of higher activity enzymes than the other organisms tested. Indeed, the trichomonads were the only organisms tested which appeared to possess all the enzymes that would be required to break down the carbohydrate chains of mucin.

The discovery of the vast array of glycosidases produced by the trichomonads could simply have reflected adaptation to their environment. One could then hypothesise that all mucin dwellers would possess similar ranges and activities of glycosidases. The amount and type of glycosidase produced would reflect the specific nature of the mucin itself since mucin is known to differ in different environments [20]. However, the results presented suggest that, in fact, the trichomonads are different from the other protozoans tested and that mucin degradation by glycosidases is not a common feature of all mucin dwelling organisms.

Mucin degradation has been described in other mucosal inhabiting micro-organisms [21]. Certain bifidobacterial and ruminococcal strains in the human colon have been found to degrade the carbohydrate chains of mucin by the production of extracellular glycosidases.

In this study, due to the limited availability of samples, we did not investigate the activities of neuraminidases in the organisms. Since mucins may contain variable amounts of sialic acid, this enzyme would be crucial in the degradation of mucin. However, neuraminidase is present at high levels in the trichomonads and a trans-sialidase has been described in trypanosomes [22].

Despite knowledge of alterations in the tracts caused by parasitic activity, it is still difficult to determine their significance to health and well-being of the host. It is also necessary to be aware and consider the limitations of the assay which may not detect different specificities of the glycosidases in question. It is known that, for example, fucosidases in *T. foetus* cannot be detected using para-nitrophenyl-sugar substrates (Greenwell, unpublished observations). Thus, a more detailed study involving more natural substrates is required.

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