Galactosamine-synthesizing enzymes are induced when *Giardia* encyst

By: P. Timothy Macechko, Paul A. Steimle, Donald G. Lindmark, Stanley L. Erlandsen, and Edward L. Jarroll

Macechko, P.T., Steimle, P.A., Lindmark, D.G., Erlandsen, S.L., Jarroll, E.L. (1992) Galactosamine synthesizing enzymes are induced when *Giardia* encyst. *Molecular and Biochemical Parasitology*. 56:301-310.

Abstract:

Galactosamine, a *Giardia* filamentous cyst wall specific-sugar, is below the limits of detection in non-encysting trophozoites. Radiolabeling studies suggest that *Giardia* synthesize galactosamine primarily from endogenous glucose rather than salvage it from the environment. Enzymes responsible for galactosamine synthesis from glucose are induced during encystment and have been characterized in crude homogenates and in supernatant (soluble) fractions. These enzymes (specific activity; time after encystment is induced for maximal activity; x-fold increase) include glucosamine 6-phosphate isomerase (in the deaminating direction, 167 mU mg protein^{-1}; 20 h; x 182-fold; in the aminating direction, 258 mU mg protein $^{-1}$; 20 h; x 13-fold), glucosamine 6-phosphate N-acetylase (11 mU mg protein^{-1}; 20 h; x 20-fold), phosphoacetylglucosamine mutase (160 mU mg protein^{-1}; 20 h; x 12-fold), UDP-N-acetylglucosamine pyrophosphorylase (22 mU mg protein^{-1}; 48 h; x 8-fold), and UDP-N-acetylglucosamine 4'-epimerase (13 mU mg protein^{-1}; 48 h; x 4000-fold). This represents the first report of these enzymes and of an inducible carbohydrate-synthesizing pathway in any protozoan.
Key words: *Giardia;* Protozoan metabolism; Encystment; Inducible enzymes; Galactosamine synthesis
Abbreviations: F, fructose; Glc, glucose; UDP-Glc, uridine diphosphoglucose; G1cN, glucosamine; Gal, galactose; Ga1N, galactosamine; Man, mannose; UDP-G1cNAc, uridine-5'- diphospho-N-acetylglucosamine; UDP-Ga1NAc, uridine-5'-diphospho-N-acetylgalactosamine; UDP-Gal, uridine diphosphogalactose; Rib, ribose; AP, acid phosphatase; ME, malate enzyme (malate dehydrogenase decarboxylating); GF 6-PAT, L-glutamine D-fructose 6-phosphate amidotransferase; G1cN 6- PI, glucosamine 6-phosphate isomerase; GlcN 6-PNA, glucosamine 6-phosphate N-acetylase; PAG1cNM, phosphoacetylglucosamine mutase; UDP-G1cNAcPP, UDP-N-acetylglucosamine pyrophosphorylase; UDP-G1cNAcE, UDP-N-acetylglucosamine 4'-epimerase.

Article:

Introduction

The *Giardia* cyst wall is composed of an outer frlamentous and an inner membranous portion [1,2]. The inner membranous portion (inner cyst wall) consists of 2 membranes: the inner membrane which borders the peritrophic space (the space between the cyst wall and trophozoites within), and the outer membrane which appears to serve as an attachment site for the outer cyst wall. The outer cyst wall is approximately 0.3-0.5 pm thick and is composed of filaments which measure 7-20 nm in diameter and which are arranged in a tightly packed meshwork [2]. These frlaments appear unchanged by treatment with sodium dodecyl sulfate (SDS) [3] or with a variety of proteases [4]. However, SDS treatment followed by amylase digestion removes virtually all of the trophozoite material and the inner cyst wall [3,4].

Biochemical analyses of in vitro encysting *Giardia* by Ortega-Barria et al. [5] showed the presence of glucosamine (perhaps as N-acetylglucosamine) in detergent-extracted membranes and intracellular glycoproteins of cysts and trophozoites. Biochemical analyses of in vivo derived cysts by Jarroll et al. [3] and Manning et al. [4] indicated that approximately 43% of the dry weight of the *Giardia* outer filamentous cyst wall is made of carbohydrate, 86% of which is galactosamine (perhaps as *N*- acetylgalactosamine). Unlike glucosamine which appears in both trophozoites [5] and cysts [4,5], galactosamine is considered an outer filamentous cyst wall specific sugar because (1) it is below the limits of detection in non-encysting trophozoites

[3,5], (2) its concentration remains unchanged by treatments of cysts that leave only the outer filamentous cyst wall [4], and (3) *Phaseolus limensis* lectin, which has affinity for N-acetylD-galactosamine, binds exclusively to the outer filamentous portion of the cyst wall [3].

Because *Giardia* is well known for its salvage of essential nutrients from the growth medium [6], it is valid to ask if this parasite also salvages its major cyst wall sugar, galactosamine, during cyst wall synthesis. With the recent ability to complete the *Giardia* life cycle in vitro [7,8] comes the opportunity to study the differentiation of encystment biochemically. Understanding encystment is potentially important, not only from a parasite control perspective, but also from a cell and molecular biology perspective since ribosomal gene sequence comparisons suggest that *Giardia* is the most primitive eucaryotic cell type studied to date [9]. Thus, the purpose of this study is to evaluate the biochemical origin of galactosamine in the *Giardia* cyst wall during encystment to determine if it is synthesized or salvaged.

Materials and Methods

Materials. All chemicals were of reagent grade and were purchased from Sigma (St. Louis, MO) unless otherwise indicated.[U-14C]glucose (308 mCi $mmol^{-1}$) (Glc),$^{-14}$ j C]glucosamine (45 mCi $mmol^{-1}$) (G1cN), [$^{-14}$ u C]galactose (108 mCi $mmol^{-1}$) (Gal), and [U-14C]mannose (300 mCi $mmo1^{-1}$) (Man)were from ICN (Costa Mesa, CA); [1-14C] galactosamine (55.4 mCi $mmol^{-1}$) (GalN) was from Amersham (Arlington Heights, IL); UDP-N-acetyl-D41^{-14}C]glucosamine (283.8 mCi $mmol^{-1}$) (UDP-G1cNAc), UDP-N-acetyl-D-[1-14C]galactosamine (55 mCi $mmol^{-1}$) (UDP-Ga1NAc) and UDP-D[U-14C]galactose (272.8 mCi $mmol^{-1}$) (UDPGal) were from New England Nuclear (Boston, MA). All radioisotopes were at least 97% pure, but they were further purified by preparative thin layer chromatography (TLC) prior to use. Sugar standards for chromatography were purchased from Supelco (Bellefonte, PA). Whatman silica gel and Merck PEI cellulose-F TLC plates were purchased from Curtin Matheson (Cleveland, OH).

Organism. Giardia duodenalis (strain MR4) trophozoites were grown axenically in TYI-S33 medium either lacking added bile (non- encysting) [3] or supplemented with 1 mg $m1^{-1}$ (non-encysting) [10], or 10 mg ml¯ (encysting) [8]. Trophozoite growth and encystment in these protozoans is asynchronous, and the percentage of encystment normally ranged from 18 to 40%.

Incorporation of galactosamine precursors. Radiolabeled Glc, Gal, Man, GlcN and GalN were added to the growth medium of non-encysting trophozoites at a fInal concentration of 0.1 µCi ml-1at the initiation of the culture. Trophozoites were harvested after 96 h growth by chilling the tubes on ice for 15 min and then centrifuging the medium at 750 *g.* Harvested cells were washed 3 x with 0.1 M phosphate-buffered saline **(PBS,** pH 7.2).

Incorporation of ^{14}C-sugars into encysting trophozoites was assessed using all of the precursors previously listed with the addition of UDP-14C-GlcNAc. These labeled sugars were added to trophozoite cultures at 0.1 Ci $m1^{-1}$at the time encystment was induced. The cells were allowed to encyst in the presence of the ^{14}C-sugar for 72 h [8] after which the cells were harvested as described above.

Non-encysting trophozoites were grown in medium containing ^{14}C-Glc (0.1 ,uCi $m1^{-1}$) for 72 h, removed from this medium, and induced to encyst [8] in medium containing no radio- labeled Glc. After 48 h in the encystment medium, encysting cells were harvested as described above and enriched for cyst forms by washing the cells in distilled water [8]. In some of these encystment experiments, enriched preparations of cyst walls were made by treating the cells with SDS [3].

Hydrolysis, sugar analyses and chromatography. Radiolabeled cells were hydrolyzed in 4 N HCl under nitrogen and sugars were collected [3]. Sugars were separated by silica gel TLC in a solvent system of isopropanol:acetone:0.1 M lactic acid (4:4:2, v/v/v) and were visualized by staining the plates with diphenylamine—aniline—phosphoric acid [11]. Verification of these sugars was accomplished by eluting the sugars from

parallel TLC plates, making trimethylsilane derivatives, and analyzing them by gas chromatography (GC) and mass spectrometry (MS) [3]. Sugar spots were scraped from the TLC plate and the incorporation of radiolabel was assessed by counting in a liquid scintillation counter (Beckman Instruments, Palo Alto, CA).

Enzyme assays. Enzyme specific activities and pH optima reported in this paper were assessed in homogenates of encysting or non- encysting *Giardia* with and without Triton X100. Cells were homogenized with a Potter-Elvehjem tissue homogenizer, fractions were prepared by differential sedimentation resulting in a nuclear fraction (containing nuclei, fragments of flagella, adhesive discs, and cysts after sedimentation at 2500 rpm for 4 min), and a particle fraction (containing lysosomes was sedimented at 19 000 rpm for 60 min). The soluble fraction was the supernatant remaining after sedimenting the particles, and protein concentrations were determined [12,13]. Acid phosphatase (EC 3.1.3.2) and malate enzyme (malate dehydrogenase decarboxylating) (EC 1.1.1.37), marker enzymes for the particle and soluble (S) fractions, respectively, were assayed [12,14]. All enzyme activities were assessed in encysting trophozoites at fixed time points during the encystment process.

L-glutamine D-fructose 6-phosphate amidotransferase (EC 2.6.1.16; GF 6-PAT) [15], glucosamine 6-phosphate isomerase (EC

TABLE I

Representative incorporation of *N*-acetylgalactosamine precursors into sugars from hydrolysates of *Giardia duodenalis*

^{14}C-Sugar	*p*mol[a] (mg dry weight)$^{-1}$	Recovery[b] *p*mol of sugar	Percent incorporation as cellular			
			Glc	GalN	GlcN	Rib
Non-encysting trophozoites[c]						
Glc	11	6	66	13	12	9
GlcN	31	17	78	4	13	5
In vitro encysting trophozoites[d]						
Glc	10	1	–	–	–	–
GlcN	1	1	–	–	–	–
Trophozoites labeled for 72 h prior to encystment						
Glc	6	5	54	12	24	10
SDS generated cyst walls						
Glc	15	8	10	50	5	35

Glc = glucose; GalN = galactosamine; GlcN = glucosamine; Rib = ribose.
[a] Total incorporation excluding gaseous end products.
[b] Recovery excludes non-sugar end products.
[c] Exogenous radiolabeled GalN, galactose and mannose were not detected in cellular sugars recovered from incorporation studies.
[d] Incorporation of exogenous radiolabeled GalN, galactose, mannose and UDP-GlcNAc were below the limits of detection.

5.3.1.10; GlcN 6-PI) [16-18], glucosamine 6- phosphate N-acetylase (EC 2.3.1.4; GIcN 6PNA) [19], phosphoacetylglucosamine mutase (EC 2.7.5.2; PAG1cNM) [20], and uridine diphosphoacetylglucosamine pyrophosphorylase (EC 2.7.7.23; UDP-G1cNAcPP) [21] activities were assessed spectrophotometrically. Uridine diphosphate N-acetylglucosamine 4'- epimerase (EC 5.1.3.7; UDP-G1cNAcE) activity was measured using radiolabeled UDPG1cNAc or UDP-Ga1NAc [22,23]. The activity of uridine diphosphoglucose 4'-epimerase (EC 5.1.3.2; UDP-G1cE) was assessed using radio- labeled UDP-Glc [23].

For, UDP-G1cNAcPP and UDP-G1cNAcE, the pH optima were assessed in 0.2 mM sodium phosphate buffer. The pH optimum for G1cN 6-PNA was assessed in 0.1 M acetate—borate—citrate buffer, and the pH optima for G1cN 6-PI and PAG1cNM were assessed in 0.1 M Tris buffer.

The subcellular localization of each enzyme was assessed following differential sedimentation [13]. Enzyme kinetics were determined from Lineweaver—Burk plots of data resulting from at least 3 experiments using S-fractions from differential sedimentations as enzyme and varying the concentration of substrate(s)

TABLE II

Characteristics of the inducible enzymes of galactosamine synthesis in the soluble fractions of encysting *Giardia duodenalis*

Enzyme	Substrate	Apparent		Optimal pH	Maximal Sp. Act.	Time (h) for maximal Sp. Act.	Fold increase*
		K_m	V_{max}				
GlcN6PId	GlcN6P	0.15	515	8.5	167	20	182
GlcN6PIa	F6P	6.06	1320				
	NH_4Cl	100	7100	7–9	258	20	13
GlcN6PNA	GlcN6P	7.5	145				
	ACoA	15	429	5	11	20	20
PAGlcNM	GlcNAc1P	0.7	148				
	Glc1, 6diP	0.03	9				
	Mg^{2+}	1.2	50	8	160	20	12
UDP-GlcNAcPP	UDP-GlcNAc	0.83	33.3				
	PPi	1	37				
	Mg^{2+}	0.24	32.3	6–8	22	48	8
UDP-GlcNAcE	UDP-GalNAc	0.0114	0.1				
	UDP-GlcNAc	0.0011	0.14				
	UDP-Glc	–	–				
	UDP-Gal	–	–	7	13	48	4000

Enzymes: GlcN6PI = glucosamine 6-phosphate isomerase (d = deaminating; a = aminating); GlcN6PNA = glucosamine 6-phosphate *N*-acetylase; PAGlcNM = phosphoacetylglucosamine mutase; UDP-GlcNAcPP = UDP-*N*-acetylglucosamine pyrophosphorylase; UDP-GlcNAcE = UDP-*N*-acetylglucosamine 4′-epimerase. L-glutamine D-fructose 6-phosphate amidotransferase and UDP-glucose 4′-epimerase activities were not detected in encysting or non-encysting trophozoites.
Substrates: GlcN6P = glucosamine 6-phosphate; F6P = fructose 6-phosphate; ACoA = acetyl CoA; GlcNAc1P = *N*-acetylglucosamine 1-phosphate; Glc1,6diP = glucose 1,6-diphosphate; UDP-GlcNAc = UDP-*N*-acetylglucosamine; UDP-GalNAc = UDP-*N*-acetylgalactosamine; UDP-Glc = UDP-glucose; UDP-Gal = UDP-galactose; PPi = pyrophosphate.
K_m concentrations are given in mM; V_{max} values are in nmoles min^{-1} mg $protein^{-1}$.
Sp. Act. = specific activity in mU mg $protein^{-1}$.
* Fold increase represents the increase in activity of the enzyme in encysting cells above that in non-encysting cells.

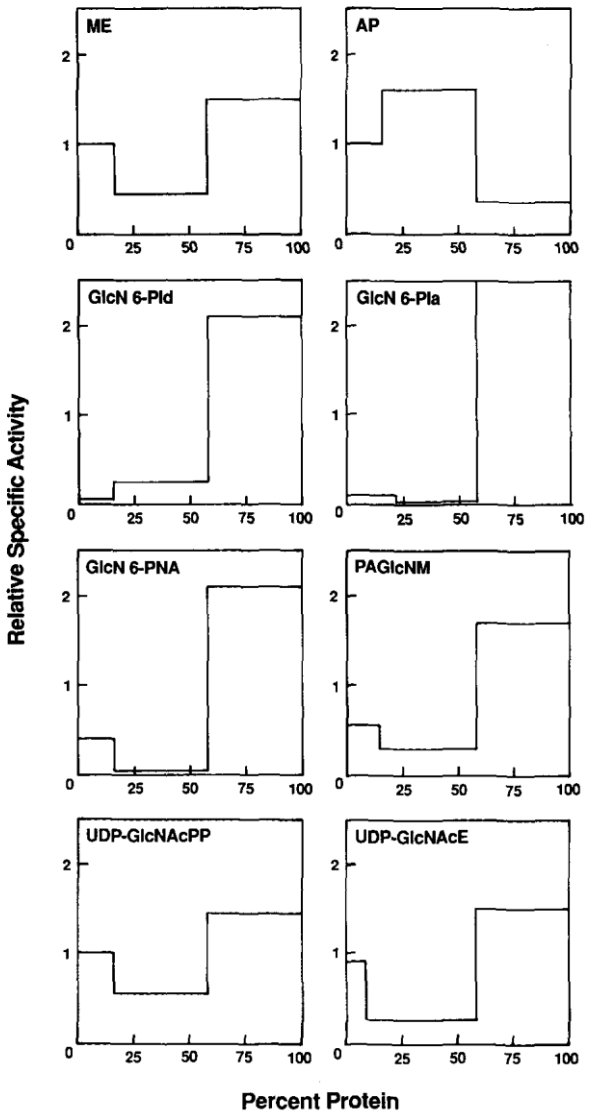

Fig. 1. Representative distribution of enzymes after differential centrifugation of encysting *Giardia duodenalis* trophozoite homogenates. Relative specific activity was plotted against cumulative percentage of protein recovered in each fraction. In the figure, the direction from left to right corresponds to increasing centrifugal force. The far right block represents the final supernatant (S fraction). Centrifugation conditions are described elsewhere [17]. Recoveries were 114% for malate enzyme (ME), 113% for acid phosphatase (AP), 102% for glucosamine 6-phosphate isomerase deaminating (GlcN 6-PId), 131% for glucosamine 6-phosphate isomerase aminating (GlcN 6-PIa), 98% for glucosamine 6-phosphate *N*-acetylase (GlcN 6-PNA), 136% for phosphoacetylglucosamine mutase (PAGlcNM), 79% for UDP-*N*-acetylglucosamine pyrophosphorylase (UDP-GlcNAcPP), and 153% for UDP-*N*-acetylglucosamine 4′-epimerase (UDP-GlcNAcE), and 124% for protein.

in the assay mixture.

With the exception of GlcN 6-PI, and preliminary time and temperature optima characterizations, all assays were performed at 37°C for 30 min (G1cN 6-PI assays were for 10 min). One unit of enzyme activity is defined as the micromoles of substrate used or product formed per minute. Enzyme specific activity is either in units mg protein^{-1} or in equivalent nanomoles min^{-1}mg protein^{-1} (mU (mg protein)$^{-1}$) for V_{max}.

Results

Incorporation of galactosamine precursors. Table I shows the results of incorporation patterns of ^{14}C-labeled sugars into non- encysting and encysting trophozoites, and SDS-generated cyst walls of *G. duodenalis.* Incorporation of ^{14}C-Glc into cellular ribose was detected in all samples. Only ^{14}C-Glc and ^{14}C-GlcN were recovered as sugars in non- encysting and encysting trophozoites. In the non-encysting trophozoites, 66% of the ^{14}C- Glc was recovered as cellular Glc and 78% of the ^{14}C-G1cN was recovered as cellular Glc. While ^{14}C-Glc was incorporated into encysting trophozoites to the same extent as in non- encysting trophozoites, little was present as recoverable sugar; incorporation of ^{14}C-GlcN by encysting trophozoites was much lower than that for non-encysting cells. Incorporation of all other exogenous, labeled GalNAc precursors used into cellular sugars was not detected in any case.

When non-encysting cells were grown in ^{14}C-Glc for 72 h prior to induction of encystment and encystment was induced in the absence of ^{14}C-Glc, there was a decrease in the amount of ^{14}C-Glc recovered as cellular Glc (54%) while there was an increase in the amount recovered as cellular GlcN (24%). When encysting cells, labeled with ^{14}C-Glc prior to encystment, were enriched for cyst walls by SDS treatment, 50% of the ^{14}C-Glc was recovered as cellular GalN, 10% was recovered as cellular Glc and only 5% as cellular GlcN. The incorporation of exogenous

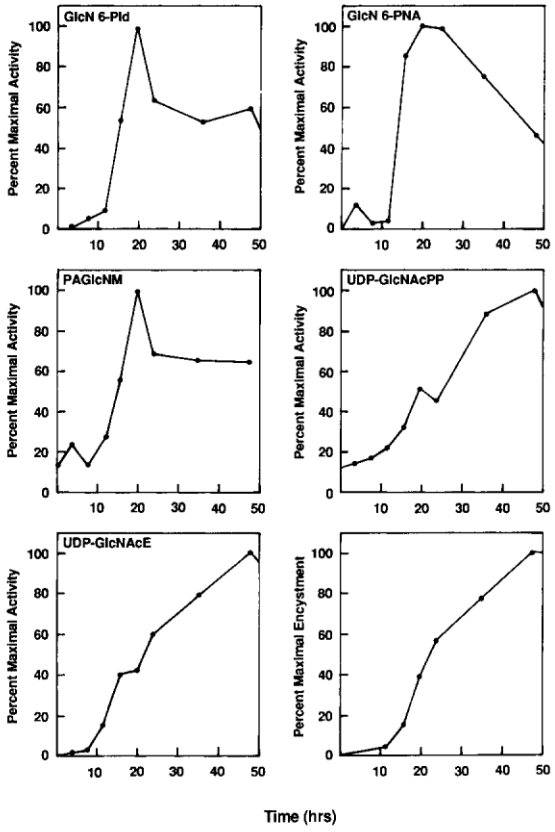

Fig. 2. Representative plot of changes in enzyme activity during encystment of *Giardia duodenalis* trophozoites. The percent maximal activity for the enzymes and the percent maximal encystment are plotted against the hours that trophozoites were in encystment medium. Maximal activities: glucosamine 6-phosphate isomerase deaminating (GlcN 6-PId), 167 mU mg protein^{-1}; glucosamine 6-phosphate *N*-acetylase (GlcN 6-PNA), 11 mU mg protein^{-1}; phosphoacetylglucosamine mutase (PAGlcNM), 142 mU mg protein^{-1}; UDP-*N*-acetylglucosamine pyrophosphorylase (UDP-GlcNAcPP), 22 mU mg protein^{-1}; UDP-*N*-acetylglucosamine 4′-epimerase (UDP-GlcNAcE), 13 mU mg protein^{-1}. In this experiment, 100% maximal encystment represents an actual percent encystment of 21% of the trophozoites induced to encyst.

radiolabeled GalN, Gal, Man, and UDP- GlcNAc into cellular sugars was not detected.

Enzymes. Table II shows the characterization of inducible enzymes detected in encysting *G. duodenalis* trophozoites. The activity of GF 6- PAT and that of UDP-G1cE were not detected in either encysting or non-encysting *G. duodenalis* trophozoites.

GlcN 6-PId and GlcN 6-PIa activities were non-sedimentable (Fig. 1) and exhibited pH optima of 8.5 and 7-9, respectively. GlcN 6-PId activity was detectable in non-encysting trophozoites at about 0.9 mU mg protein^{-1}, but the activity increased 182-fold by 20 h after encystment began (Fig. 2). GlcN 6-PIa was detectable in non-encysting trophozoites at approximately 20 mU mg protein^{-1}but its activity increased 13-fold by 20 h after encystment was induced. Whether these isomerase activities (deaminating and aminating) represent the same enzyme or different enzymes remains to be determined.

GlcN 6-PNA non-sedimentable activity (Fig. 1) was optimal at pH 5. This enzyme exhibited at least a 20-fold increase in activity 20 h after encystment was induced (Fig. 2); GlcN 6-PNA activity in non-encysting trophozoites was < 0.2 mU mg protein $^{-1}$. N-acetylation of glucosamine was also detected with a specific activity of < 0.5 mU mg protein $^{-1}$in non-encysting cells and ca. 1 mU mg protein^{-1}in encysting trophozoites. Whether these represent the same enzyme or different enzymes remains to be determined.

PAG1cNM requires Mg^{2+} , Glc 1,6-diphosphate, and diethyldithiocarbamate. The optimal pH was 8.0 for this non-sedimentable enzyme activity (Fig. 1). The activity of this enzyme increased 8- to 12-fold by 20 h into the encystment process (Fig. 2).

Non-sedimentable activity of UDPG1cNAcPP (Fig. 1) exhibited a broad pH optimum between 6 and 8. Activity of this enzyme was detected in non-encysting trophozoites at ca. 1.5 mU mg protein^{-1}, but this increased 8-fold by 48 h after the induction of encystment (Fig. 2). Non-specific pyrophosphorylase activity was not detected.

UDP-G1cNAcE activity was non-sedimentable (Fig. 1) and it exhibited a pH optimum of 7. UDP-Glc and UDP-Gal were unable to substitute for UDP-G1cNAc and UDP-GalNAc. Peak activity for the epimerase was at 48 h after induction of encystment (Fig. 2), and this represented a 4000-fold increase in epimerase activity when compared to that in non-encysting trophozoites.

While Fig. 2 shows the increase in activity of these enzymes for the first 48 h of encystment, all were detected for up to 72 h, but in all cases their activities had decreased from the maximal values obtained (data not shown). Low levels of some of these enzymes were detected in homogenates that were not induced to encyst. These low activities were detected in the presence or absence of 1 mg m1^{-1} added bile.

Discussion

Data presented in this paper show that encysting *G. duodenalis* synthesize rather than salvage GaIN. That salvage is not a major source of Ga1N for *Giardia* cyst wall formation was shown by the lack of detectable incorporation of Ga1N into the sugars of either encysting or non-encysting trophozoites. When non- encysting trophozoites were labeled with ^{14}C- Glc prior to encystment, and these trophozoites were induced to encyst in isotope-free medium, intense labeling of the GaIN recovered from enriched cyst wall preparations (SDS generated) was detected. This intense labeling of GaIN from the incorporated ^{14}C- Glc suggests that much of the Ga1N for cyst wall synthesis is derived synthetically from endogenous Glc. It is not surprising that Glc is a major source of GaIN since Glc is the most abundant endogenous cyst and trophozoite sugar (ca. 72 nmol 10^{-6} cysts; ca. 131 nmol 10^{-6} trophozoites) [3,4] and it is the only exogenous sugar shown to stimulate respiration in *Giardia* trophozoites [12,24-26]. The apparent incorporation of exogenous GlcN into cellular Glc may be accounted for by the presence of a reversible G1cN 6-PI. The activities of G1cN and GlcNAc kinase which catalyze the conversion of G1cN and GlcNAc to GlcN 6-phosphate and GlcNAc 6-phosphate, respectively, have not yet been demonstrated in encysting *Giardia.* Work is in progress to assess these activities. However, radiolabeling studies in non-encysting trophozoites suggest that GleN kinase activity is present since much of the G1cN incorporated by these trophozoites is apparently converted to Glc. Since GlcN can be converted to

GlcNAc, whether by a specific enzyme or as a consequence of GlcN 6-PNA activity, it is conceivable that GlcNAc kinase activity might also be present.

Although Glc was incorporated by encysting cells to approximately the same extent as in non-encysting cells, the low level of labeled Glc detected as sugar suggests that Glc may have been taken up by cells early in the encystment process and perhaps incorporated as amino acids [27,28]. The low level of G1cN uptake by encysting trophozoites suggests that GlcN may not normally be taken up as early from the culture medium as Glc or that GlcN transport in encysting trophozoites may be altered prior to incorporation of significant amounts of GlcN. Incorporation of radiolabeled Glc into ribose is not surprising since *Giardia* have an active pentose phosphate pathway [25].

The second line of evidence that *G. duodenalis* synthesize rather than salvage GaIN comes from the fact that the enzymes necessary for that synthesis have been detected in encysting *G. duodenalis* trophozoites. This paper represents the first report of these enzymes in protozoa. The fact that all of the enzymes in this pathway appear inducible (i.e., the specific activity of the enzyme increases in response to an inducer) during encystment is indeed interesting since it represents the first report of inducible carbohydrate synthesizing enzymes in any protozoan.

The specific activity of the enzymes reported here may be deceptively low since they are reported with reference to total protein. Since the trophozoites are not growing or encysting synchronously and since only from 18 to 40% of the trophozoites encyst, use of total protein as a reference may artificially decrease the speciíic activity. Furthermore, the lack of synchronous growth and encystment of these trophozoites produces some variation in the time when each enzyme activity peaks. The results presented, however, are typical of the most common times when the activities reach a maximal level. However, the enzyme activities early in the synthetic pathway are apparently induced earlier than those later in the pathway. Characterization of these enzymes in crude

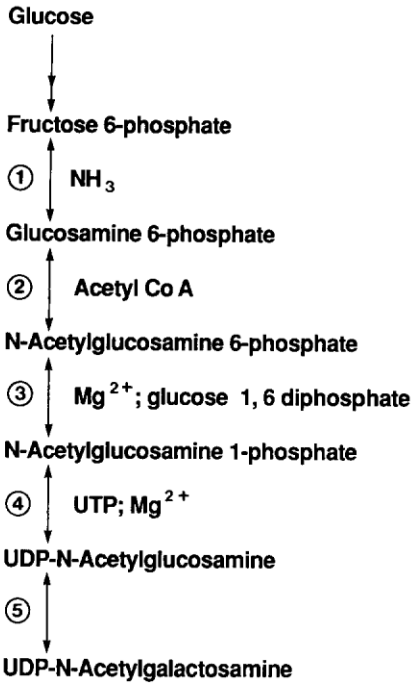

Fig. 3. Proposed pathway of UDP-*N*-acetylgalactosamine synthesis in encysting *Giardia* trophozoites. The enzymes involved include (1) glucosamine 6-phosphate isomerase, (2) glucosamine 6-phosphate *N*-acetylase, (3) phosphoacetylglucosamine mutase, (4) UDP-*N*-acetylglucosamine pyrophosphorylase, and (5) UDP-*N*-acetylglucosamine 4′-epimerase.

homogenates and S-fractions is presented here as part of a preliminary analysis of these enzymes. More accurate characterization of these enzymes must await their purification which is currently underway.

Based on the above, the following pathway for the synthesis of UDP-N-acetylgalactosamine in encysting *Giardia* is proposed (Fig. 3): The enzymes responsible for converting Glc to F 6-phosphate have not been included in this study or in Fig. 3 since the presence of an active Embden—Meyerhof—Parnas pathway in *Giardia* has been demonstrated elsewhere [12,25,29].

The exact nature of the inducer in this system is presently unknown, but presumably it (they) is (are) one or more of the components of bile or its degradation products. Regulation of these inducible enzymes could be either by control of transcription of the genes for these enzymes or by controlled conversion of the inactive protein to the functional enzyme. Although studies are in progress to determine the nature of the inducer and which of these regulatory mechanisms is operating in encysting *Giardia*, the answers to these questions for encysting *Giardia* remain, at present, unresolved.

In summary, encysting *Giardia* trophozoites synthesize their major cyst wall sugar, galactosamine, primarily from endogenous glucose via a pathway of enzymes the activities of which are induced within 20-48 h following induction of encystment.

Acknowledgements

We wish to thank Ms. M.J. Robles and Ms. K.M. Schmidt for their technical assistance. Support for this research came from the OBOR Academic and Research Challenge Programs in Molecular Parasitology and from NIHAID grant No. AI29591 (E.L.J.).

References

1 Feely, D.E., Erlandsen, S.L. and Chase, D.G. (1984) Structure of the trophozoite and cyst. In: *Giardia* and Giardiasis (Erlandsen, S. and Meyer, E., eds.), pp. 3-31, Plenum Press, NY.

2 Erlandsen, S.L., Bemrick, W. and Pauley, J. (1989) High-resolution electron microscopic evidence for the filamentous structure of the cyst wall of *Giardia muris* and *Giardia duodenalis.* J. Parasitol. 75, 787-797.

3 Jarroll, E.L., Manning, P., Lindmark, D.G., Coggins, J.R. and Erlandsen, S.L. (1989) *Giardia* cyst wall specific carbohydrate: evidence for the presence of galactosamine. Mol. Biochem. Parasitol. 32, 121-132.

4 Manning, P., Erlandsen, S.L. and Jarroll, E.L. (1992) Carbohydrate and amino acid analyses of *Giardia muris* cysts. J. Protozool. 39, 290-296.

5 Ortega-Barria, E., Ward, H., Evans, J.E. and Pereira, M.E.A. (1990) N-acetylglucosamine is present in cysts and trophozoites of *Giardia lamblia* and serves as receptor for wheatgerm agglutinin. Mol. Biochem. Parasitol. 43, 151-166.

6 Jarroll, E.L. and Lindmark, D.G. (1990) *Giardia* metabolism. In: Giardiasis (Meyer, E.A., ed.), pp. 6176, Elsevier, Amsterdam.

7 Gillin, F.D., Reiner, D.S., Gault, M., Douglas, H., Das, S., Wunderlich, A. and Sauch, J. (1987) Encystation and expression of cyst antigens by *Giardia lamblia* in vitro. Science 235, 1040-1043.

8 Schupp, D., Januschka, M., Sherlock, L., Stibbs, H., Meyer, E., Bemrick, W. and Erlandsen, S. (1988) Production of viable *Giardia* cysts in vitro: determination by fluorogenic dye staining, excystation and animal infectivity in the mouse and Mongolian gerbil. Gastroenterology 95, 1-10.

9 Sogin, M.L., Gunderson, J.H., Elwood, J., Alonso, R.A. and Peattie, D.A. (1989) Phylogenetic meaning of the kingdom concept: an unusual ribosomal RNA from *Giardia lamblia.* Science 243, 75-77.

10 Keister, D.B. (1983) Axenic culture of *Giardia lamblia* in TYI-S-33 medium supplemented with bile. Trans. R. Soc. Trop. Med. Hyg. 77, 487-488.

11 Churms, S.C. (1982) Carbohydrates. In: Handbook of Chromatography (Zweig, G. and Sherma, J. eds.), CRC Press, Boca Raton, FL.

12 Lindmark, D.G. (1980) Energy metabolism of the anaerobic protozoan *Giardia lamblia.* Mol. Biochem. Parasitol. 1, 1-12.

13 Lindmark, D.G. (1988) *Giardia lamblia:* localization of hydrolase activities in lysosome-like organelles in trophozoites. Exp. Parasitol. 65, 141-147.
14 Muller, M. (1973) Biochemical cytology of trichomonad flagellates. I. Subcellular localization of hydrolases, dehydrogenases, and catalase in *Tritrichomonas foetus.* J. Biol. Chem. 254, 1526-1533.
15 Kornfeld, R. (1967) Studies of L-glutamine D-fructose 6-phosphate amidotransferase. J. Biol. Chem. 242, 3135-3141.
16 Clarke, J.S. and Pasternak, C.A. (1962) The regulation of amino sugar metabolism in *Bacillus subtilis.* Biochem.
J. 84, 185-191.
17 Bates, C.J. and Pasternak, C.A. (1965) Further studies on the regulation of amino sugar metabolism in *Bacillus subtilis.* Biochem. J. 96, 147-154.
18 Gopal, P., Sullivan, P. and Sheperd, M. (1982) Enzymes of N-acetylglucosamine metabolism during germ-tube formation in *Candida albicans.* J. Gen. Microbiol. 128, 2319-2326.
19 Davidson, E.A., Blumenthal, H.J. and Roseman, S.
(1954) Glucosamine metabolism. H. Studies on glucosamine 6-phosphate N-acetylase. J. Biol. Chem. 226, 125-133.
20 Reissig, J.L. (1956) Phosphoacetylglucosamine mutase of *Neurospora.* J. Biol. Chem. 219, 753-767.
21 Strominger, J.L. and Smith, M.S. (1959) Uridine diphosphoacetylglucosamine pyrophosphorylase. J. Biol. Chem. 234, 1822-1827.
22 Merrill, C.R., Das, A.K., LaPolla, R.J. and Prissovsky, I. (1976) Microassay for UDP-galactose 4'-epimerase activity. Anal. Biochem. 72, 606-613.
23 Kingsley, D.M., Kozarsky, K.F., Hobbie, L. and Krieger, M. (1986) Reversible defects in o-linked glycosylation and LDL receptor expression in UDPGal/UDP-GalNAc 4'-epimerase deficient mutant. Cell 44, 749-759.
24 Weinbach, E.C., Claggett, C.E., Keister, D., Diamond, L. and Kon, H. (1980) Respiratory metabolism in *Giardia lamblia.* J. Parasitol. 66, 347-350.
25 Jarroll, E.L., Muller, P., Meyer, E.A. and Morse, S.A. (1981) Lipid and carbohydrate metabolism of *Giardia lamblia.* Mol. Biochem. Parasitol. 2, 187-196.
26 Paget, T.A., Jarroll, E.L., Manning, P., Lindmark, D.G. and Lloyd, D. (1989) Respiration in the cysts and trophozoite forms of *Giardia muris.* J. Gen. Microbiol. 135, 145-154.
27 Paget, T., Kelly, M., Jarroll, E., Lindmark, D. and Lloyd, D. (1993) The effects of oxygen on fermentation in the intestinal protozoal parasite *Giardia lamblia.* Mol. Biochem. Parasitol. 57, 65-72.
28 Edwards, M., Gilroy, F., Jimenez, B. and O'Sullivan, W. (1989) Alanine is a major end product of metabolism by *Giardia lamblia:* a proton nuclear magnetic resonance study. Mol. Biochem. Parasitol. 37, 19-26.
29 Mertens, E. (1990) Occurrence of pyrophosphate: fructose 6-phosphate 1-phosphotransferase in *Giardia lamblia* trophozoites. Mol. Biochem. Parasitol. 40, 147150.