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ORIGINAL

Exploring Glycogen Processing Enzymes in Trichomonas vaginalis: Cloning, Characterization and Implications for Carbohydrate Metabolism and Cervical Cancer Risk

Exploración de las enzimas que procesan el glucógeno en Trichomonas vaginalis: clonación, caracterización e implicaciones para el metabolismo de los carbohidratos y el riesgo de cáncer de cuello uterino

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ABSTRACT

Trichomonas vaginalis, often known as T. vaginalis, is a protozoan that causes trichomoniasis, a prevalent sexually transmitted disease (STD) and a serious human pathogen. Glycogen is a branching polymer of glucose that the individual has been identified to accumulate for an elongated instance and then use when carbohydrates are insufficient. The crucial enzymes involved in glycogen production and investigate; Trichomonas vaginalis glycogen (TVG) syntheses and glycogen phosphorylase were considered. Research found that their regulatory features were different from known enzymes in animals and fungi. Bacterial recombinant synthesis of the open reading frames (ORF) TVAG 276310 produced proteins exhibiting branch and de-branch activity. Specifically, transgenic TVAG_276310 shows a preference for polysaccharides with long outside branches, which can include a substance and amylose undergrowth. Three proteins encompassed in the glucosidase formulation were identified by fluid chromatography and tandem mass spectrometry. The most notable was a putative-amylase, encoded by the TVAG_080000 ORF. Glycogen increase and branching were standardized after transgenic production of yeast cells devoid of enzyme movement that branches or de-branch glycogen.

Keywords: Trichomonas Vaginalis (T.vaginalis); Glycogen; TVAG 276310; TVAG_080000 ORF.

RESUMEN

Trichomonas vaginalis, a menudo conocido como T. vaginalis, es un protozoo que causa la tricomoniasis, una enfermedad de transmisión sexual (ETS) muy prevalente y un patógeno humano grave. El glucógeno es un polímero ramificado de glucosa que el individuo acumula durante un periodo prolongado y luego utiliza cuando los carbohidratos son insuficientes. Se investigaron las enzimas cruciales que intervienen en la

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producción de glucógeno: la síntesis de glucógeno de Trichomonas vaginalis (TVG) y la glucógeno fosforilasa. La investigación reveló que sus características reguladoras eran diferentes de las enzimas conocidas en animales y hongos. La síntesis recombinante bacteriana de los marcos de lectura abiertos (ORF) TVAG 276310 produjo proteínas que mostraban actividad de ramificación y desramificación. Concretamente, el TVAG_276310 transgénico muestra una preferencia por los polisacáridos con ramificaciones externas largas, que pueden incluir una sustancia y un subproducto de amilosa. Se identificaron tres proteínas incluidas en la formulación de glucosidasa mediante cromatografía líquida y espectrometría de masas en tándem. La más notable fue una amilasa putativa, codificada por el ORF TVAG_080000. El aumento y la ramificación del glucógeno se estandarizaron tras la producción transgénica de células de levadura desprovistas de movimiento enzimático que ramifica o desramifica el glucógeno.

Palabras clave: Trichomonas Vaginalis (T. vaginalis); Glucógeno; TVAG 276310; ORF TVAG_080000.

INTRODUCTION

Attaining vaginal mucosal homeostasis is a state of optimal reproductive health requires a eubiotic microbiota, which is defined as a harmonious community of beneficial bacteria and host components.[1] A dyspeptic vaginal microbiome is defined by an ever-changing community of bacteria and viruses that up-regulates the mucosal inflammatory tone and has various clinical effects. In addition to increasing the risk of sexually transmitted diseases (STDs), microbial symbiosis can lead to urogenital tract diseases and obstetric problems.[2] Although traditional techniques of diagnosis have failed to differentiate between the many functional subtypes, this difference has become crystal obvious due to modern ∝ omics technologies.[3] The most prevalent protozoan pathogenic illness that can be spread during oral, anal and vaginal intercourse is trichomoniasis, a urogenital infection. Worldwide, trichomoniasis has an alarmingly high incidence rate.[4] The endosomal membrane is invigorated to create exosomes, a subclass of extracellular vehicles (EVs) that are discharged into the extracellular environment. Their presence was detected in the fluid released by reticulocytes.[5] The lipid bilayer membrane encases a variety of cellular specialized lipids and nucleic acids, including metabolites, proteins and exosomes. These exosome payloads assist in cellular communication and the delivery of bimolecular with specialized activities.[6] The human body harbors a wide variety of microorganisms that form symbiotic interactions with various parts of the host. All of these microbes and the genetic information it contains is called the micro-biome or macrobiotic.[7] Research during the last few decades has shown that macrobiotics has an essential role in human immunology, hormone control and metabolic balance. Lactobacilli are the most common kind of bacteria in the human vaginal macrobiotic and their interactions with the uro genital protozoal parasite Trichomonas vaginalis (T. vaginalis) were of particular interest to the laboratory.[8] Factors such as age, geography and behavioral traits influence the composition of the human vaginal macrobiotic. Compared to the gut macrobiotic, the vaginal micro biome's design and function have received less attention.[9] Lactobacilli are the most common bacteria in the human vaginal micro-biome and focused on their interactions with T. vaginalis, a protozoal infection that affects the genital area.[10]The symptom brought on by a transvaginal is infection is described inwith the present understanding of the biology of the parasite and the mechanisms it uses to infect hosts.[11]Any relationships between stress, estrogen and glycogen levels as well as the species composition of the vaginal bacterial populations in a group of black teenage girls were identified.[12] An evaluation was made of the connections between the make-up of these communities, estradiol, glycogen and psychological stress. T. vaginalis, a micro aerophilic human parasite, as a leading cause of urogenital tract infections and a common STD globally described.[13] To keep oxygen-sensitive metabolic enzymes like Pyruvate: Ferredoxin Oxido reductase (PFOR) from becoming inactive, its anaerobic metabolism necessitates the rapid removal of intracellular oxygen. T. vaginalis iron-containing superoxide dismutase (TvSOD) was the primary protein represented due to its vital role for removing toxic reactive oxygen species (ROS) and converting oxidant to oxygen and hydrogen peroxide.[14] Modern techniques for determining the presence of T. vaginalis include culture, immune fluorescence, wet accumulate microscopy, and the enzyme-linked immune sorbent test and polymerase chain reaction was discussed.[15] The sexually transmitted parasite T. vaginalis attaches itself to cells lining the urogenital canal was examined and it was quite common.[16] Despite the importance of adherence to the host's survival for this extracellular parasite, more needs to be understood about the processes or molecules that play a role in this adherence. T. vaginalis grows in cold environments but recovers when exposed to the standard 37°C culture temperature was revealed.[17] However, it was unidentified how T. vaginalis controls this adaptation process. Vaginal bacteria metabolize glycogen because there's evidence linking human and bacterial enzymes to the process were represented.[18] A biochemical characterization of six glycogen-degrading enzymes (GDEs) from vaginal bacteria was presented that promote the growth of Lactobacillus crispatus, an amylase-deficient bacterium, on glycogen. Investigation described the protozoan

parasite T. vaginalis, which causes trichomoniasis, a widely distributed and prevalent sexually transmitted illness.[19] Since T. vaginalis does not possess the synthesis routes for purines and pyrimidines, one potential therapeutic target is nucleoside metabolism. The potential for cellulose polymerization, glycogen digestion and Raffinose family oligosaccharide (RFOs) accumulation were determined in the research.[20] There was an increase in the medium-chain fatty acid (MCFA) capric acid, whereas there was a significant decrease in the majority of the C18 fatty acids found.

METHOD

Analyzing the branching pattern of the TVG and Determination of the length of the surface chain

Measurement of ∝1,6-Glucosidase Activity: The branching pattern of *Trichomonas vaginalis* glycogen (TVG) was analyzed by measuring glucose release through ∝1,6-glucosidase activity. This activity indicates the extent of glycogen branching, as the enzyme hydrolyzes ∝1, 6-glycosidic bonds.

Quantification of Glucose-1-Phosphate Production: Phosphorylase activity was measured to determine glucose-1-phosphate production, which reflects glycogen debranching. The Illingworth method was used to estimate the average distance between glycogen branch points.

Sample Preparation and Enzyme Reaction

- TVG was dissolved in sodium phosphate buffer.
- Phosphorylase enzyme was added to initiate glycogen degradation.
- Samples were allowed to undergo enzymatic processing by being incubated at 30°C.

Assessment of Phosphate Release: After incubation, aliquots were collected, and phosphate release was measured using below.

- Glucose-6-phosphate dehydrogenase and phosphoglucomutase for liberated phosphate detection.
- Spectrophotometry at 340 nm to quantify NADH generation.
- Sugar-1-phosphate was calculated as the percentage of glucose released.

Determination of Surface Chain Length

Debranching Enzyme Treatment: The glycogen structure was further examined by incubating glycogen samples treated with phosphorylase with a glycogen de-branching enzyme.

Measurement of Released Glucose: The amount of glucose released by ∝1,6-glucosidase activity was measured.

- Hexokinase and glucose-6-phosphate dehydrogenase for enzymatic glucose quantification.
- The analysis provided structural insights into the branching pattern and chain length of TVG.

Colorimetric method

Risk Factors for Hyperemesis Gravidarum (HG): To develop hyperemesis during pregnancy, there are risk factors to consider. There is evidence that larger placental mass is associated with a greater prevalence of HG in mothers who are carrying multiple babies or who are carrying more than one baby at a time. In addition, pregnant women who, for reasons unrelated to pregnancy, have vomiting and nausea as a side effect of taking medicines that include oestrogen, being in an environment with a lot of motion, Women who have experienced migraines in the past are more likely to experience these symptoms once more while pregnant. In addition, a number of studies have shown that women who have first-degree relatives, who have HG, such as their mother or sister, have a higher risk of developing the condition themselves.

Absorbance Spectra of Glycogen: The structural features of the glycogen mixture were analysed by measuring its absorbance spectra. The polyglucan outer branch length and the wavelength of utmost absorption are correlated, with shorter branches corresponding to lower wavelengths.

Glycogen Extraction and Enzymatic Analysis in T. vaginalis

Obtaining glycogen through T. vaginalis

T. vaginalis cultures were grown in a glucose medium reaching a cell density of 2X106 cells/mL.Before being used again, the cell pellet was kept at 80°C and frozen in liquid nitrogen. The extraction process involved.

- Cell reconstruction and ethanol precipitation.
- Water suspension to dissolve glycogen.
- Enzymatic assay to measure glucose release and glycogen yield.

Cells were further cultivated in Tryptone Yeast Extract Glucose medium (TYG) medium, reaching a similar density before being harvested via centrifugation. Chromosomal DNA was extracted, and the gene TVAG_276310 was identified as a potential enzyme involved in glycogen branching.

Expression and Cloning of T. vaginalis Glycogen Enzyme

The yeast expression vector (pPICZαA) was used for cloning. Pichia pastoris GS115 strains were transformed with TVAG_276310 pPICZαA vectors via electroporation. Cultivation was performed in YPD medium, and protein expression was induced using methanol. Cells were lysed using homogenization buffer and benzonase nuclease for protein extraction.

Glycogen Branching and debranching activity

The enzymatic activity of TVAG_276310 in glycogen branching and debranching requires extensive biochemical analysis. By catalytic efficiency and substrate specificity, researchers can illuminate glycogen metabolism and its regulatory role in energy storage and consumption.

Affinity Gel Electrophoresis

TVAG_276310's interaction with glycogen and amylopectin affinity gel electrophoresis was conducted using the following condition.

- *Buffer:* Ph 8,3 with 250 Mm oyster glycogen and 25 Mm waxy maize amylopectin.
- Gel Composition: 7,5 % (v/w) polyacrylamide gels
- Protein addition: Purified-modified TVAG_276310 protein and an equivalent amount of bovine serum albumin.

Following polymerization, the gels were incubated in Tris-HCl (25 mM) and glycine (250 mM) and analysed for protein mobility and dissociation constants of the TVAG_276310/glycogen and amylopectin complexes.

Dissociation Constant Calculation

The interaction of TVAG_276310 with glycogen and amylopectin was analysed using the Equation 1. Where Q_n: Mobility relative to bovine serum albumin. Q_p: Mobility of TVAG_276310 without polysaccharide binding. [T]: Concentration of amylopectin or glycogen. L: Enzyme-substrate complex dissociation constant. Plotting 1/ Q_n against [T] produces a linear relationship with the dissociation constant defined by the x-axis intercept.

$$\frac{1}{Q_n} = \frac{1}{Q_p}\left(1 + \frac{[T]}{L}\right) \quad (1)$$

Protein Identification and Structural Analysis

To further analyze glycosidase activity Native PAGE (Polyacrylamide gel electrophoresis) and protein precipitation were applied.

Replication of ß-amylase with TVAG_080000 ORF

To investigate the replication of ß-amylase in T. vaginalis, total RNA was extracted using the TRIzol reagent method, followed by DNase I treatment to eliminate genomic DNA contamination. cDNA synthesis was performed using the SuperScript III Reverse Transcriptase to ensure high-fidelity cDNA generation.

Gene Amplification and Sequencing

- The 5′-end sequence of TVAG_080000 was identified using the next-generation sequencing (NGS).
- Primers WW129 and WW128 were designed quantitative real-time PCR (qRT-PCR) to confirm transcript expression levels.
- For sequencing, third-generation sequencing was used instead of Sanger sequencing to obtain full-length, high-accuracy reads.

Vector Construction and Bacterial Expression

- To facilitate transcription and cloning, Gibson Assembly was used to insert the gene into the bacterial expression vector pPICZαA instead of restriction enzyme-based cloning.
- The recombinant vector was transformed into Pichia pastoris X-33, a yeast expression system known for its ability to perform post-translational modifications and secrete recombinant proteins.
- The transformed yeast was cultured in Buffered Glycerol-Complex Medium (BMGY) at 30°C for cell growth, followed by induction in Buffered Methanol-Complex Medium (BMMY) for ß-amylase expression.
- The culture supernatant was collected, and secreted ß-amylase was purified directly from the medium to avoid cell lysis.

Protein Purification and Storage

- Ion-exchange chromatography (IEC) was used for protein purification.

- The purified protein was further refined using size-exclusion chromatography (SEC) and concentrated using ammonium sulfate precipitation.

RESULTS

The temperature was incubated and the pure TvBE containing amylose was 30°C. The reaction aliquots were added to a pH-adjusted iodine/KI mixture that included HCl at a concentration of about 2,4. The absorbance at 660,00 nm decreased linearly after 20 minutes as compared to a process without TvBE, as illustrated in Figure 1. Results showed a shift of 1,8 units/min/mg protein in the absorbance rate.

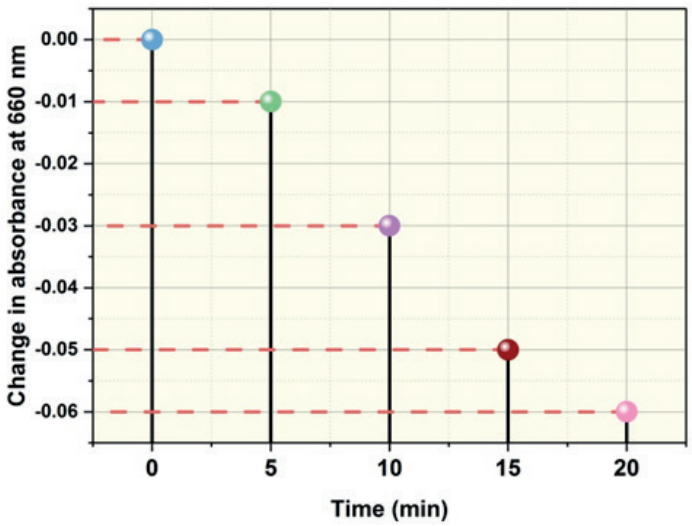

Figure 1. Evaluation of the combined TVAG 276310 protein's branching activity quantitatively.

Features of Crystal-clear substance

TVAG_080000 refers to a specific gene that encodes a protein in *Trichomonas vaginalis*, a protozoan parasite. The gene is identified by its unique code (TVAG_080000), and the protein it encodes is a part of the *Trichomonas vaginalis* proteome.

From the Native PAGE (Polyacrylamide Gel Electrophoresis) analysis presented in table 1, here is what each lane represents:

- Lane 1 (Molecular weight marker): This lane contains standard protein markers, used to determine the molecular weight of the proteins in the other lanes.
- Lane 2-4 (TVAG_080000 expression): These lanes show the expression of the TVAG_080000 protein. The key observation here is that the protein has a high molecular weight and is expressed as an insoluble protein, which suggests that the protein may aggregate or not be fully soluble under the conditions used for the analysis.

This kind of protein behavior could indicate that TVAG_080000 is involved in structural or membrane-associated functions, and it might require specific conditions or cofactors to be soluble. The high molecular weight could also suggest that the protein is part of a larger complex or has a functional role that requires it to interact with other proteins or cellular components.

Table 1. TLC Analysis of Glycogen Digestion by Purified Glucosidase

Incubation Time	Observed Digestive Products	Enzyme Activity Interpretation
5 min (A)	Maltose detected	Early-stage digestion
20 min (B)	Maltose remains prominent	Increased breakdown of glycogen
23 hr (C)	Larger sugar molecules detected	Presence of other enzyme activities (α-amylase)

TVAG_ 080000 in the Native PAGE

Table 2 presents the results of the Native PAGE analysis for the expression of the TVAG_080000 protein. The experiment involved running different samples on a gel to assess protein size, solubility, and integrity in its native (non-denatured) state.

Lane Descriptions & Observations

Lane 1 – Molecular Weight Marker

- A standard protein ladder was loaded in this lane.
- It serves as a reference to estimate the molecular weight of other protein bands by comparing their migration distances.

Lanes 2-4 – TVAG_080000 Protein Expression Samples

- These lanes contain the expressed TVAG_080000 protein.
- The presence of high molecular weight insoluble protein suggests that the expressed protein might have aggregated, forming inclusion bodies.
- This result indicates that the expressed protein may require solubilization or refolding strategies to obtain a functional form.

Table 2. Native PAGE Analysis of TVAG_080000 Protein Expression

Lane	Sample	Observations
1	Molecular weight marker	Standard protein bands
2-4	TVAG_080000 expression	High molecular weight insoluble protein

Table 3 presents the results of enzyme activity analysis for TVAG_080000, comparing its digestion products with those of commercially available α- and ß-amylases. The goal is to determine whether TVAG_080000 functions as a ß-amylase based on the digestion products it generates.

Table 3. Enzyme Activity Analysis of TVAG_080000

Enzyme Source	Digestive Products	Interpretation
Commercial ß-amylase	Maltose	Consistent with ß-amylase activity
Commercial α-amylase	Glucose, maltotriose, α-limit dextrins	Expected α-amylase activity
Recombinant TVAG_080000	Maltose	Identified as a ß-amylase

Glycogen processing enzymes in Trichomonas vaginalis, particularly Glycogen Synthase and Glycogen Phosphorylase, play essential roles in the organism's energy metabolism. Glycogen Synthase facilitates the synthesis of glycogen from glucose, allowing the protozoan to store energy for times when carbohydrates are scarce. Conversely, Glycogen Phosphorylase is responsible for breaking down glycogen into glucose-1-phosphate, which can be further processed for energy production. The regulation of these enzymes is crucial, as it ensures that T. vaginalis can efficiently mobilize energy reserves in response to varying environmental conditions. Understanding the activity and regulation of these enzymes provides insights into the organism's metabolic flexibility and adaptability, which are vital for its survival as an obligate human parasite.

CONCLUSION

The ability of T. vaginalis to store large amounts of glycogen during invitro development has been known for quite some time. Further research into the branched nature of TVG led to the discovery that two ORFs encode functional enzymes TvDBE for glycogen debranching and TvBE for branching as well as building upon earlier work. The Trachoma vaginalis's reconstructed genomic contains several glucosidases and more than 20 distinct ß and ∝ amylases, at least some of which might have glycogen-degrading activity. Glycogen metabolism in eukaryotic organisms has been studied in relation to model mammalian and fungal systems. Although there are limits, the research on Trichomonas vaginalis glycogen-digesting enzymes has provided important insights. Research in the future could aim to understand better the host-parasite relationship by identifying pharmacological targets, deciphering the complexities of glycogen metabolism and establishing links to the parasite's virulence mechanisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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