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Research paper

Glycogen synthase from the parabasalian parasite *Trichomonas vaginalis*: An unusual member of the starch/glycogen synthase family

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ABSTRACT

Trichomonas vaginalis, a parasitic protist, is the causative agent of the common sexually-transmitted infection trichomoniasis. The organism has long been known to synthesize substantial glycogen as a storage polysaccharide, presumably mobilizing this compound during periods of carbohydrate limitation, such as might be encountered during transmission between hosts. However, little is known regarding the enzymes of glycogen metabolism in *T. vaginalis*. We had previously described the identification and characterization of two forms of glycogen phosphorylase in the organism. Here, we measure UDP-glucose-dependent glycogen synthase activity in cell-free extracts of *T. vaginalis*. We then demonstrate that the TVAG_258220 open reading frame encodes a glycosyltransferase that is presumably responsible for this synthetic activity. We show that expression of TVAG_258220 in a yeast strain lacking endogenous glycogen synthase activity is sufficient to restore glycogen accumulation. Furthermore, when TVAG_258220 is expressed in bacteria, the resulting recombinant protein has glycogen synthase activity *in vitro*, transferring glucose from either UDP-glucose or ADP-glucose to glycogen and using both substrates with similar affinity. This protein is also able to transfer glucose from UDP-glucose or ADP-glucose to maltose and longer oligomers of glucose but not to glucose itself. However, with these substrates, there is no evidence of processivity and sugar transfer is limited to between one and three glucose residues. Taken together with our earlier work on glycogen phosphorylase, we are now well positioned to define both how *T. vaginalis* synthesizes and utilizes glycogen, and how these processes are regulated.

1. Introduction

The parasitic protist *Trichomonas vaginalis* is the causative agent of trichomoniasis, a highly-prevalent sexually-transmitted infection with almost 200 million cases reported annually [1]. In addition to the obvious clinical importance of the organism, *T. vaginalis* has received considerable attention due to certain unusual aspects of its biochemistry and physiology [2]. Trichomonads are grouped with the parabasalids, a large collection of anaerobic, flagellated protists, highly divergent from fungi, plants, and metazoan, and characterized by the presence of hydrogenosomes, mitochondrion-derived organelles that are a site of ATP generation [3].

Despite the clinical and evolutionary significance of *T. vaginalis* and the rich history of study of the organism, many fundamental questions relating to the basic biology of the protist remain.

Glycogen, a branched polymer of glucose, is used as a storage form of both carbon and energy by a wide variety of organisms. It has long been known that a variety of trichomonad species, including *T. vaginalis*, synthesize glycogen and that this compound can constitute up to 15% of the dry weight of the organisms [4–6]. Such an abundance of glycogen implies that this compound plays an important role in the physiology of trichomonads. Indeed, synthesis of glycogen in the presence of excess extracellular carbohydrate and its utilization upon carbohydrate depletion has been demonstrated [7]. *T. vaginalis* would likely experience periods of carbohydrate limitation during transmission from person to person and it is conceivable that glycogen stores may be important for the maintenance of viability at such times.

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The mechanisms of glycogen synthesis and degradation have been well studied in both fungi and mammals [8–10]. In both cases, synthesis begins with the self-glucosylating initiator protein glycogenin (EC 2.4.1.186). Glycogenin transfers glucose residues from UDP-glucose to specific tyrosine residues within its own sequence, forming a short oligosaccharide primer. Glycogen synthase (EC 2.4.1.11) then elongates this primer, adding glucose residues in α1→4 glycosidic linkage, again using UDP-glucose as a sugar donor. Glycogen synthase is capable of synthesizing only linear α1→4 chains and the α1→6 branch points, which are characteristic of the mature glycogen molecule, are introduced via the branching enzyme (EC 2.4.1.18).

Glycogen degradation requires the activity of a different set of enzymes [8,10]. Glycogen phosphorylase (EC 2.4.1.1) catalyzes the sequential removal of glucose residues from the non-reducing ends of the α1→4 linked glucose chains while debranching enzyme handles the α1→6 branches. In both fungi and mammals, debranching enzyme is a bifunctional molecule, which carries both 4-α-glucanotransferase (EC 2.4.1.25) and amylo-α-1,6-glucosidase (EC 3.2.1.33) activities that act in concert to remove branches [11,12].

Synthesis and degradation of bacterial glycogen differs markedly from the processes that occur in mammals and fungi [10]. There is an absence of glycogenin-like genes in sequenced bacterial genomes and experimental evidence that bacterial glycogen synthase both elongates the α1→4 linked glucose chains and forms the primer required for this elongation process by glucosylating itself [13]. Thus, there does not appear to be a requirement for a glycogenin-like molecule to initiate glycogen synthesis. Furthermore, the glucose donor used for glycogen synthesis in the clear majority of cases is ADP-glucose rather than UDP-glucose, although there are reports of some bacterial enzymes that can utilize either nucleotide [10]. Lastly, there is no evidence of a role for glucose 6-phosphate or reversible phosphorylation in the regulation of bacterial glycogen synthases and control of the synthesis of glycogen appears to lie at an earlier step of the pathway, with the generation of the glucose donor ADP-glucose [10].

Regarding their lack of a requirement for a separate primer protein, sugar nucleotide preference, and regulatory properties the bacterial glycogen synthases resemble the well-characterized starch synthases of green plants. Here, the sugar donor is again ADP-glucose, the oligosaccharide primer that 'seeds' starch granule formation is thought to be synthesized by a particular isoform of starch synthase, and control appears to rely upon the regulation of the supply of ADP-glucose to starch synthases, rather than through allosteric or posttranslational control of the synthases themselves (reviewed in Refs. [14,15]).

Our overarching goal is to determine the role that glycogen plays in the lifecycle of *T. vaginalis*. Previously, we have cloned and characterized two isoforms of glycogen phosphorylase [16]. Additionally, inspection of the *T. vaginalis* genome indicates the presence of open reading frames that are predicted to encode branching enzyme (TVAG_276310 and TVAG_453180) and bifunctional debranching enzyme (TVAG_143490 and TVAG_330630) [17,18]. The genome sequence does not include any sequence that has a high degree of similarity to the known glycogenin proteins from mammals and fungi, nor does it contain any sequences that closely resemble mammalian or fungal glycogen synthases. However, the open reading frame TVAG_258220 encodes a large protein of 1572 amino acids, the C-terminal ~475 amino acids of which are clearly related to the plant and bacterial starch/glycogen synthases [17,18].

The glycogen synthases of fungi and mammals belong to glycosyltransferase family 3 and those whose enzymatic activities have been characterized employ UDP-glucose as a glucose donor [19]. In contrast the starch synthases of green plants, and the majority of bacterial glycogen synthases, belong to glycosyltransferase family 5. Here, ADP-glucose is the glucose donor most commonly employed, although some enzymes do have broader substrate specificity and are able to use either ADP-glucose or UDP-glucose, or indeed have a preference for UDP-glucose (see for example [20–23]).

The TVAG_258220 open reading frame encodes a protein that is considerably larger than the well-characterized glycogen/starch synthases from most other species and the starch/glycogen synthase-like domain is confined to the C-terminus of molecule (Fig. 1). Proteins with similar architecture to TVAG_258220 are present in social amoebae (*Dictyostelium* sp., *Acytostelium subglobosum, Polyspondilium pallidum*), intestinal amoebae (*Entamoeba* sp.) and ciliated protists (*Stylonchia lemnae*, *Oxytricha trifallax, Paramecium tetraurelia*). A detailed phylogenetic analysis of glycogen/starch synthase domains conducted by Deschamps et al. demonstrated a clear relationship between the domains of these protist proteins and the starch/glycogen synthase domains found in red algae (*Galdieria sulphuraria* and *Cyanidoschyzon merolae*) and the glaucophyte *Cyanophora paradoxa* [24]. There was compelling evidence that, despite their close relationship to the ADP-glucose utilizing GT5 glycogen/starch synthases, several red algal synthases and a glaucophyte synthase preferentially utilized UDP-glucose as a glucose donor [22,24,25]. Thus, it was hypothesized that UDP-glucose was the glucose donor for this protist/red algal group of proteins, which were designated as 'UDP-glucose using starch/glycogen synthases' [24,26].

Here, we provide direct biochemical evidence that TVAG_258220 encodes a glycosyltransferase that is presumably responsible for the synthesis of glycogen by *T. vaginalis*. We cloned the TVAG_258220 open reading frame and found that its expression could restore glycogen storage to a yeast strain devoid of glycogen synthase activity. Furthermore, we expressed recombinant TVAG_258220 in bacteria and determined that the purified protein had glycogen synthase activity *in vitro*, transferring glucose from either UDP-glucose or ADP-glucose into glycogen and having a similar affinity for both sugar nucleotides. Since neither *T. vaginalis* nor *S. cerevisiae* express ADP-glucose pyrophosphorylase but both express UDP-glucose pyrophosphorylase, we conclude that the physiological substrate of the *T. vaginalis* glycogen synthase is UDP-glucose.

2. Materials and methods

2.1. Reagents

Unless otherwise stated, all reagents were purchased from Sigma-Aldrich Corp., St. Louis, MO, and were of the highest grade available.

2.2. Growth and maintenance of trichomonads

Trichomonas vaginalis G3 (ATCC PRA-98) was obtained from the American Type Culture Collection (Manassas, VA). Stock cultures were grown and maintained in trypticase-yeast extract-maltose medium supplemented with 10% v/v horse serum (TYM) as previously described [16].

2.3. Cloning of the TVAG_258220 open reading frame and construction of bacterial and yeast expression vectors

Total RNA was isolated from *T. vaginalis* G3 and cDNA was synthesized using a combination of the SV Total RNA Isolation System and ImProm-II Reverse Transcription System (both from Promega, Madison, WI) following the manufacturer's instructions. The cDNA