Molecular & Biochemical Parasitology 181 (2012) 53–56

Contents lists available at SciVerse ScienceDirect

Molecular & Biochemical Parasitology

Short communication

UDP-xylose and UDP-galactose synthesis in *Trichomonas vaginalis*

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ARTICLE INFO

Article history:
Received 1 August 2011
Received in revised form
21 September 2011
Accepted 3 October 2011
Available online 8 October 2011

Keywords:
UDP-xylose
UDP-galactose
Trichomonas vaginalis

ABSTRACT

The presence of xylose and galactose residues in the structure of trichomonad lipoglycans was indicated by previous studies and the modification of any glycoconjugate with either monosaccharide requires the respective presence of the nucleotide sugars, UDP-xylose and UDP-galactose. Biosynthesis of UDP-xylose *de novo* is mediated by UDP-xylose synthase (UXS; UDP-glucuronic acid decarboxylase), which converts UDP-glucuronic acid to UDP-xylose, whereas UDP-galactose can be generated from UDP-glucose by UDP-galactose epimerases (GalE). *Trichomonas vaginalis* cDNAs, encoding proteins with homology to these enzymes from other eukaryotes, were isolated. The recombinant *T. vaginalis* UDP-xylose synthase and UDP-galactose epimerase were expressed in *Escherichia coli* and tested via high pressure liquid chromatography to demonstrate their enzymatic activities. Thereby, in this first report on enzymes involved in glycoconjugate biosynthesis in this organism, we demonstrate the existence of xylose and galactose synthesising pathways in *T. vaginalis*.

Trichomonas vaginalis is a parasitic flagellated protozoan which causes human trichomoniasis, one of the most common sexually transmitted diseases in humans. Despite its wide spread and high prevalence, with more than 200 million affected people and at least three million new cases per year in the USA [1,2], it has proven to be an underestimated disease. Indeed, an infection with *T. vaginalis* causes not only vaginitis, exocervicitis and urethritis, it is also implicated in miscarriages and occurrence of human immunodeficiency virus. Cytopathogenicity starts with the adhesion of the protozoan to the host cell and indeed glycoconjugates such as a lipoglycan covering its cell surface are important for the parasite's interaction with its host [3]. The structure of this lipoglycan [4,5], as well as of its protein-linked N-glycans [20], have been recently determined to contain monosaccharides such as xylose and galactose. In order to perform the relevant xylosylation and galactosylation reactions necessary for the biosynthesis of these glycan structures *in vivo*, the organism requires the relevant nucleotide sugars, UDP-xylose and UDP-galactose.

UDP-xylose is the product of a two-step conversion from UDP-glucose: first, dehydrogenation of UDP-glucose is catalyzed by UDP-glucose dehydrogenase (UGD, EC 1.1.1.22) thus forming UDP-glucuronic acid (UDP-GlcA). Then, UDP-glucuronic acid decarboxylase (UDP-xylose synthase; UXS, EC 4.1.1.35) acts on UDP-GlcA to form UDP-xylose [6]. Depending on the organism, UXS may be cytosolically or lumenally located. In plants, the biosynthesis of UDP-xylose by different UXS isoforms occurs both in the cytosol and in membrane-bound compartments [7,8]. Mammals and nematodes on the other hand express only one UXS, which is located in the golgi apparatus [9,10], whereas the fungus *Cryptococcus* expresses only one, probably cytosolic, form [11]. In bacteria such as *Micromonospora echinospora* and *Sinorhizoboium meliloti* UDP-xylose is also synthesised from UDP-glucuronic acid [12,13]. On the other hand, the *de novo* biosynthesis of UDP-galactose from UDP-glucose is mediated by the cytosolic UDP-galactose epimerase (GalE; EC 5.1.3.2); the relevant GalE genes have been identified from a number of organisms and in *T. brucei* GalE is essential for growth [14].

Considering that xylose and galactose are components of several *Trichomonas* glycoconjugates we expected that this organism possesses at least one UXS and one GalE gene. Homology searching of the *T. vaginalis* genome from the G3 strain [15] was performed and the annotation suggested the presence of two putative homologues of UDP-galactose epimerase (GalE1, TVAG_186740 and TVAG_101620) and one putative homologue of UDP-xylose synthase (TVAG_178290). In the case of the UDP-xylose synthase the relevant reading frame is predicted to encode a protein of 313 residues lacking a transmembrane domain; therefore, the *T. vaginalis* enzyme is proposed to be a cytosolic protein as is the case with the fungal and some plant isoforms. Using RNA extracted from *T. vaginalis* (C1 strain; ATCC 30001), the UXS and one of the GalE reading frames were isolated by two-step RT-PCR using the primer pairs Tv-UXS-for-NcoI (catgccatggtgagtacacctaccaagagtac)

Abbreviations: GalE, UDP-galactose-4′-epimerase; UDP-GlcA, UDP-glucuronic acid; UXS, UDP-xylose synthase.

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doi:10.1016/j.molbiopara.2011.10.001

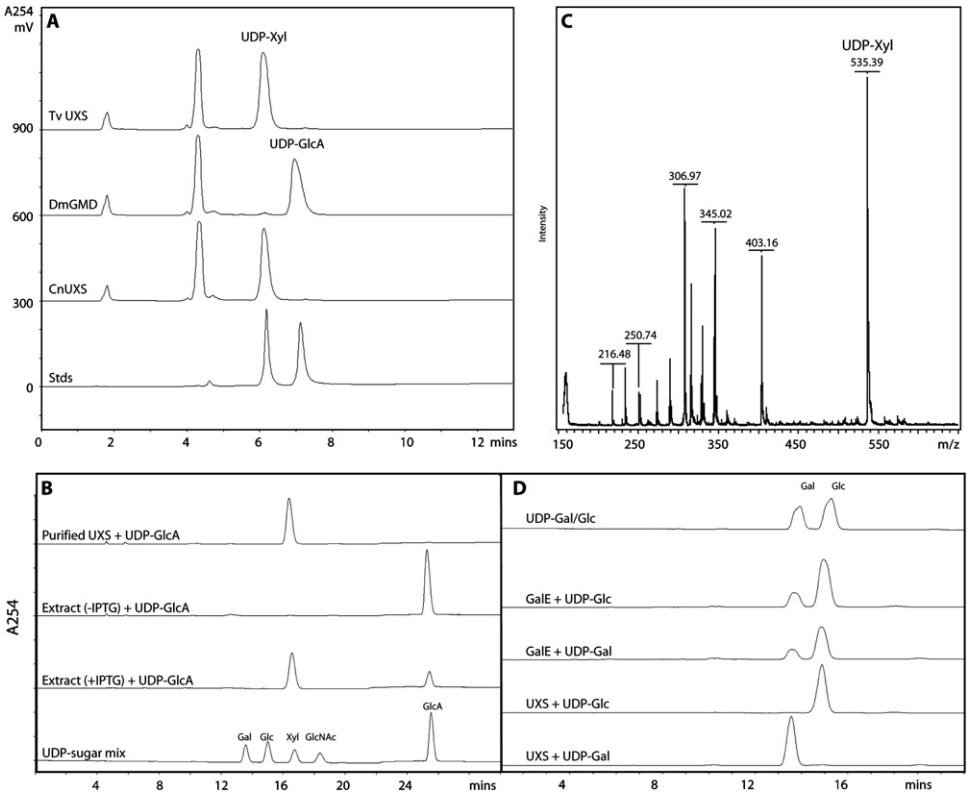

Fig. 1. Test of UDP-xylose synthase and UDP-galactose epimerase activities. UDP-xylose synthase (UXS) and UDP-galactose/glucose epimerase (GalE1) activities were assayed by incubating the enzyme (1 μl purified enzyme or 5 μl *E. coli* crude extract) in the presence of the relevant 3 mM UDP-sugar and 3 mM NAD^+ in 80 mM Tris–Cl, pH 7.7 at 30 or 37 °C (final volume 50 μL). (A) SAX-HPLC of 4 h assays performed using UDP-glucuronic acid as substrate and lysates of bacteria expressing *T. vaginalis* UXS, *Drosophila* GMD (negative control) or *Cryptococcus* UXS (positive control). After injection onto a Hypersil column (0.5 × 25 cm), the column was washed for 10 min with buffer A (2 mM ammonium formate, pH 3.2; the flow rate was 1.5 ml/min) prior to elution with a linear gradient from 0 to 40% buffer B (600 mM ammonium formate, pH 3.2) as described [7]; absorbance at 254 nm was recorded. Standard UDP-xylose elutes at around 6 min as compared to the UDP-glucuronic acid at 7 min (Stds). The peak at 4.5 min corresponds to imidazole present in the lysis buffer. (B) Ion-pair RP-HPLC of assays of recombinant UXS in either purified form or in an *E. coli* extract (+IPTG) showing conversion of UDP-GlcA to UDP-Xyl. A control extract of *E. coli* transformed with the UXS plasmid but not induced (-IPTG) showed no such activity. Analysis was performed using a Cosmosil C18-AR-II column (250 mm × 4.6 mm; Nacalai Tesque, Kyoto, Japan): buffer A was 20 mM triethylamine-acetate (pH 7) and buffer B was 20 mM triethylamine-acetate (pH 7) containing 10% acetonitrile [19]. After isocratic elution with 100% buffer A, a gradient of 1% per minute (buffer B) was applied after 15 min. (C) Negative-mode MALDI-TOF MS of pooled UXS products; the *m*/*z* of UDP-xylose $[M\text{-}H]^-$ of 535.3 compares to the calculated molecular mass of 536.2. For analysis, 1 μl of an aliquot of UDP-Xyl was spotted onto a MALDI plate, vacuum dried prior to application of 1 μl 2,5-dihydroxybenzoic acid (DHB; 2% in 30% acetonitrile/70% 50 mM $(NH_4)_2SO_4$); the dried and crystallized sample was analysed by MALDI-TOF MS using a Bruker Ultraflex instrument. (D) Ion-pair RP-HPLC of GalE1 assays; purified recombinant *T. vaginalis* GalE converted UDP-Gal partly into UDP-Glc and also UDP-Glc partly into UDP-Gal, whereas purified recombinant *T. vaginalis* UXS displayed no such activity.

and Tv-UXS-rev-SacI (ccgagctctagtaacatttagaaaatgtttta) or Tv-GalE1-for-BamHI (cgcggatccatgtctatcctcatacaggc) and Tv-GalE1-rev-HindIII (cccaagcttttaagctctgtagccatttgg) prior to ligation into the pET30a vector. The recombinant proteins were expressed in *Escherichia coli* (DE3) pLysS Gold cells upon induction with isopropyl-β-D-thiogalactopyranoside at 25 or 37 °C for 3 h; cells were lysed and the recombinant proteins isolated by purification on Ni/nitrilotriacetate resin and elution with 250 mM imidazole. The purified forms of the recombinant proteins were analysed by SDS–PAGE and Western blotting and displayed molecular masses of ~40 kDa (data not shown) in agreement with the size predicted from the amino acid sequences.

In the UXS sequence, two 'silent' nucleotide alterations were observed in the UXS cDNA cloned from the C1 strain; the amino acid sequence is thereby identical with that predicted from the genomic sequence available from the G3 strain (data not shown; Genbank/EBI accession number HE575670). The trichomonad UXS protein is 57% identical to the human sequence over 307 amino acids and 50% identical to predicted, but uncharacterised, proteins from *Trypanosoma cruzi* (XP_820252 and XP_806161). UDP-xylose synthase activity of UXS was verified by assaying with UDP-glucuronic acid (UDP-GlcA) as substrate and NAD^+ as a cofactor. The negative control (with *Drosophila melanogaster* GDP-mannose dehydratase) displayed no conversion of UDP-GlcA to UDP-Xyl, whereas incubations with the positive control (UXS from *Cryptococcus neoformans* [11]) and the UXS from *T. vaginalis* showed the generation of a clear UDP-xylose peak as judged by either SAX (Fig. 1A) or RP-HPLC (Fig. 1B). Substrate conversion was dependent on incubation time and the amount of protein, whereas no product was formed after heat inactivation of the protein (data not shown). The UDP-xylose produced by the purified *T. vaginalis* enzyme was collected from a number of HPLC runs prior to analysis by MALDI-TOF MS and NMR. The *m*/*z* value of 535.4 for the $[M-H]^-$ molecular ion (Fig. 1C) is as expected, whereas the in-source fragment of 403.1 corresponds to loss of a pentose to yield UDP. Key chemical shifts in the ^1H and ^{31}P NMR spectra, as compared to literature data [16], confirm the identity of the UXS enzymatic product as UDP-xylose (Table 1).

The purified UXS was examined further in terms of its pH and temperature optima. The enzyme exhibited a good activity over a broad pH range (5.5–8.0) with an optimum of around pH 7.0 (Fig. 2A). This optimum compares well to that for *Cryptococcus* UXS [6,11], *Sinorhizobium* UXS [12] and endomembrane-associated *Arabidopsis* UXS2 [17], but is higher than that for the plant UXS3 [7]. The activity of the enzyme was not significantly affected by either $MgCl_2$ or $MnCl_2$ (data not shown). The expressed protein was active at temperatures from 4 to 50 °C whereas its activity was nearly abolished at 70 °C (Fig. 2B) and the highest activity could be achieved at 37 to 50 °C, consistent with efficient function within a mammalian