Published in final edited form as:
Biochem Biophys Res Commun. 2009 December 25; 390(4): 1414–1418. doi:10.1016/j.bbrc.2009.11.007.

Characterization of Mouse UDP-glucose Pyrosphosphatase, a Nudix Hydrolase encoded by the *Nudt14* Gene

Candy A. Heyen[#], **Vincent S. Tagliabracci**, **Lanmin Zhai**, and **Peter J. Roach**[§]
Department of Biochemistry and Molecular Biology, Indiana University School of Medicine

Abstract

Recombinant mouse UDP-glucose pyrophosphatase (UGPPase), encoded by the *Nudt14* gene, was produced in *Escherichia coli* and purified close to homogeneity. The enzyme catalyzed the conversion of [β-^{32}P]UDP-glucose to ^{32}P-glucose-1-P and UMP, confirming that it hydrolyzed the pyrophosphate of the nucleoside diphosphate sugar to generate glucose-1-P and UMP. The enzyme was also active towards ADP-ribose. Activity is dependent on the presence of Mg^{2+} and was greatest at alkaline pH above 8. Kinetic analysis indicated a K_m of ~4 mM for UDP-glucose and ~0.3 mM for ADP-ribose. Based on V_{max}/K_m values, the enzyme was ~20-fold more active towards ADP-ribose. UGPPase behaves as a dimer in solution and can be cross-linked to generate a species of Mr 54,000 from a monomer of 30,000 as judged by SDS-PAGE. The dimerization was not affected by the presence of glucose-1-P or UDP-glucose. Using antibodies raised against the recombinant protein, Western analysis indicated that UGPPase was widely expressed in mouse tissues, including skeletal muscle, liver, kidney, heart, lung, fat, heart and pancreas with a lower level in brain. It was generally present as a doublet when analyzed by SDS-PAGE, suggesting the occurrence of some form of post-translational modification. Efforts to interconvert the species by adding or inhibiting phosphatase activity were unsuccessful, leaving the nature of the modification unknown. Sequence alignments and database searches revealed related proteins in species as distant as *D. melanogaster* and *C. elegans*.

Keywords

Nudt14; UDP-glucose; pyrophosphatase; Nudix hydrolase; ADP-ribose; glycogen

Introduction

Enzyme activities capable of hydrolysing UDP-glucose to generate glucose-1-P and UMP were first reported many years ago [1-3]. More recently, Yagi et al. [4] encountered such an activity in tissue extracts as interfering with assays of UDP-glucose pyrophosphorylase in the direction of pyrophosphorylysis (see Fig. 1A). These investigators went on to purify the enzyme to homogeneity from pig kidney and ultimately to clone a human cDNA. From its predicted amino acid sequence, the enzyme fell into the category of Nudix (nucleoside diphosphate linked to some other group, X) hydrolases [5,6]. Based on surveying the activity of the enzyme towards a variety of potential substrates, Yagi et al. [4] concluded that the preferred substrate was UDP-

§ To whom correspondence should be addressed. 317 274 1582; FAX 317 274 4686; proach@iupui.edu.
[#]Present address: Maternal Fetal Medicine, St. Vincent Women's Hospital, Indianapolis, IN 46260.

glucose although significant activity towards ADP-ribose was also observed. In their studies, the V_{max}/K_m for UDP-glucose was approximately four times that for ADP-glucose. Taking also into account the fact that UDP-glucose concentrations, in the range 0.1 - 1 mM [7-9], are thought to be significantly greater than the micromolar ADP-ribose levels in cells [10], these authors therefore proposed that the enzyme be named UDP-glucose pyrophosphatase.

In mammalian cells, a major role of UDP-glucose is as the glucosyl donor for the synthesis of the storage polysaccharide glycogen [11,12]. Therefore, an active UDP-glucose pyrophosphatase would have the potential to influence or even regulate the flux of glucose into glycogen (Fig. 1). Although such a mechanism to regulate UDP-glucose would be energetically expensive, there are many examples where benefit to the cell must outweigh the energetic expense of operating so-called “futile cycles”. In this regard, it would be of interest to know the distribution of the UGPPase in mammalian tissues, one goal of the present study. Some support for an involvement of Nudix hydrolase in polysaccharide metabolism comes from other organisms. In plants, glucose is stored as starch, a polysaccharide chemically similar to glycogen, but in this case the glucosyl donor is ADP-glucose [13-16]. Recent studies have identified an ADP-glucose pyrophosphatase that, analogously to UGPPase, has been proposed to control ADP-glucose levels, and hence starch accumulation, in some tissues [17,18]. In *Escherischia coli*, an ADP-glucose pyrosphatase, encoded by the NudF (aspP) gene, has been reported to regulate ADP-glucose levels linked to bacterial glycogen synthesis [19].

As a first step in characterizing the role of mammalian UGPPase, we cloned and analyzed recombinant mouse UGPPase. Using [β-^{32}P]UDP-glucose as substrate, UGPPase catalyzed the formation of ^{32}P-glucose-1-P, proving the hydrolysis of the pyrophosphate of UDP-glucose. While confirming the earlier report that both UDP-glucose and ADP-ribose were substrates for UGPPase, the results with the mouse enzyme suggested preferential activity towards ADP-ribose. This outcome does not preclude a role for UGPPase in influencing UDP-glucose metabolism and indeed Western blotting with antibodies raised against the recombinant protein indicated that the enzyme was widely distributed in mammalian tissues, including skeletal muscle, liver and kidney, the major repositories of glycogen in the body.

Materials and Methods

Cloning and Expression of UGPPase

A full length cDNA encoding UGPPase was amplified by PCR from mouse skeletal muscle cDNA (Biochain) using primers specific for the gene encoding the mouse UGPPase, *UGPP* (NCBI accession number NM_025399): 5′-GCTCGGATCCCATATGGAGCGCATCGACGGGGTG-3′ (forward) and 5′-TGGAATTCGGCCTCTCGAGCCCCAAGGTCTCACTG-3′ (reverse). The cDNA was subsequently cloned into a TA vector (Invitrogen), creating pUGPP, and introduced into *Escherichia coli* DH5-α competent cells. For protein expression, the *Nde*1/*Xho*1 fragment of pUGPP was subcloned into the pET28a expression vector (Novagen). *E. coli* BL21-DE3 transformed with the pET-UGPP plasmid were grown at 37°C in 500 mL 2 × YT medium containing 30 μg/ml kanamycin until an A_{600} reading of 0.4-0.6 was reached. The culture was induced with 1 mM isopropyl β-D-thiogalactoside, grown 2 h, harvested by centrifugation, and resuspended in 10 mL of lysis buffer (50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl, 10 mM imidizole). Cells were lysed by incubating 30 minutes on ice with 0.5 mg/ml lysozyme followed by sonication. After centrifugation at 20,000 × g at 4°C for 30 minutes, the supernatant was applied to a column containing 2 ml of 50% Ni-NTA resin (Qiagen), washed with 35 mM imidizole buffer, eluted with 250 mM imidizole buffer, and dialyzed into 1L 50 mM Tris-HCl pH 8.0, 1 mM dithiothreitol. Aliquots were stored at -80°C with 46%(v/v) ethylene glycol.

Enzyme Assays

Routine measurements of UGPPase activity against UDP-glucose used the two step spectrophotometric determination described by Rodriguez-Lopez *et al.* [20] except that step one contained 50 mM Tris-HCl, pH 8.8, 10 mM $MgCl_2$, 8 mM UDP-glucose and purified recombinant protein. After incubation at 37°C, reactions were boiled 2 minutes and added to a step two reaction mix containing 50 mM HEPES pH7.0, 2 mM $MgCl_2$, 1 mM EDTA, 15 mM KCl, 0.6 mM $NADP^+$, 1 unit phosphoglucomutase, and 1 unit glucose-6-phosphate dehydrogenase in a total volume of 300 μl. The solution was incubated at 37°C for 20 minutes and the $OD_{340\ nm}$ was recorded.

For determination of UDP-glucose kinetic parameters, a coupled assay was developed with the reaction containing 50 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, 1 mM EDTA, 0.6 mM $NADP^+$, 1 unit phosphoglucomutase, 1 unit glucose-6-phosphate dehydrogenase and purified recombinant protein. For determination of activity towards ADP-ribose, the assay monitored the appearance of inorganic phosphate released from the reaction product ribose-5-P. The assay mixture contained 50 mM Tris-HCl pH 8.0, 10 mM Mg^{2+}, 0-8 mM ADP-ribose, and 2 units of alkaline phosphatase and purified recombinant protein. The reaction was terminated by the addition of 20 mM EDTA. The phosphate was determined by the molybdate assay and quantified by comparison with a standard curve. One unit of enzyme is defined as the amount of enzyme required to convert 1 μmol of substrate into product per minute. Kinetic parameters were calculated by curve-fit analysis to the Mechalis-Menten equation.

Synthesis of [β^{32}P]UDP-glucose

[β^{32}P]UDP-glucose was synthesized and purified essentially as in [21,22]. Briefly, 500 μCi of [γ-^{32}P]ATP (3000 Ci/mmol) was dried under N_2 gas and resuspended in a buffer containing 45 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, 0.8 mM UTP, 9 mM glucose, 0.9 mM DTT, 3 units of UDP-glucose pyrophosphorylase (Sigma), 3 units of inorganic pyrophosphatase (Roche) and 6 units of phosphoglucomutase (Roche). The reaction was initiated by the addition of 5.6 units of hexokinase (Roche), incubated at 30°C for 3 hours and terminated by boiling in a water bath for 10 min. The denatured protein was removed by centrifugation and the supernatant was added to 5 mg activated charcoal. After centrifugation the pellet was washed 3 times with 1ml ice cold water and the [β^{32}P]UDP-glucose was eluted with 200 μl of a 50% (v/v) ethanol solution containing 0.16 M ammonium hydroxide. The eluent was dried under reduced pressure and the [β^{32}P]UDP-glucose resuspended in 50% ethanol and stored at -80°C. Ascending chromatography was performed in a solvent consisting of n-propanol:ethyl acetate:H_2O (7:1:4) using HPTLC silica gel plates (Alltech).

Antibody Production and Western Analysis

Polyclonal antibodies were raised in rabbits (Cocalico Biologicals) immunized with recombinant mouse UGPPase and affinity-purified using a column of antigen coupled to Affigel 15 support (BioRad). Bound antibodies were eluted with 100 mM glycine HCl (pH 2.4)/ 150 mM NaCl and aliquots stored in PBS/0.2% sodium azide at -20°C until use. Tissue samples were homogenized in 25-30 volumes of buffer containing 50 mM Tris-HCl pH 7.8, 10 mM EDTA, 2 mM EGTA, 100mM NaF, 2 mM benzamidine-HCl, 0.1 mM TLCK, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml aprotinin, using a polytron homogenizer (Kinematica) for 20 s. After a low speed centrifugation, 600 μl of soluble fractions were cleared to minimize non-specific interactions by incubating with 30 μl of protein A agarose for 10 minutes. Cleared supernatant was immunoprecipitated by incubating 2 hours with anti-UGPPase antibodies or buffer alone as control, followed by 1 hour with protein A agarose resin (Sigma-Aldrich). Samples were centrifuged and washed twice with homogenization buffer. After a final wash, the immunoprecipitated complex was resuspended in 30 μl of 5× SDS loading buffer containing 250 mM Tris-HCl (pH 6.8), 50% glycerol, 10

mM EDTA, 10% sodium dodecyl sulfate (SDS), 720 mM β-mercaptoethanol, and 0.05% bromophenyl blue. After boiling for 2 minutes, samples were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and incubated with antibodies to recombinant UGPPase. Horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence were used for detection.

Cross-linking

Recombinant UGPPase was incubated with 0.02% glutaraldehyde in 50 mM Hepes buffer, pH 8.2, 10 mM $MgCl_2$ and 0-8 mM UDP-glucose. Reactions were incubated for 5 minutes at 37° C before addition of SDS-loading buffer and boiling for 2 minutes. Samples were separated on 10% SDS-PAGE gels and visualized by Western analysis.

Results and Discussion

UGPPase characterization

UGPPase was expressed in *E. coli* as a protein with an NH_2-terminal His tag and was purified to homogeneity using a Ni-NTA agarose column to yield 50-70 mg enzyme per liter of culture. On SDS-PAGE, the protein ran with apparent Mr ~30,000 compared with a predicted size of 26,700. The standard assay (see Experimental Procedures) monitors the formation of glucose-1-P. As further proof that the enzyme hydrolyzed the pyrophosphate moiety of UDP-glucose, we synthesized [β-^{32}P]UDP-glucose. Exposure of this substrate to UGPPase resulted in the complete loss of radioactivity running in correspondence to UDP-glucose and its appearance as a species with the mobility of glucose-1-P (Fig. 1B).

The reaction had an absolute requirement for Mg^{2+} and was optimal at alkaline pH, greater than pH 8 (Fig. 2). At low UDP-glucose concentrations (<1 mM), we observed a lag in glucose-1-P production and so we developed a continuous coupled assay so that the linear rate established after the lag could be quantitated to analyze UDP-glucose kinetics. At higher UDP-glucose concentrations the lag was not observed. The enzyme was active towards both UDP-glucose and ADP-ribose, as has been previously reported [4], with V_{max} values in a similar range (Fig.2C). The reaction followed a hyperbolic dependence on either substrate concentration. However, we observed a significantly lower K_m for ADP-ribose and higher Km for UDP-glucose than previously reported, so that the V_{max}/K_m values in our study suggested preferential ADP-ribose hydrolysis. Otherwise, the results obtained were consistent with the previous analysis of the pig kidney and human enzymes.

Earlier gel filtration studies had suggested that UGPPase behaved as a dimer in solution [4]. We sought to confirm this observation by cross-linking the subunits using glutaraldehyde (Fig. 3A). As analyzed by SDS-PAGE, a species of apparent Mr ~54,000 was generated in the presence of glutaraldehyde in a concentration dependent manner, consistent with the presence of dimers. At higher glutaraldehyde concentrations, both the monomer and dimer tended to run as less discrete bands, possibly due to the occurrence of intra-subunit cross-linking producing more compact molecules even in the presence of SDS. Interestingly, this phenomenon was accentuated in the presence of Mg^{2+} ions (not shown), suggesting that Mg^{2+} binding may affect the protein structure to enhance intra-subunit cross-linking. Because of the observed lag in the UDP-glucose hydrolysis reaction at low substrate concentrations, we wondered whether the dimerization might be affected by the presence of reactants. Cross-linking was therefore performed in the presence of UDP-glucose and/or glucose-1-P together with Mg^{2+}. Cross-linking was totally unaffected by the presence of these compounds (not shown).

Tissue distribution of UGPPase

With the idea that UGPPase expression might have an impact on UDP-glucose, and thus glycogen, metabolism, we were interested in analyzing the levels of UGPPase protein in different mouse tissues. Although direct Western blotting using antibodies raised against recombinant UGPPase detected species around the expected size in several tissues, liver, kidney, lung and others, we found that the analysis was more sensitive after immunoprecipitation. From this study, we identified species in the M_r range 28-30 kDa in all tissues analyzed (Fig. 3B). Of note, UGPPase was detected in liver, skeletal muscle and kidney, the most important glycogen accumulating tissues. The apparent molecular weight by SDS-PAGE was greater than the 24.4 kDa predicted for the full length UGPPase, consistent with the reduced electrophoretic mobility observed with the recombinant protein made in *E. coli*. The antibodies generally detected a doublet, with the relative prominence of the two bands dependent on the tissue analyzed. In the limited literature on UGPPase, there is no mention of multiple isoforms. At the Ensembl Web site (http://www.ensembl.org), the mouse Nudt14 gene is listed as having a single transcript and the human gene two splice variants. However, both human transcripts have the same coding sequence. At the EMB-EBI Alternative Splicing Database Project (http://www.ebi.ac.uk/asd/), four variant transcripts are listed for the mouse gene, besides the full length 222 amino acid form. Three would encode much smaller proteins but one corresponds to a 201 amino acid polypeptide, with a modified NH_2-terminus and a 2.6 kDa smaller M_r. The other major possibility to explain the presence of multiple protein species derived from the same gene is some form of post-translational covalent modification, of which the most common is phosphorylation. We tested for the latter by preparing extracts in the presence or absence of fluoride, a serine/threonine protein phosphatase inhibitor, and by incubating extracts with either purified type 1 protein phosphatase catalytic subunit or alkaline phosphatase. None of these conditions affected the ratio of the intensities of the two bands. These negative results provide no evidence for the occurrence of phosphorylation but do not definitively rule it out. Likewise, we cannot exclude the presence of other covalent modifications. Therefore, the basis for the heterogeneity of UGPPase awaits further investigation.

Phylogenetic Analysis of UGPPase

The Nudix hydrolase family (InterPro IPR000086; Pfam PF00293) represents a functionally diverse group of enzymes whose commonality resides in the ability to catalyze pyrophosphorylysis of a wide range of generally nucleoside containing substrates [5,6]. The enzymes are found from bacteria to humans. In humans, there are 24 Nudix hydrolase genes [5,6]. The common catalytic activity is linked to a conserved 23 amino acid Nudix box (Fig. 4A) in which several residues are highly conserved, including the RExxEE motif which is important for the binding of divalent cations. From database searches, UGPPase-like sequences are identified in mammals, chicken, zebrafish, worm and fly (Supplementary Fig. 1;Fig. 4B). Related sequences were also well represented in bacteria, and sequences for *Helicobacter hepaticus* and *Campylobacter jejuni* are included in our analysis, the latter being the most distantly related. As expected, all had an identifiable Nudix box but with some notable differences in consensus residues. UGPPase lacked the Glu residue at position 7 and the Arg residue at position 13. The latter was replaced by a Lys in mammals but was Glu or Gln in the other species. UGPPase had the known variant Ala instead of a hydrophobic residue at position 13. In addition, the UGPPase Nudix box was characterized by a conserved Asp at position 4, Ser at position 9 and an almost completely conserved Cys at position 21. The four residues preceding the classic Nudix box, Glu-Leu-Cys-Ala, were also highly conserved. Residues outside of the Nudix box are believed to determine substrate specificity of Nudix hydrolases [5,6] and significant sequence similarity is seen among these putative UGPPase sequences. Two notable highly conserved regions NH_2-terminal to the Nudix box are ^{15}SxYU[RK][PG]

xxUxYxx[DN]G[29] and [55]UUUV[KR]QFRPAU[FY][66] where U is a hydrophobic residue and the superscripts refer to the mouse sequence.

Conclusion

Our study of mouse UGPPase broadly confirms the biochemical data obtained with the human and pig enzymes [4], which exhibit 82% and 80% amino acid sequence identity respectively to the murine enzyme. The major difference is that our results indicated that the mouse UGPPase was more specific for ADP-ribose hydrolysis than UDP-glucose hydrolysis, raising questions as to the physiological role of the enzyme. We cannot exclude a role in glycogen metabolism, as was proposed, and indeed, our screening indicated that the enzyme is widely distributed in mouse tissues including those important for glycogen storage. Further studies will be needed to clarify this issue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by NIH Grant DK27221 to PJR and an American Heart Fellowship to VST.

References

1. Schliselfeld LH, Vaneys J, Touster O. The Purification and Properties of a Nucleotide Pyrophosphatase of Rat Liver Nuclei. J Biol Chem 1965;240:811–818. [PubMed: 14275140]
2. Skidmore J, Trams EG. Nucleotide pyrophosphatase activity of rat liver plasma membranes. Biochim Biophys Acta 1970;219:93–103. [PubMed: 4319696]
3. Bachorik PS, Dietrich LS. The purification and properties of detergent-solubilized rat liver nucleotide pyrophosphatase. J Biol Chem 1972;247:5071–5078. [PubMed: 4403504]
4. Yagi T, Baroja-Fernandez E, Yamamoto R, Munoz FJ, Akazawa T, Hong KS, Pozueta-Romero J. Cloning, expression and characterization of a mammalian Nudix hydrolase-like enzyme that cleaves the pyrophosphate bond of UDP-glucose. Biochem J 2003;370:409–415. [PubMed: 12429023]
5. McLennan AG. The Nudix hydrolase superfamily. Cell Mol Life Sci 2006;63:123–143. [PubMed: 16378245]
6. Mildvan AS, Xia Z, Azurmendi HF, Saraswat V, Legler PM, Massiah MA, Gabelli SB, Bianchet MA, Kang LW, Amzel LM. Structures and mechanisms of Nudix hydrolases. Arch Biochem Biophys 2005;433:129–143. [PubMed: 15581572]
7. Laughlin MR, Petit WA Jr, Dizon JM, Shulman RG, Barrett EJ. NMR measurements of in vivo myocardial glycogen metabolism. J Biol Chem 1988;263:2285–2291. [PubMed: 3123491]
8. Seoane J, Trinh K, O'Doherty RM, Gomez-Foix AM, Lange AJ, Newgard CB, Guinovart JJ. Metabolic impact of adenovirus-mediated overexpression of the glucose-6-phosphatase catalytic subunit in hepatocytes. J Biol Chem 1997;272:26972–26977. [PubMed: 9341134]
9. Manchester J, Skurat AV, Roach P, Hauschka SD, Lawrence JC Jr. Increased glycogen accumulation in transgenic mice overexpressing glycogen synthase in skeletal muscle. Proceedings of the National Academy of Sciences of the United States of America 1996;93:10707–10711. [PubMed: 8855244]
10. Guida L, Zocchi E, Franco L, Benatti U, De Flora A. Presence and turnover of adenosine diphosphate ribose in human erythrocytes. Biochem Biophys Res Commun 1992;188:402–408. [PubMed: 1417862]
11. Skurat, AV.; Roach, PJ. Regulation of glycogen biosynthesis. In: LeRoith, D.; Olefsky, JE.; Taylor, S., editors. Diabetes Mellitus: A fundamental and clinical text. J. B. Lippincott Company; Philadelphia: 2003. p. 317-333.
12. Roach PJ. Glycogen and its metabolism. Curr Mol Med 2002;2:101–120. [PubMed: 11949930]

13. Ball S, Guan HP, James M, Myers A, Keeling P, Mouille G, Buleon A, Colonna P, Preiss J. From glycogen to amylopectin: a model for the biogenesis of the plant starch granule. Cell 1996;86:349–352. [PubMed: 8756717]

14. Preiss J, Romeo T. Physiology, biochemistry and genetics of bacterial glycogen synthesis. Adv Microb Physiol 1989;30:183–238. [PubMed: 2700539]

15. Zeeman SC, Smith SM, Smith AM. The diurnal metabolism of leaf starch. Biochem J 2007;401:13–28. [PubMed: 17150041]

16. Smith AM, Zeeman SC, Smith SM. Starch degradation. Annu Rev Plant Biol 2005;56:73–98. [PubMed: 15862090]

17. Munoz FJ, Baroja-Fernandez E, Moran-Zorzano MT, Alonso-Casajus N, Pozueta-Romero J. Cloning, expression and characterization of a Nudix hydrolase that catalyzes the hydrolytic breakdown of ADP-glucose linked to starch biosynthesis in Arabidopsis thaliana. Plant Cell Physiol 2006;47:926–934. [PubMed: 16774931]

18. Baroja-Fernandez E, Munoz FJ, Akazawa T, Pozueta-Romero J. Reappraisal of the currently prevailing model of starch biosynthesis in photosynthetic tissues: a proposal involving the cytosolic production of ADP-glucose by sucrose synthase and occurrence of cyclic turnover of starch in the chloroplast. Plant Cell Physiol 2001;42:1311–1320. [PubMed: 11773523]

19. Moreno-Bruna B, Baroja-Fernandez E, Munoz FJ, Bastarrica-Berasategui A, Zandueta-Criado A, Rodriguez-Lopez M, Lasa I, Akazawa T, Pozueta-Romero J. Adenosine diphosphate sugar pyrophosphatase prevents glycogen biosynthesis in Escherichia coli. Proc Natl Acad Sci U S A 2001;98:8128–8132. [PubMed: 11416161]

20. Rodriguez-Lopez M, Baroja-Fernandez E, Zandueta-Criado A, Pozueta-Romero J. Adenosine diphosphate glucose pyrophosphatase: A plastidial phosphodiesterase that prevents starch biosynthesis. Proc Natl Acad Sci U S A 2000;97:8705–8710. [PubMed: 10890880]

21. Orellana A, Neckelmann G, Norambuena L. Topography and Function of Golgi Uridine-5[prime]-Diphosphatase from Pea Stems. Plant Physiol 1997;114:99–107. [PubMed: 12223692]

22. Dhugga KS, Ray PM. Purification of 1,3-beta-D-glucan synthase activity from pea tissue. Two polypeptides of 55 kDa and 70 kDa copurify with enzyme activity. Eur J Biochem 1994;220:943–953. [PubMed: 8143748]

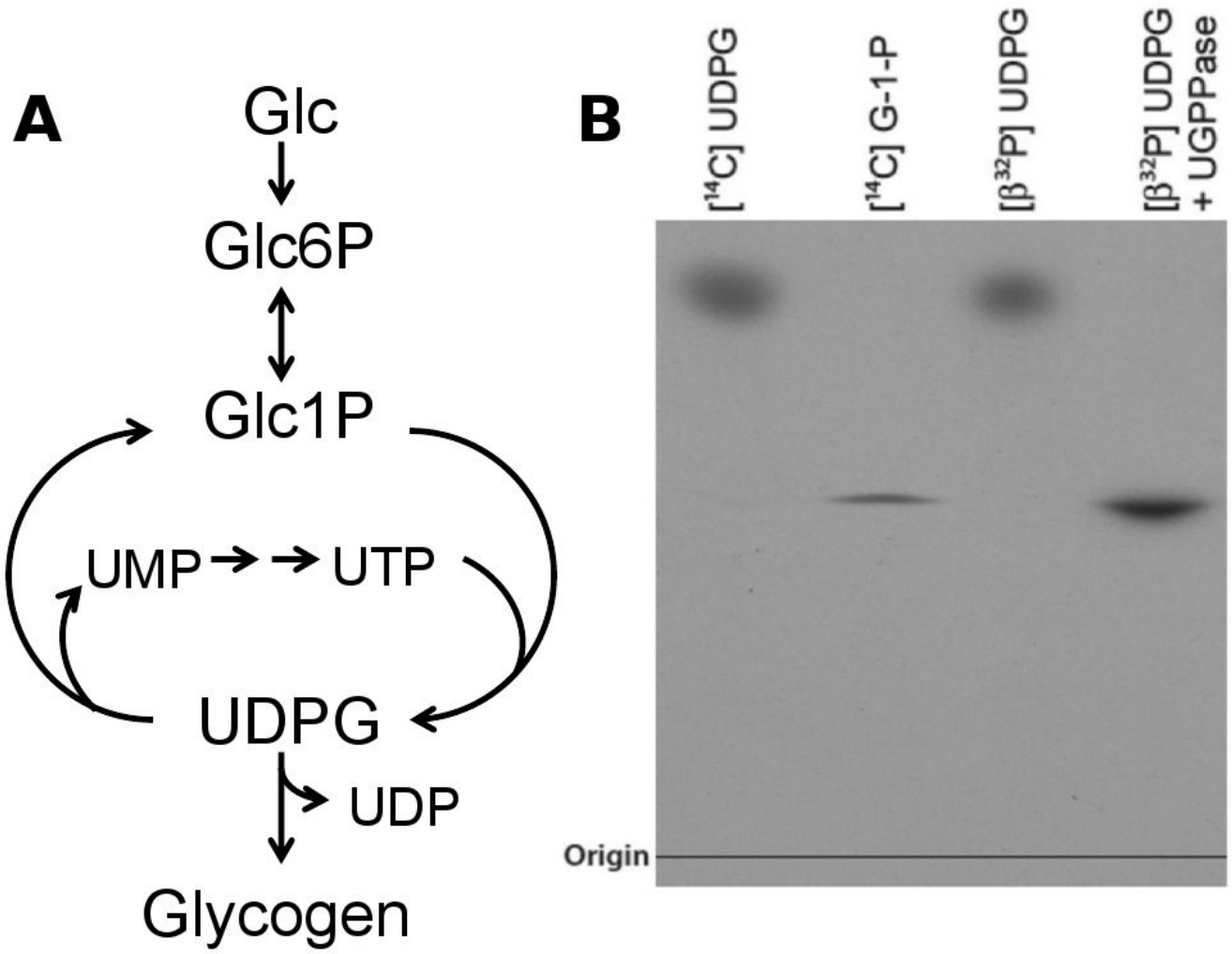
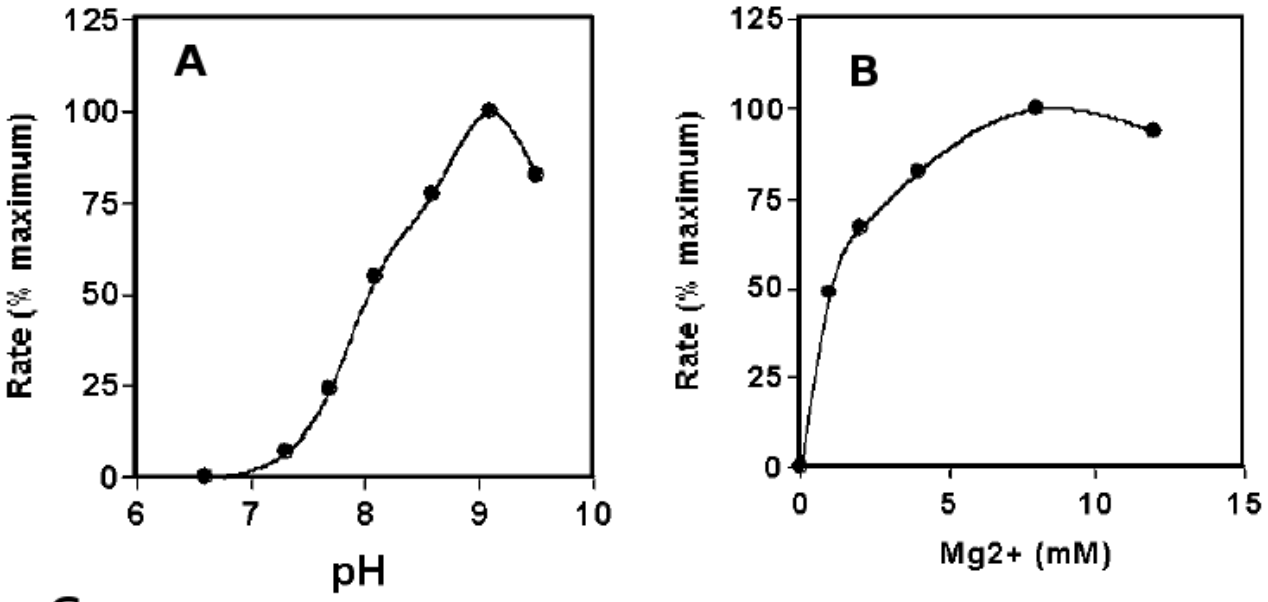


Figure 1.
UDP-glucose metabolism and UGPPase reaction. A. The conversion of glucose to glycogen via UDP-glucose is shown, together with the reaction catalyzed by UGPPase. B. Conversion of [β^{32}P]UDP-glucose to [^{32}P]glucose-1-P by the action of UGPPase. ^{14}C-labeled UDP-glucose and glucose-1-P standards were included.



C

Substrate	K_m (mM)	V_{max} (units/mg)	V_{max}/K_m
UDP-glucose	4.1 ± 1.7	6.5 ± 1.3	1.6
ADP-ribose	0.30 ± 0.02	10.6 ± 1.1	35

Figure 2.

Kinetic analysis of recombinant UGPPase. Using the methods described under "Experimental Procedures", the dependence of the reaction on pH (A) and Mg^{2+} concentration (B) are shown. The K_m and V_{max} values towards UDP-glucose (three independent experiments, two enzyme preparations) and ADP-ribose (four independent experiments, two enzyme preparations) are tabulated (C).

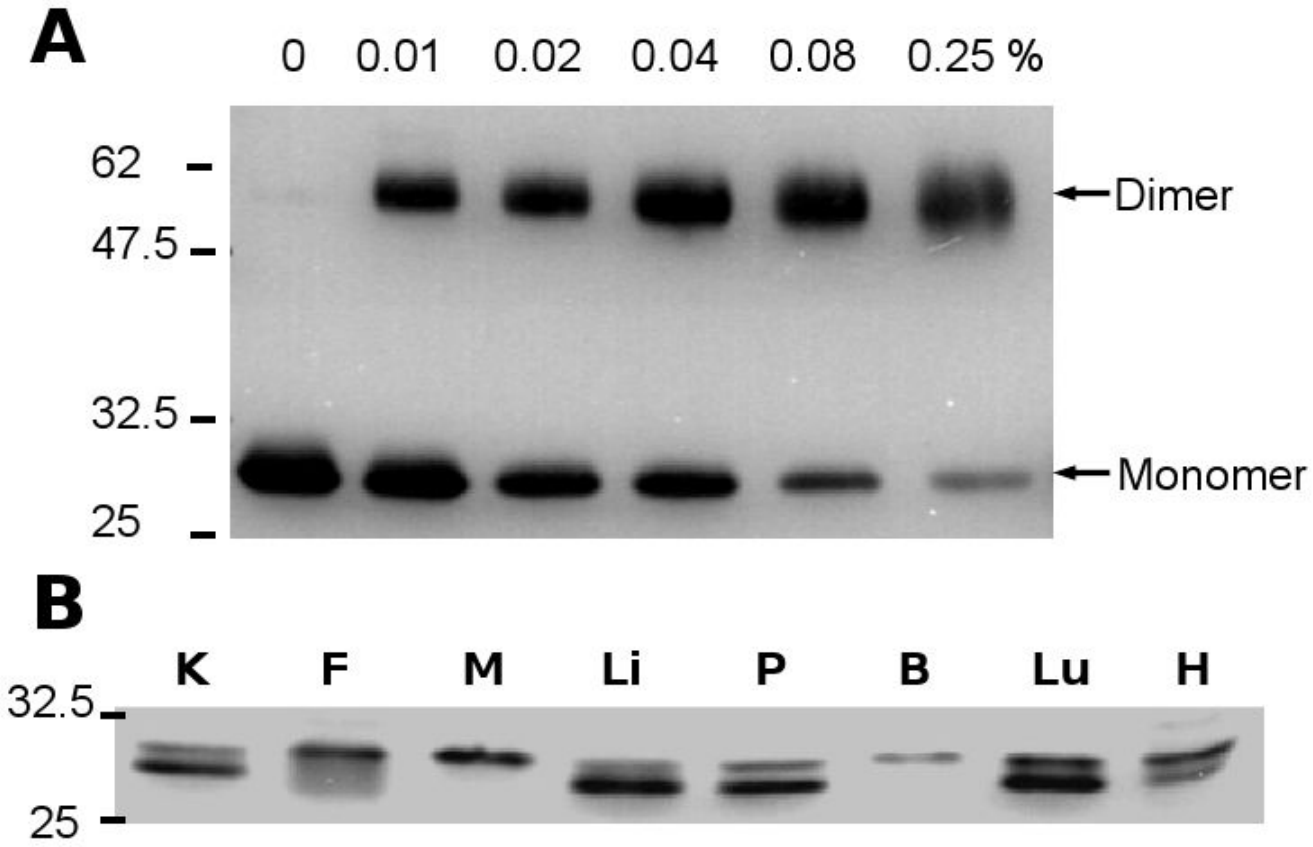


Figure 3.

A. Cross-linking of recombinant UGPPase with glutaraldehyde. The glutaraldehyde concentration was increased from 0 to 0.25%(v/v) as indicated above the corresponding lane and UGPPase was visualized by Western blotting. B. UGPPase was immunoprecipitated from mouse tissues extracts and then visualized by Western blotting, as described under "Experimental Procedures". K, kidney; F, fat; M, skeletal muscle; Li, liver; P, pancreas; B, brain; Lu, lung; H, heart.

A

	A
McLennan:	[1]GxxxxxExxxxxUxRExxEExGU[23]
Mildvan:	GxxxxxExxxxxxxREUxEExGU
UGPPase:	ELCAGxxDxxxxSxxxAxxExxEECGY

B

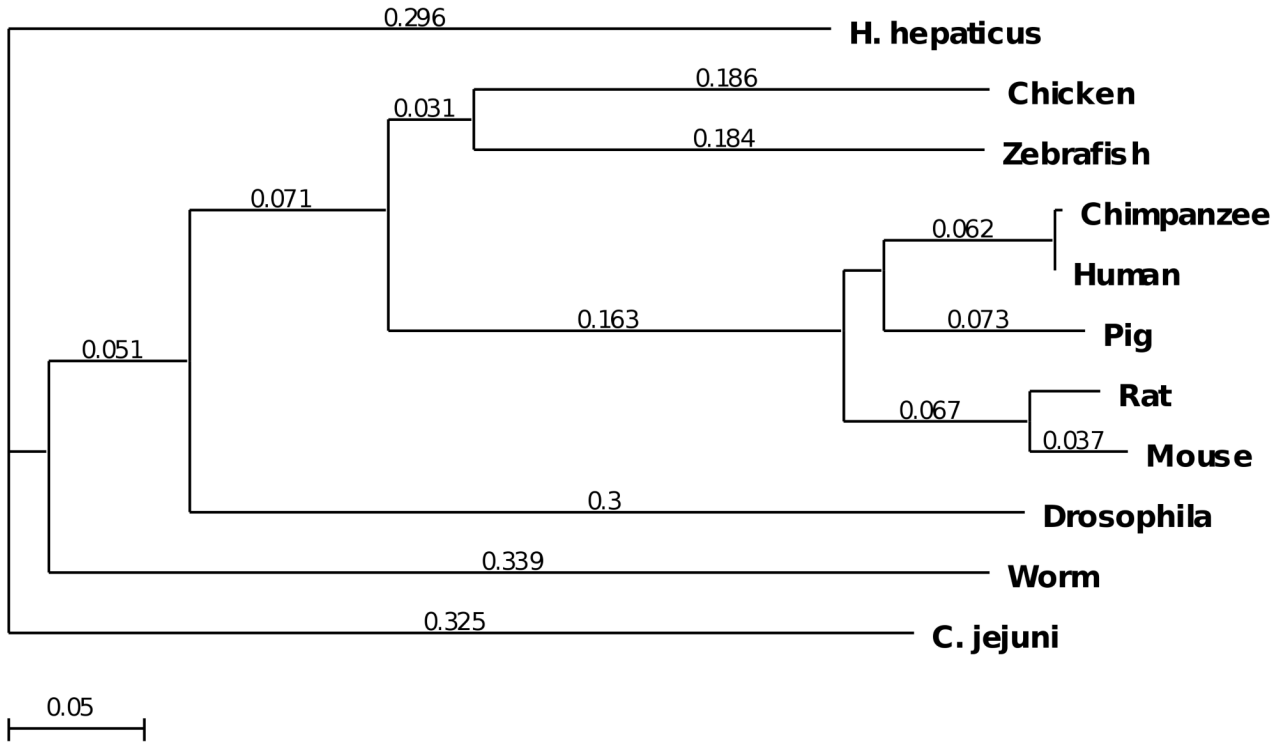


Figure 4.

Nudix box and phylogenetic analysis of UGPPase from different organisms. A. Nudix box consensus sequences compared with UGPPase Nudix box. B. The indicated UGPPase sequences were aligned by Clustal and a phylogentic tree generated using MacVector™ 8.0.