Sequence of a putative glucose 6-phosphate translocase, mutated in glycogen storage disease type Ib

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Abstract We report the sequence of a human cDNA that encodes a 46 kDa transmembrane protein homologous to bacterial transporters for phosphate esters. This protein presents at its carboxy terminus the consensus motif for retention in the endoplasmic reticulum. Northern blots of rat tissues indicate that the corresponding mRNA is mostly expressed in liver and kidney. In two patients with glycogen storage disease type Ib, mutations were observed that either replaced a conserved Gly to Cys or introduced a premature stop codon. The encoded protein is therefore most likely the glucose 6-phosphate translocase that is functionally associated with glucose-6-phosphatase.

Key words: Glucose-6-phosphatase; Membrane protein; Translocase; Glycogen storage disease; Endoplasmic reticulum

1. Introduction

Glycogen storage disease type I (GSD I) is due to a deficiency of glucose-6-phosphatase. This enzyme is normally present in the liver and the kidney, where it is located in the endoplasmic reticulum. According to the substrate-transport model, the enzymatic system comprises a hydrolase, whose catalytic site faces the lumen of the organelle, and various translocases responsible for the transport of glucose 6-phosphate, Pi and glucose [1–7]. The cDNAs encoding the hydrolase [8] and the glucose translocase [9] have been cloned, and the isolation of the phosphate transporter T2 has also been reported [10]. The putative glucose 6-phosphate transporter has not yet been characterized at the molecular level. Its existence has even been questioned by some authors, who interpret the peculiar kinetic properties of glucose-6-phosphatase by a conformational model [11–13]. The most common form of GSD I, called type Ia, is due to mutations in the gene encoding the hydrolase [14,15], whereas a second form (GSD Ib) has been attributed to a defect in the putative glucose 6-phosphate translocase [16–18].

Bacteria are able to metabolize externally added phosphorylated compounds such as hexose 6-phosphates, glycerol 3-phosphate and phosphoglycerate due to their ability to synthesize appropriate transporters (UhpT, GlpT and PgtP, respectively). These transporters all belong to the same family [19], which is itself a cluster in the superfamily of transmembrane facilitators with 12 transmembrane helices [20] and could therefore be homologous to liver and kidney glucose 6-phosphate translocase. By comparison of these sequences with liver ESTs (expressed sequence tags), we identified a cDNA sequence encoding a protein of the endoplasmic reticulum that is mutated in GSD Ib and is, therefore, most likely a glucose 6-phosphate transporter.

2. Materials and methods

2.1. Materials

Radioactive compounds and Thermosequenase were from Amersham and *Taq* polymerase and *Pwo* polymerase, from Boehringer. M-MLV reverse transcriptase was from Gibco-BRL and pcDNAI/Amp from Invitrogen.

2.2. Human tissues

Surgical biopsy specimens were obtained from two female patients when they were 22 (GL) or 10 (VK) years old respectively, and were kept at −80°C before use. The two patients displayed typical clinical and laboratory symptoms of GSD Ib including neutropenia. The diagnosis was confirmed by the finding that glucose-6-phosphatase activity was normal in detergent-treated extracts, but reduced in homogenates from fresh liver and by the fact that [U-^{14}C]glucose 6-phosphate uptake [18] was reduced to about 10% of control values. Fragments of control liver, from injured subjects, were obtained from the liver transplantation department (Cliniques Universitaires St-Luc).

2.3. Methods

The ≈440 bp probe used in the screening of the human cDNA library and in the Northern blots was obtained by PCR amplification of mouse liver cDNA with *Taq* polymerase and with two primers designed from the EST with accession number AA261251 (GenBank): 5′-GATCCAGGCACTAAAGAGAGCTAGC-3′ and 5′-CTTGTGCCGGACCATTAGGAACCCA-3′. The amplified product was purified by electrophoresis in agarose gel and labelled with [α-^{32}P]dCTP by random priming [21]. About 180 000 clones of a human bladder tumor (LB831-BLC) cDNA library (oriented, in pcDNAI/Amp, kindly provided by M. Guéguen and B. Van den Eynde, Ludwig Institute, Brussels) were screened. Four positive independent clones were obtained after primary and secondary screening. Restriction fragments were subcloned in pBlueScript for sequencing. RNA extraction and Northern blotting were performed as previously described [22].

For the search of mutations, total RNA extracted from the liver of patients and of controls was reverse transcribed with M-MLV reverse transcriptase. The 5′ and 3′ parts of the open reading frame of the human translocase were amplified by two successive rounds of amplification with nested primers (p1, p2, p5 and p6 for the 5′ end; p3, p4, p8 and p9 for the 3′ end, see Fig. 1) and with *Pwo* polymerase, a polymerase with proofreading activity. The amplified products were cloned in the *Eco*RV site of pBlueScript and sequenced.

cDNAs were sequenced completely in both directions by the dideoxy method [23] with T7 Thermosequenase and IR-dye labelled primers. Products were analyzed using an automated laser fluorescence DNA sequencer 4000L from LI-COR. Multiple sequence alignment was performed using the program PILEUP, Wisconsin Package version 9.0, Genetics Computer Group, Madison, WI.

The QIAAmp Blood kit (QIAGEN) was used to isolate genomic

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The nucleotide sequence of the cDNA described in this article is deposited in the EMBL database under accession number Y15409.

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DNA from liver homogenates prepared in 100 mM NaCl, 0.5% SDS, 0.1 mg/ml proteinase K, 10 mM Tris-HCl and 25 mM EDTA, pH 8.0. PCR was carried out with *Taq* polymerase.

3. Results

3.1. Cloning and sequencing of the human cDNA

Using the sequence encoding the first 215 amino acids of the *Lactobacillus lactis* hexose-phosphate transporter (GenBank no. X71493), we identified a 445 bp EST from mouse liver (no. AA261251) encoding a homologous protein.

Primers derived from this sequence were used to PCR-amplify cDNA from mouse liver, kidney, heart and brain. A fragment with the expected size (≈440 bp) was obtained in all cases, though it was more abundant with liver and kidney cDNA than with cDNA from the other two tissues. The fragment amplified from liver cDNA was used as a probe to screen a human cDNA library from a bladder tumor. Four different clones were obtained, the sequence of the longest is shown in Fig. 1. The second ATG codon of this sequence is most likely the one that is utilized for initiation of translation, since it fits much better with Kozak's consensus [24] than the first one. Furthermore, comparison of the human and the mouse nucleotide sequences indicated that a high degree of conservation (85% identity) started just before this ATG codon, whereas the upstream sequence was much less conserved (≈51% identity), as expected for untranslated sequences.

The cDNA thus encodes a 429 residue protein with a calculated molecular mass of 46 329 Da. As shown in Fig. 2, the predicted protein is homologous to a series of bacterial transporters, such as GlpT (25% identity), PgtP (21%) and UhpT (20%), as well as with UhpC (26%), a membrane protein known to be involved in the control of UhpT expression and which most likely serves as a glucose 6-phosphate receptor [25]. The putative human translocase is a very hydrophobic protein, with a hydropathy profile nearly superimposable to those of GlpT and UhpT (not shown). Thus, its structure is

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CAGGCTTAATGATTGTCCAGAAGGCGGCTATAAAGGGAGCCTGGGAGGCTGGGTGGAGGA   60
GGGAGCAGAAAAAACCCAACTCAGCAGATCTGGGAACTGTGAGAGCGGCAAGCAGGAACT  120 p1
GTGGTCAGAGGCTGTGCGTCTTGGCTGGTAGGGCCTGCTCTTTTCTACCATGGCAGCCCA  180 p2
                                                M  A  A  Q    4
GGGCTATGGCTATTATCGCACTGTGATCTTCTCAGCCATGTTTGGGGGCTACAGCCTGTA  240
  G  Y  G  Y  Y  R  T  V  I  F  S  A  M  F  G  G  Y  S  L  Y   24
TTACTTCAATCGCAAGACCTTCTCCTTTGTCATGCCATCATTGGTGGAAGAGATCCCTTT  300
  Y  F  N  R  K  T  F  S  F  V  M  P  S  L  V  E  E  I  P  L   44
GGACAAGGATGATTTGGGGTTCATCACCAGCAGCCAGTCGGCAGCTTATGCTATCAGCAA  360
  D  K  D  D  L  G  F  I  T  S  S  Q  S  A  A  Y  A  I  S  K   64
GTTTGTCAGTGGGGTGCTGTCTGACCAGATGAGTGCTCGCTGGCTCTTCTCTTCTGGGCT  420
  F  V  S  G  V  L  S  D  Q  M  S  A  R  W  L  F  S  S  G  L   84
GCTCCTGGTTGGCCTGGTCAACATATTCTTTGCCTGGAGCTCCACAGTACCTGTCTTTGC  480
  L  L  V  G  L  V  N  I  F  F  A  W  S  S  T  V  P  V  F  A  104
TGCCCTCTGGTTCCTTAATGGCCTGGCCCAGGGGCTGGGCTGGCCCCCATGTGGGAAGGT  540
  A  L  W  F  L  N  G  L  A  Q  G  L  G  W  P  P  C  G  K  V  124
CCTGCGGAAGTGGTTTGAGCCATCTCAGTTTGGCACTTGGTGGGCCATCCTGTCAACCAG  600
  L  R  K  W  F  E  P  S  Q  F  G  T  W  W  A  I  L  S  T  S  144
CATGAACCTGGCTGGAGGGCTGGGCCCTATCCTGGCAACCATCCTTGCCCAGAGCTACAG  660
  M  N  L  A  G  G  L  G  P  I  L  A  T  I  L  A  Q  S  Y  S  164
CTGGCGCAGCACGCTGGCCCTATCTGGGGCACTGTGTGTGGTTGTCTCCTTCCTCTGTCT  720
  W  R  S  T  L  A  L  S  G  A  L  C  V  V  V  S  F  L  C  L  184  p3
CCTGCTCATCCACAATGAACCTGCTGATGTTGGACTCCGCAACCTGGACCCCATGCCCTC  780 p4
  L  L  I  H  N  E  P  A  D  V  G  L  R  N  L  D  P  M  P  S  204
TGAGGGCAAGAAGGGCTCCTTGAAGGAGGAGAGCACCCTGCAGGAGCTGCTGCTGTCCCC  840
  E  G  K  K  G  S  L  K  E  E  S  T  L  Q  E  L  L  L  S  P  224  p5
TTACCTGTGGGTGCTCTCCACTGGTTACCTTGTGGTGTTTGGAGTAAAGACCTGCTGTAC  900 p6
  Y  L  W  V  L  S  T  G  Y  L  V  V  F  G  V  K  T  C  C  T  244
TGACTGGGGCCAGTTCTTCCTTATCCAGGAGAAAGGACAGTCAGCCCTTGTAGGTAGCTC  960
  D  W  G  Q  F  F  L  I  Q  E  K  G  Q  S  A  L  V  G  S  S  264
CTACATGAGTGCCCTGGAAGTTGGGGGCCTTGTAGGCAGCATCGCAGCTGGCTACCTGTC 1020
  Y  M  S  A  L  E  V  G  G  L  V  G  S  I  A  A  G  Y  L  S  284
AGACCGGGCCATGGCAAAGGCGGGACTGTCCAACTACGGGAACCCTCGCCATGGCCTGTT 1080
  D  R  A  M  A  K  A  G  L  S  N  Y  G  N  P  R  H  G  L  L  304
GCTGTTCATGATGGCTGGCATGACAGTGTCCATGTACCTCTTCCGGGTAACAGTGACCAG 1140
  L  F  M  M  A  G  M  T  V  S  M  Y  L  F  R  V  T  V  T  S  324
TGACTCCCCCAAGCTCTGGATCCTGGTATTGGGAGCTGTATTTG@TTTCTCCTCGTATGG 1200 p7
  D  S  P  K  L  W  I  L  V  L  G  A  V  F  @  F  S  S  Y  G  344
CCCCATTGCCCTGTTTGGAGTCATAGCCAAC@AGAGTGCCCCTCCCAACTTGTGTGGCAC 1260
  P  I  A  L  F  G  V  I  A  N  @  S  A  P  P  N  L  C  G  T  364
CTCCCACGCCATTGTGGGACTCATGGCCAATGTGGGCGGCTTTCTGGCTGGGCTGCCCTT 1320
  S  H  A  I  V  G  L  M  A  N  V  G  G  F  L  A  G  L  P  F  384
CAGCACCATTGCCAAGCACTACAGTTGGAGCACAGCCTTCTGGGTGGCTGAAGTGATTTG 1380
  S  T  I  A  K  H  Y  S  W  S  T  A  F  W  V  A  E  V  I  C  404
TGCGGCCAGCACGGCTGCCTTCTTCCTCCTACGAAACATCCGCACCAAGATGGGCCGAGT 1440
  A  A  S  T  A  A  F  F  L  L  R  N  I  R  T  K  M  G  R  V  424
GTCCAAGAAGGCTGAGTGAAGAGAGTCCAGGTTCCGGAGCACCATCCCACGGTGGCCTTC 1500 p8
  S  K  K  A  E                                                 429  p9
CCCCTGCACGCTCTGCGGGGAGAAAAGGAGGGGCCTGCCTGGCTAGCCCTGAACCTTTCA 1560
CTTTCCATTTCTGCGCCTTTTCTCTCACCCGGGTGGCGCTGGAAGTTATCAGTGGCTAGT 1620
GAGGTCCCAGCTCCCTGATCCTATGCTCTATTTAAAAGATAACCTTTGGCCTTAGACTCC 1680
GTTAGCTCCTATTTCCTGCCTTCAGACAAACAGGAAACTTCTGCAGTCAGGAAGGCTCCT 1740
GTACCCTTCTTCTTTTCCTAGGCCCTGTCCTGCCCGCATCCTACCCCATCCCCACCTGAA 1800
GTGAGGCTATCCCTGCAGCTGCAGGGCACTAATGACCCTTGACTTCTGCTGGGTCCTAAG 1860
TCCTCTCAGCAGTGGGCGACTGCTGTTGCCAATACCTCAGACTCCAGGGAAAGAGAGGAG 1920
GCCATCATTCTCACTGTACCACTAGGCGCAGTTGGATATAGGTGGGAAGAAAAGGTGACT 1980
TGTTATAGAAGATTAAAACTAGATTTGATACTGAAAAAAAAAAAAAAAAAAAAAAAAAAA 2040
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Fig. 1. Sequence of the human cDNA encoding the putative transporter and deduced amino acid sequence. The stop codon and the retention signal for the endoplasmic reticulum are underlined. The primers are numbered p1 to p9 in the right margin, and their position and direction are indicated by arrows under the nucleotide sequence. The two mutations ($G^{1184}T$ resulting in $Gly^{339}Cys$ and $G^{1232}T$ resulting in $Glu^{355}stop$) are in shadowed characters.

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                                h1                                  h2
UhpC MLPFLKAPAD APL-MTDKYE IDARYRYWRR HILLTIWLGY ALFYFTRKSF NAAVPEILAN GVLSRSDIGL LATLFYITYG VSKFVSGIVS DRSNARYFMG  99
GlpT MLSIFKPAPH KAR-LPAA-E IDPTYRRLRW QIFLGIFFGY AAYYLVRKNF ALAMPYLVEQ G-FSRGDLGF ALSGISIAYG FSKFIMGSVS DRSNPRVFLP  97
PgtP MLTILKTGQS AHK-VPPE-K VQATYGRYRI QALLSVFLGY LAYYIVRNNF TLSTPYLKEQ LDLSATQIGL LSSCMLIAYG ISKGVMSSLA DKASPKVFMA  98
UhpT MLAFLNQVRK PTLDLPLEVR RKMWFKPFM- QSYLVVFIGY LTMYLIRKNF NIAQNDMIST YGLSMTQLGM IGLGFSITYG VGKTLVSYYA DGKNTKQFLP  99
Hums ---------- ---------M AAQGYGYYRT VIFSAMFGGY SLYYFNRKTF SFVMPSLVEE IPLDKDDLGF ITSSQSAAYA ISKFVSGVLS DQMSARWLFS  81

          h3                                h4                                  h5
UhpC IGLIATGIIN ILF-GF---- STSLWAFAVL WVLNAFFQGW GSPVCARLLT AWYS----RT ERGGWWALWN TAHNVGGALI PIV-MAAAAL HYGWR---AG 186
GlpT AGLILAAAVM LFM-GFVPWA TSSIAVMFVL LFLCGWFQGM GWPPCGRTMV HWWS----QK ERGGIVSVWN CAHNVGGGIP PLLFLLGMAW FNDWH---AA 189
PgtP CGLVLCAIVN VGL-GF---- SSAFWIFAAL VVFNGLFQGM RRP-----LV YYYCKLVPRR ERGRVGAFWN ISHNVGGGIV APIVGAAFAI LGSEHWQSAS 188
UhpT FMLILSAICM LGFSASMGSG SVSLFLMIAF YALSGFFQST GGSCSYSTIT KW----TPRR KRGTFLGFWN ISHNLGGAGA AGVALFGANY LFDGH-VIGM 194
Hums SGLLLVGLVN IFFA-----W SSTVPVFAAL WFLNGLAQGL GWPPCGKVLR KWF----EPS QFGTWWAILS TSMNLAGGLG PIL----ATI LAQSYSWRST 168

          h6                                                    h7
UhpC MMIAGCMAIV VGIFLCWRLR DRPQALGLPA VGEWRHDALE IAQ----QQE GAGLTRKEIL TKYVLLNPYI WLLSFCYVLV YVVRAAINDW GNLYMSETLG 282
GlpT LYMPAFCAIL VALFAFAMMR DTPQSCGLPP IEEYKNDYPD DYN----EKA EQELTAKQIF MQYVLPNKLL WYIAIANVFV YLLRYGILDW SPTYLKEVKH 285
PgtP YIVPACVAVI FALIVLVLGK GSPRKEGLPS LEQMMPEEKV VLKTKNTAKA PENMSAWQIF CTYVVRNKNA WYISLVDVFV YMVRFGMISW LPIYLLTVKH 288
UhpT FIFPSIIALI VGFIGLRYGS DSPESYGLGK AEELFGEE-- -ISEEDKETE STDMTKWQIF VEYVLKNKVI WLLCFANIFL YVVRIGIDQW STVYAFQELK 291
Hums LALSGALCVV VSFLCLLLIH NEPADVGLRN LDPMPSEGK- ----KGSLKE ESTL------ -QELLLSPYL WVLSTGYLVV FGVKTCCTDW GQFFLIQEKG 256

                h8                                  h9                                  h10
UhpC VDLVTANTAV TMFELGGFIG ALVAGWGSDK L--------F NGNRGPMNLI FAAGILLSVG SLWLMPFASY VMQATCFF-- TIGFFVFGPQ MLIGMAAAEC 372
GlpT FALDKSSWAY FLYEYAGIPG TLLCGWMSDK V--------F RGNRGATGVF FMTLVTIATI VYWMNPAGNP TVDMICMI-- VIGFLIYGPV MLIGLHALEL 375
PgtP FSKEQMSVAF LFFEWAAIPS TLLAGWLSDK L--------F KGRRMPLAMI CMALIFVCLI GYW--KSESL LMVTIFAA-- IVGCLIYVPQ FLASVQTMEI 376
UhpT LSKAVAIQGF TLFEAGALVG TLLWGWLSD- L--------A NGRRGLVACI ALALIIATLG VY--QHASNE YIYLASLF-- ALGFLVFGPQ LLIGVAAVGF 379
Hums QSALVGSSYM SALEVGGLVG SIAAGYLSDR AMAKAGLSNY GNPRHGLLLF MMAGMTVSMY LFRVTVTSDS PKLWILVLGA VFGFSSYGPI ALFGVIANES 356
                                                                                    C                   *
                h11                                 h12
UhpC SHKEAAGAAT GFVGLFAY-L GASLAGWP-- ----LAKVLD TWHWSGFFVV ISIAAG--IS ALLLLPFLNA QTPREA                          439
GlpT APKKAAGTAA GFTGLFGY-L GGSVAASAI- ----VGYTVD FFGWDGGFMV MIGGSI--LA VILLIVVMIG EKRRHEQLLQ ERNGG                 452
PgtP VPSFAVGSAV GLRGFMSYIF GASLGTSLF- ----C                                                                     406
UhpT VPKKAIGAAD GIKGTFAYLI GDSFAKLGLG MIADGTPVFG LTGWAGTFAA LDIAAIGCIC LMAIVAVMEE RKIRREKKIQ QLTVA               463
Hums APPNLCGTSH AIVGLMAN-V GGFLAGLPFS TIA------K HYSWSTAFWV AEVICAASTA AFFLLRNIRT KMGRVSKKAE                       429
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Fig. 2. Sequence comparison of the human transporter with bacterial transporters for monophosphate esters. The following sequences are shown: glucose 6-phosphate receptor (UhpC, from *E. coli* [25]); transporters for glycerol 3-phosphate (GlpT, from *E. coli* [26]), for phosphoglycerate (PgtP, from *Salmonella typhimurium* [27]) and for hexose 6-phosphates (UhpT, from *E. coli* [28]); human transporter (Hums). Bars above the alignments indicate the positions of the transmembrane helices predicted for UhpC [25]. Strictly conserved residues are in bold. The positions of the two mutations found in the patients are indicated by a C (for cysteine) and by an asterisk (stop).

most likely that of a membrane protein with 12 helical transmembrane domains [25,29].

Remarkably, the encoded protein possesses at its carboxy terminus two lysine residues in positions −3 and −4. This sequence has been shown to be a signal necessary to maintain transmembrane proteins in the endoplasmic reticulum [30,31]. A similar motif is observed in the sequence of glucose-6-phosphatase [8]. One single potential glycosylation signal is found, but it is present in the loop between helices 10 and 11, which is predicted to be on the cytosolic side if the same topology applies as for UhpT and UhpC [25].

3.2. Northern blots

Northern blot performed on rat tissues (Fig. 3) showed that a major 2.2 kb mRNA was much more abundant in liver and in kidney than in other tissues. A second 1.7 kb mRNA was mainly found in liver whereas small RNA species with sizes >2.2 kb were present in other tissues. Human liver contained a ≈ 2 kb mRNA species (not shown), which suggested that the cDNA shown in Fig. 1 is complete.

3.3. Presence of mutations in patients with GSD Ib

cDNA was prepared from the liver of two patients with GSD Ib and the coding region of the presumed transporter was amplified by PCR using sets of primers spaced by about 700 nucleotides. The amplification products were cloned and sequenced on both strands. One patient (GL) had a mutation converting an extremely conserved glycine residue (Gly^{339}) to a cysteine (Figs. 1 and 2). This mutation was found in all five clones that were sequenced. The second patient (VC) had in addition to the Gly^{339}Cys mutation (found in two clones out of six), a mutation that replaced Glu^{355} by a stop codon (in the four other clones). These mutations were not found in cDNA amplified from four controls.

To confirm the presence of these mutations at the genomic level, we PCR-amplified genomic DNA from the patients and from four controls using primer p9 (Fig. 1) and either a mutated or a non-mutated primer (p7) with its 3′ end corresponding to the position of the mutated nucleotide in the 339th codon. Amplification was observed only with the non-mutated primer in the case of controls, only with the mutated primer with patient 1 and with both primers in the case of patient 2. Patient 1 appears therefore to be homozygous and patient 2 heterozygous, for the Gly^{339}Cys mutation. In all cases, the size of the amplified product was 500 bp, which indicated the presence of a ≈ 150 bp intron in this region.

The second mutation found in patient 2 was expected to introduce a *MaeI*/*Bfa*I restriction site. Its presence was confirmed by the finding that restriction with *Bfa*I generated a ≈ 70 bp fragment from the PCR product of genomic DNA amplified with the non-mutated primer p7, though not from that obtained with mutated p7. No such restriction was found with fragments amplified from four controls or from patient 1. These results indicated that the two mutations found in patient 2 were allelic.

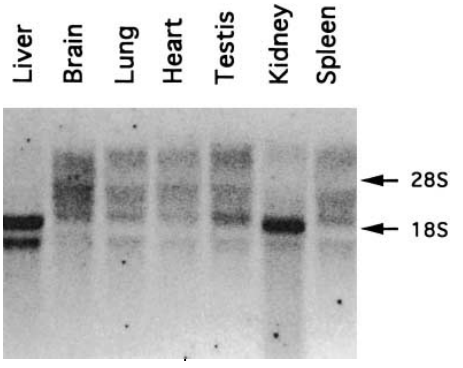

Fig. 3. Northern blot of rat tissues. Each lane was loaded with 25 μg total RNA from the indicated tissues. Representative of three experiments performed on different animals.

4. Discussion

The cDNA reported in the present paper encodes a protein homologous to several bacterial proteins that are transporters (UhpT, GlpT and PgtP) or receptor (UhpC) for monophosphate esters, and which actually shares most of the residues that are common to these bacterial proteins. This human protein is, therefore, most likely a transporter or a receptor for a monophosphate ester. Due to the fact that its two closest homologs, UhpC and GlpT, have affinity for glucose 6-phosphate and glycerol 3-phosphate, respectively, it is impossible to deduce its specificity solely on the basis of the sequence data. However, the tissular distribution of this protein and the presence of a targeting sequence for the endoplasmic reticulum are easily accounted for if one assumes that it is the glucose 6-phosphate translocator (or receptor) that is functionally associated with glucose-6-phosphatase.

The best proof for its identity comes from the finding that this protein is mutated in two patients with glycogen storage disease type Ib. Due to the high degree of conservation of Gly^{339} (it is one of the only 30 residues that are strictly conserved in the sequence comparison shown in Fig. 2), its replacement by a cysteine residue is probably incompatible with correct folding or function. In addition, the presence of an additional cysteine residue may create abnormal disulfide bridges. The stop codon introduced by the second mutation found in patient 2 not only removes the last two helices of the protein but also its targeting signal. Thus, even if the protein is functional in the absence of the last two helices, it wouldn't be located in the appropriate compartment for the function of glucose-6-phosphatase.

The availability of the cDNA reported here will be of great help to define the precise function of the putative glucose 6-phosphate transporter, i.e. if it truly acts as a transporter and if it is responsible for the specificity of glucose-6-phosphatase. It also opens the possibility of diagnosing GSD Ib directly at the gene level.

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