Identification of the cDNA encoding human 6-phosphogluconolactonase, the enzyme catalyzing the second step of the pentose phosphate pathway[1]

François Collard[2], Jean-François Collet[2], Isabelle Gerin, Maria Veiga-da-Cunha, Emile Van Schaftingen*

Laboratory of Physiological Chemistry, Université catholique de Louvain and Christian de Duve Institute of Cellular Pathology, 7539 Avenue Hippocrate, B-1200 Brussels, Belgium

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Abstract We report the sequence of a human cDNA encoding a protein homologous to *devB* (a bacterial gene often found in proximity to the gene encoding glucose-6-phosphate dehydrogenase in bacterial genomes) and to the C-terminal part of human hexose-6-phosphate dehydrogenase. The protein was expressed in *Escherichia coli*, purified and shown to be 6-phosphogluconolactonase, the enzyme catalyzing the second step of the pentose phosphate pathway. Sequence analysis indicates that bacterial devB proteins, the C-terminal part of hexose-6-phosphate dehydrogenase and yeast Sol1–4 proteins are most likely also 6-phosphogluconolactonases and that these proteins are related to glucosamine-6-phosphate isomerases.

Key words: Glucose-6-phosphate dehydrogenase; 6-Phosphogluconolactonase; Glucosamine-6-phosphate isomerase; Pentose phosphate pathway

1. Introduction

The second step of the pentose phosphate pathway is the hydrolysis of 6-phosphogluconolactone, a spontaneous reaction that is greatly accelerated by a specific 6-phosphogluconolactonase widely distributed in the living world [1–4]. This enzyme has been purified to homogeneity from bovine erythrocytes [3] and bass liver [5] and shown to be a $\approx$30 kDa monomer. 6-Phosphogluconolactonase hydrolyzes both the δ- and γ-forms of 6-phosphogluconolactone [3] and, in contrast to other lactonases, does not depend on Mg^{2+} for its activity [2]. Until now, the sequence of this enzyme has not been published.

Many bacterial genomes contain, in proximity to the gene encoding glucose-6-phosphate dehydrogenase, a gene called *devB*, which is homologous to the C-terminal part of the hexose-6-phosphate dehydrogenase present in the endoplasmic reticulum of mammalian cells [6,7]. These findings led us to hypothesize that the protein encoded by *devB* is 6-phosphogluconolactonase. In this paper, we report the sequence of a new human cDNA homologous to *devB* and show that it encodes indeed a 6-phosphogluconolactonase.

*Corresponding author. Fax: (32) (2) 7647598.
E-mail: vanschaftingen@bchm.ucl.ac.be

[1] The nucleotide sequence for human 6-phosphogluconolactonase has been deposited in the EMBL database under EMBL accession number AJ243972.

[2] The first two authors contributed equally to this work.

2. Materials and methods

2.1. Materials

6-Phosphogluconate dehydrogenase (type V, from *Torula* yeast), antipain, leupeptin and Tris were from Sigma. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase and *Pwo* polymerase were from Roche. DEAE-Sepharose, Sephacryl S-200 and Thermosequenase were from Amersham Pharmacia Biotech. IMAGE clone 329009 was ordered from HGMP (Hinxton, UK). Poly(ethyleneglycol) 6000 was from UCB. Molecular weight standards were from Bio-Rad.

2.2. Bioinformatics

ESTs homologous to the protein encoded by the *devB* gene of *Actinobacillus actinomycetemcomitans* (D88189, [8]) were searched with the tblastn function of the BLAST 2.0 program [9,10]. Multiple sequence alignments were made using the PILEUP program of the GCG package (Wisconsin Package version 10.0, Genetics Computer Group, Madison, WI, USA).

2.3. Molecular biology techniques

The insert of clone 329009 was subcloned in pBluescript SK− and completely sequenced in both directions by the dideoxy method [11] with T7 Thermosequenase and primers labelled with an infra-red dye (IRD 41). Products were analyzed using an automated laser fluorescence DNA sequencer 4000L from LI-COR. cDNA prepared from human liver RNA [12,13] with M-MLV reverse transcriptase and an oligodT primer served as template to amplify the region corresponding to the open reading frame of the *devB* homolog. The PCR reaction was carried out with *Pwo* polymerase, a primer (5′-CAT**ATG**-GCCGCGCCGGCCCCGGGCCTCA-3′) containing the ATG codon (bold) in a *Nde*I restriction site (underlined) and another (5′-GC-GGGATCCCTCTGGCCAG**CTA**CAAAGTGG-3′) containing the stop codon (bold) and a *Bam*HI restriction site (underlined). The product was cloned in pBluescript and sequenced. A *Nde*I-*Bam*HI fragment was excised from this plasmid and inserted into pET3a [14].

The chromosomal localization of the gene encoding the *devB* homolog was determined using the low-resolution Genebridge 4 radiation hybrid panel (Research Genetics). The occurrence of the human gene encoding the *devB* homolog was determined by PCR with *Taq* polymerase using the first primer mentioned above and a second one corresponding to nucleotides 171–191 of the cDNA sequence. Mapping was computed by the RH Mapper program available on-line at the Whitehead Institute for Genome Research, Massachussetts Institute of Technology.

2.4. Expression of the protein

BL21(DE3)pLysS cells were transformed with the expression plasmid and aerobically grown in M9 medium at 37°C until an A_{600} of 0.6 was reached. Isopropylthiogalactoside (IPTG) was then added to a final concentration of 0.4 mM and the culture was further incubated for 18 h at the same temperature. Bacteria were collected and extracts were prepared as described [15], with a lysis buffer containing 20 mM potassium phosphate, pH 7.4, 5 mM EDTA, 1 mM dithiothreitol, 5 μg/ml leupeptin, 5 μg/ml antipain, 0.5 mM PMSF and 1 mg/ml hen egg lysozyme.

For the purification of the lactonase, an extract prepared from a 4 l culture that was incubated for 18 h with IPTG was centrifuged for 40 min at 16 000 × *g* and 4°C and 33 g of poly(ethyleneglycol) 6000 was dissolved in the resulting supernatant (200 ml). The mixture was

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maintained for 15 min on ice and centrifuged for 15 min at 12 000×*g*. The supernatant, which contained most (>80%) of the 6-phosphogluconolactonase activity, was diluted 2-fold in buffer A (10 mM Tris, pH 8.5, 1 mM dithiothreitol, 1 μg/ml leupeptin and 1 μg/ml antipain) and applied onto a DEAE-Sepharose column (1.6×10 cm). The column was washed with 100 ml of buffer A and protein was eluted with a linear gradient of NaCl (0–400 mM in 2×200 ml buffer A). 6-Phosphogluconolactonase was eluted at ≈125 mM NaCl. The active fractions (14 ml) were concentrated to 1.5 ml by ultrafiltration on an Amicon YM10 membrane and loaded onto a 1.6×50 cm Sephacryl S-200 column, which was equilibrated and washed with a buffer containing 20 mM HEPES, pH 7.5, 100 mM KCl, 1 mM dithiothreitol, 1 μg/ml leupeptin and 1 μg/ml antipain. The active fractions were concentrated to a protein concentration of 1 mg/ml and stored at −80°C.

2.5. Enzyme and protein assays

For the measurement of 6-phosphogluconolactonase activity, the lactone was prepared extemporaneously by incubating 50 μM glucose-6-phosphate in the presence of 0.2 mM NADP, 25 mM HEPES, pH 7.1, 2 mM $MgCl_2$ and 1.75 U yeast glucose-6-phosphate dehydrogenase in 1 ml at 30°C. When the A_{340} reached a plateau, 0.5 U/ml 6-phosphogluconate dehydrogenase and the preparation of lactonase to be assayed (between 0.5 and 5 mU) were added and A_{340} was further measured for about 10 min. The blank corresponding to the spontaneous hydrolysis of lactone (≈0.8 nmol/min/ml) was subtracted. One unit of enzyme is the amount that hydrolyzes 1 μmol of 6-phosphogluconolactone per min under these conditions. Protein was measured according to Bradford [16] with bovine γ-globulin as a standard.

3. Results and discussion

3.1. Identification and sequence of a human cDNA encoding a devB homolog

A BLAST search in human EST databanks for homologs of devB from *A. actinomycetemcomitans* yielded about 80 sequences, which all appeared to be derived from one single gene, different from the one encoding human hexose-6-phosphate dehydrogenase [7]. Further BLAST searches with these sequences allowed us to identify a total of about 100 human ESTs derived from this gene. The clone with the most 5′ sequence was subcloned and completely re-sequenced on both strands. We also independently sequenced the region corresponding to the open reading frame, which was PCR-amplified from human liver cDNA.

```
CCGCCGCCGCCCTCGCCATGGCCGCGCCGGCCCCGGGCCTCATCTCGGTG   50
                  M  A  A  P  A  P  G  L  I  S  V    11
TTCTCGAGTTCCCAGGAGCTGGGTGCGGCGCTAGCGCAGCTGGTGGCCCA  100
 F  S  S  S  Q  E  L  G  A  A  L  A  Q  L  V  A  Q   28
GCGCGCAGCATGCTGCCTGGCAGGGGCCCGCGCCCGTTTCGCGCTCGGCC  150
  R  A  A  C  C  L  A  G  A  R  A  R  F  A  L  G    44
TGTCGGGCGGGAGCCTCGTCTCGATGCTAGCCCGCGAGCTACCCGCCGCC  200
L  S  G  G  S  L  V  S  M  L  A  R  E  L  P  A  A   61
GTCGCCCCTGCCGGGCCAGCTAGCTTAGCGCGCTGGACGCTGGGCTTCTG  250
 V  A  P  A  G  P  A  S  L  A  R  W  T  L  G  F  C   78
CGACGAGCGCCTCGTGCCCTTCGATCACGCCGAGAGCACGTACGGCCTCT  300
  D  E  R  L  V  P  F  D  H  A  E  S  T  Y  G  L    94
ACCGGACGCATCTTCTCTCCAGACTGCCGATCCCAGAAAGCCAGGTGATC  350
Y  R  T  H  L  L  S  R  L  P  I  P  E  S  Q  V  I  111
ACCATTAACCCCGAGCTGCCTGTGGAGGAGGCGGCTGAGGACTACGCCAA  400
 T  I  N  P  E  L  P  V  E  E  A  A  E  D  Y  A  K  128
GAAGCTGAGACAGGCATTCCAAGGGGACTCCATCCCGGTTTTCGACCTGC  450
  K  L  R  Q  A  F  Q  G  D  S  I  P  V  F  D  L   144
TGATCCTGGGGGTGGGCCCCGATGGTCACACCTGCTCACTCTTCCCAGAC  500
L  I  L  G  V  G  P  D  G  H  T  C  S  L  F  P  D  161
CACCCCCTCCTACAGGAGCGGGAGAAGATTGTGGCTCCCATCAGTGACTC  550
 H  P  L  L  Q  E  R  E  K  I  V  A  P  I  S  D  S  178
CCCGAAGCCACCGCCACAGCGTGTGACCCTCACACTACCTGTCCTGAATG  600
  P  K  P  P  P  Q  R  V  T  L  T  L  P  V  L  N   194
CAGCACGAACTGTCATCTTTGTGGCAACTGGAGAAGGCAAGGCAGCTGTT  650
A  A  R  T  V  I  F  V  A  T  G  E  G  K  A  A  V  211
CTGAAGCGCATTTTGGAGGACCAGGAGGAAAACCCGCTGCCCGCCGCCCT  700
 L  K  R  I  L  E  D  Q  E  E  N  P  L  P  A  A  L  228
GGTCCAGCCCCACACCGGGAAACTGTGCTGGTTCTTGGACGAGGCGGCCG  750
  V  Q  P  H  T  G  K  L  C  W  F  L  D  E  A  A   244
CCCGCCTCCTGACCGTGCCCTTCGAGAAGCATTCCACTTTGTAGCTGGCC  800
A  R  L  L  T  V  P  F  E  K  H  S  T  L           258
AGAGGGACGCCGCAGCTGGGACCAGGCACGCGGCCCATGGGGCTGGGCCC  850
CTGCTGGCCGCCACTCTCCGGGCTCTCCTTTCAAAAAGCCACGTCGTGCT  900
GCTGCTGGAAGCCAACAGCCTCCGGCCAGCAGCCCTACCCGGGGCTCAAC  950
ACACAGGCTGTGGCTCTGGACATCCGGATATTAAAAGGAGCGTTGCTGGA 1000
AAAAAAAAAA                                          1010
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Fig. 1. Sequence of the cDNA encoding the human homolog of devB (human 6-phosphogluconolactonase). The stop codon is underlined and the nucleotides missing in clone 329009 are italicized.

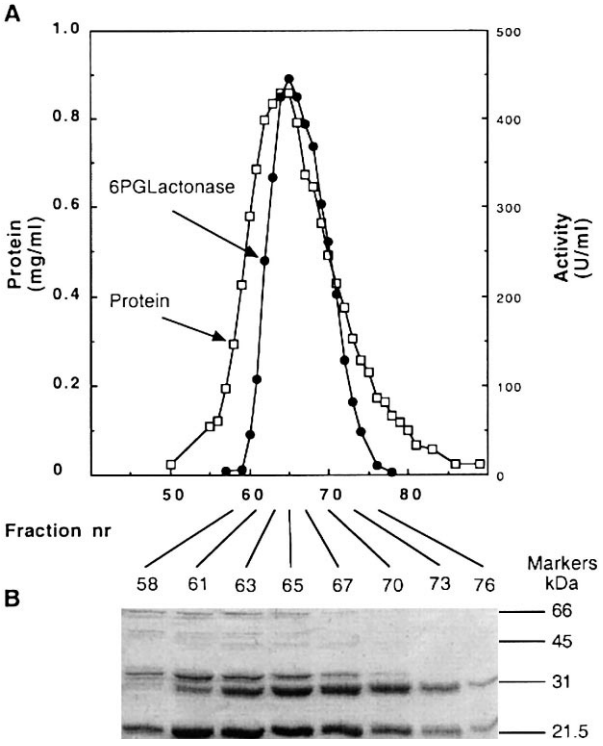

Fig. 2. Purification of 6-phosphogluconolactonase on Sephacryl S-200. Fractions from the DEAE-Sepharose column were pooled, concentrated and applied onto a Sephacryl S-200 column. Fractions of 1 ml were collected. 6-Phosphogluconolactonase activity and the protein concentration are shown in (A). The indicated fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Coomassie blue staining (B).

The cDNA sequence derived from these results is shown in Fig. 1. The first ATG codon is in an appropriate Kozak consensus sequence [17] and is conserved in the mouse cDNA, the sequence of which could be reconstituted from ESTs (not shown). In the human sequence, the initiator codon opens a reading frame predicting a protein of 258 residues, with a calculated molecular mass of 27 529.

Compared to the sequence derived from the product amplified from liver cDNA, clone 329009 presented a 20 bp deletion (Fig. 1). This deletion was only observed in two amongst the 70 human or mouse ESTs that are informative for this region of the sequence. Remarkably, the two ESTs presenting this deletion (W43017 and W43013) are derived from the same cDNA bank (from human pancreatic islet) as clone 329009. This suggests that this deletion, which changes the reading frame and therefore modifies the last 54 residues of the protein, is due to a genomic mutation present in the subject from whom the RNA was isolated to construct the library. As often

[18], this deletion occurs between two short direct repeats, GAAG in the present case.

3.2. Chromosomal localization

Using a radiation hybrid panel, we mapped the gene encoding the human *devB* homolog between markers WI-6344 and WI-1413 to 19p13.2.

3.3. Expression and characterization of the encoded protein

The open reading frame was inserted in pET3a, which was used to express the encoded protein in *Escherichia coli* BL21(DE3)pLysS [14]. Extracts were prepared from bacteria different times after induction with IPTG and the 6-phosphogluconolactonase activity was measured in supernatants. This activity, which was below the detection limit of our assay (0.02 U/mg protein) before the induction, reached a value of about 10–15 U/mg protein after 18 h at 37°C. No lactonase activity was detected when the bacteria contained a pET plasmid without insert.

The recombinant lactonase was purified about 50-fold with a ≈20% yield by a procedure involving (1) elimination of about 80% of the protein by precipitation with 16.5% poly(ethyleneglycol) 6000, (2) anion-exchange chromatography on DEAE-Sepharose and (3) gel filtration on Sephacryl S-200. In the last step (Fig. 2), the lactonase co-eluted with a polypeptide of the expected size (≈30 kDa). The two other proteins that were still present in the preparation had an elution profile that did not match that of the activity. The specific activity of the purest fraction (710 U/mg protein) was intermediary between the values reported for the enzymes purified to homogeneity from bovine erythrocytes (≈55 U/mg protein, [3]) and from bass liver (≈8300 U/mg protein, [5]). These differences could be due to the use of different assay conditions.

Comparison of the elution volume of recombinant 6-phosphogluconolactonase with those of molecular weight standards run on the same column allowed us to calculate a molecular mass of 30 kDa, indicating a monomeric structure, in agreement with previous results [3,5].

3.4. Sequence comparisons

BLAST searches performed with the human lactonase showed that the closest homologs to this enzyme were the yeast Sol1–4 proteins (33–37% sequence identity) followed by the C-terminal part of human hexose-6-phosphate dehydrogenase (26% identity), bacterial devB proteins (20–25% identity) and glucosamine-6-phosphate isomerases (17% identity in the case of the human enzyme). An alignment of some of these proteins with human 6-phosphogluconolactonase is shown in Fig. 3.

Glucosamine-6-phosphate isomerase catalyzes the conversion of glucosamine-6-phosphate and H_2O to fructose-6-phosphate and NH_3. One of its substrates (H_2O) is therefore shared with 6-phosphogluconolactonase and the other is similar in structure between C-3 and C-6 with 6-phosphogluconolactone. However, purified recombinant 6-phosphogluconolactonase was unable to deaminate glucosamine-6-phosphate, no production of fructose-6-phosphate (<0.2 μmol/min/mg protein) being observed in the presence of 1 mM glucosamine-6-phosphate, with or without 0.1 mM *N*-acetylglucosamine-6-phosphate, an allosteric effector of the isomerase [19].

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6pgl_human  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~MAAPAP    6
sol1_yeast  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~M TTTVPKVFAF HEFAGVAEAV ADHVIHAQNS ALKKGKVSRS TQMSGTSLNG NGNTESKTME   61
devb_actac  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
h6pd_human  PVRERDAHSV LLSHIFHGRK NFFITTENLL ASWNFWTPLL ESLAHKAPRL YPGGAENGRL LDFEFSSGRL FFSQQQPEQL VPGPGPAPMP SDFQVLRAKY  550
g6pi_human  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
g6pi_ecoli  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

                                                      GG                                    D R
6pgl_human  GLISVFSS.S QELGAALAQL VAQRAACCLA ..GA.R.ARF ALGLSGGSLV SMLARELPAA VAPAGPASLA RWTLGFCDER L.VPFDHAES TYGLYRTHLL  100
sol1_yeast  RVNSVRSN.A SSRGGS.... .EDGATKKLK ..KE.KERRF KIALSGGSLI QVL.HE...G LLKRDDVQWG KWDIYFADER L.VPFSSSES NYGLAKRKIF  147
devb_actac  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~M IYSQ.LNHPA HISLSGGSTP KLLFKTLAQS PY.AEQINWR NLHFWWGDDR M.VSPSDPES NYGEVQKLLF   68
h6pd_human  RESPLVSAWS EELISKLAND IEATAVRAVR RFGQ.F.... HLALSGGSSP VALFQQLATA HY.G..FPWA HTHLWLVDER C.VPLSDPES NFQGLQAHLL  641
g6pi_human  ~~~~~MKLII LEHYSQASEW AAKYIRNRII QFNPGPEKYF TLGLPTGSTP LGCYKKLIE. YYKNGDLSFK YVKTFNMDEY VGLPRDHPES YHSFMWNNFF   94
g6pi_ecoli  ~~~~~MRLIP LTTAEQVGKW AARHIVNRIN AFKPTADRPF VLGLPTGGTP MTTYKALVE. MHKAGQVSFK HVVTFNMDEY VGLPKEHPES YYSFMHRNFF   94
                          AA      I              LGL TG  P    Y  L           F     T N DEY         P S Y   M   FF

                                                                  G  G  GH  S F P
6pgl_human  SRLPIPE... SQVITINPEL ...PVEEAAE DYAKKLRQAF QG.DS..I.. PVFDLLILGV GPDGHTCSLF PDHPLLQE.. REKI..VAPI SDS.......  178
sol1_yeast  DLIDTEKYGT PKIYHIDESL INDP.QECAD NYEKILIKGF AGRDS..VKL PMFDLFLLGC APDGHIASLF PN...FQENL RENLAWVIPV ENA.......  234
devb_actac  DHIQIPAE.. ....NIH... .....RIRGE NEPHFELKRF QAELSAVISD GVFDWIILGM GADGHTSSLF PHQ.TNFDD. .ENLA.V..I AKH.......  141
h6pd_human  QHVRIPYY.. ....NIHPMP VHLQQRLCAE EDQGAQI..Y AREISALVAN SSFDLVLLGM GADGHTASLF PQSPTGLDG. .EQLV.V..L TTS.......  721
g6pi_human  KHIDIHPE.. ....NTH.IL DGNAVDLQAE ......CDAF EEKIKAA... GGIELFVGGI GPDGHIAFNE PGSSLVSRTR VKTLAMDTIL ANARFFDGEL  178
g6pi_ecoli  DHVDIPAE.. ....NIN.LL NGNAPDIDAE ......CRQY EEKIRSY... GKIHLFMGGV GNDGHIAFNE PASSLASRTR IKTLTHDTRV ANSRFFDNDV  178
                          G       E       C              G       G  G  GHI FNE P       T     L   T   AN R F

          P     R                   G  K
6pgl_human  PKPPPQRVTL TLPVLNAART VIFVATGEGK AAVLKRILED QEEN.PLPAA LV.QPHTGKL CWFLDEAAAR LLTVPFEKHS TL~~~~~~~~ ~~~~~~~~~~  258
sol1_yeast  PSGPSNRISL TIPVICHSHR VTFVVEGATK APVIKTIME. RPEK.GLPSS IVNEGAAGRV SWFVDDDALK DVFVIKKKYK FYDDENLTE~ ~~~~~~~~~~  321
devb_actac  PESGQIRISK TAKLIEQAKR ITYLVTGEGK AEILKEIQST PAENLPYPAA KIY.AKNGVT EWYLDKDAAK LL~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~  212
h6pd_human  PSQPHRRMSL SLPLINRAKK VAVLVMGRMK REITTLVSRV GHEPKKWPIS GVL.PHSGQL VWYMDYDA.. FLG~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~  791
g6pi_human  TKVPTMALTV GVGTVMDARE VMILITGAHK AFALYKAIEE GVNHM.WTVS .AFQQHP.RT VFVCDEDATL ELKVKTVKYF KGLMLVHNKL VDPLYSIKEK  275
g6pi_ecoli  NQVPKYALTV GVGTLLDAEE VMILVLGSQK ALALQAAVEG CVNHM.WTIS .CLQLHP.KA IMVCDEPSTM ELKVKTLRYF NELEAENIKG L~~~~~~~~~  266
              VP  AL   G  T            G  K A A              S      H         D

g6pi_human  ETEKSQSSKK PYSD                                                                                          289
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Fig. 3. Alignment of the sequence of human 6-phosphogluconolactonase with Sol1 from *Saccharomyces cerevisiae*, with devB from *A. actinomycetemcomitans*, with the C-terminal part of human hexose-6-phosphate dehydrogenase and with glucosamine-6-phosphate isomerases from human and from *E. coli*. The residues in bold are those that are strictly conserved either amongst 6-phosphogluconolactonases (the human enzyme and the *devB* genes found in proximity to the genes encoding glucose-6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase in the genomes of *A. actinomycetemcomitans*, *Chlamydia trachomatis*, *Helicobacter pylori*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Neisseria gonorrhoeae* and *Treponema pallidum*) or amongst glucosamine-6-phosphate isomerases (i.e. the human and *E. coli* enzymes as well as the homologs found in *nag* operons in the genomes of *Bacillus subtilis*, *Borrelia burgdorferi*, *Clostridium acetobutylicum* and *Haemophilus influenzae*). The underlined residues were concluded to interact with the substrate of glucosamine-6-phosphate isomerase based on the crystal structure of this enzyme [23].

Since two enzymes catalyzing different reactions appeared to be homologous, it was of interest to identify the residues specifically involved in both functions. To identify these residues in the 6-phosphogluconolactonases, we restricted our analysis to (1) human 6-phosphogluconolactonase and (2) the bacterial devB proteins that are encoded by operons also containing the glucose-6-phosphate dehydrogenase gene. In the absence of experimental proof that the bacterial devB proteins are 6-phosphogluconolactonases (one of them, from *Pseudomonas aeruginosa*, is mentioned as 6-phosphogluconolactonase in the databanks under accession number AF029673 but without any reference to published work), this proximity is good evidence for this catalytic function. Similarly, identification of strictly conserved residues in glucosamine-6-phosphate isomerases was obtained by comparing the sequence of the enzymes from *Homo sapiens* and *E. coli* with bacterial homologs present in *nag* (*N*-acetylglucosamine) operons.

The strictly conserved residues in each type of enzyme (Fig. 3) are more abundant in the isomerase than in the lactonase (56 versus 15), indicating that the former enzyme is more conserved than the latter. This may be because glucosamine-6-phosphate isomerase, which forms hexamers [20] and is allosterically regulated [19], is more constrained than 6-phosphogluconolactonase, a monomeric enzyme without known allosteric regulation [21]. Out of these strictly conserved residues, eight are common between both enzymes. Analysis of the crystal structure of *E. coli* glucosamine-6-phosphate isomerase bound to a substrate analog [23] has led to the conclusion that three of these eight residues (underlined in Fig. 3) interact with glucosamine-6-phosphate: D72 with the hydrogen bound to C-2 and an oxygen bound to C-1, H143 with the oxygen bound to C-5 and K208 with the phosphate group. In addition, residues 41–44, which comprise a strictly conserved glycine residue, form a loop that hydrogen-bonds with the phosphate group [22]. The residues in equivalent positions in 6-phosphogluconolactonase most likely interact in a similar fashion with 6-phosphogluconolactone.

Further comparisons indicate that R81, F159 and R185 (positions indicated for the human 6-phosphogluconolactonase) are a 'signature' for the lactonase. R81 replaces a tyrosine (Y74 in the *E. coli* isomerase) close to the conserved aspartate (D72) mentioned earlier. We speculate that R81 stabilizes the anion that forms during hydrolysis of the lactone.

Since the yeast Sol1–4 proteins share the lactonase-specific residues (except for a glycine which is replaced by an alanine in Sol1 and Sol2) and are actually even closer to human 6-phosphogluconolactonase than the bacterial devB proteins, it is likely that they also catalyze the hydrolysis of 6-phosphogluconolactone. However, it is not obvious how this lactonase activity can account for the function of *Sol1* to act as a multicopy suppressor of the *los1-1* mutation, which prevents yeast from correctly splicing a suppressor tRNA [23]. The sequence comparisons (Fig. 3) also suggest that mammalian hexose-6-phosphate dehydrogenase is a bifunctional enzyme catalyzing the first two steps of the pentose phosphate pathway in the endoplasmic reticulum. By contrast, these two reactions are catalyzed by separate enzymes in the cytosol, the lactonase being the one characterized in the present work.

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