Journal of Cellular Biochemistry 88:932–940 (2003)

Cloning and Functional Characterization of GNPI2, a Novel Human Homolog of Glucosamine-6-Phosphate Isomerase/Oscillin

Jia Zhang, Weiping Zhang, Dajin Zou, Guoyou Chen, Tao Wan, Nan Li, and Xuetao Cao*

Institute of Immunology & Department of Internal Medicine, Second Military Medical University, 800 Xiangyin Road, Shanghai 200433, People's Republic of China

Abstract The enzyme, glucosamine-6-phosphate isomerase (GNPI) or deaminase (GNPDA) (EC 5.3.1.10), catalyzes the conversion of GNP to fructose-6-phosphate and ammonia, with an aldo/keto isomerization and an amination/deamination. A hamster sperm-derived protein (Oscillin) with high similarity to bacterial GNPI has been proved to be capable of inducing calcium oscillation in eggs at fertilization. GNPI/Oscillin was supposed to be an important factor in starting embryonic development. From the cDNA library of human dendritic cells (DC), we isolated a novel full-length cDNA encoding a 276-amino acid-residue protein that shares high homology with human GNPI/Oscillin. So, the novel molecule is named as GNPI2. The GNPI2 gene consists of seven exons and six introns. It is mapped to chromosome 4. Northern blot analysis indicated that the tissue distribution of GNPI2 mRNA is different from that of human GNPI or Oscillin mRNA. GNPI2 is ubiquitously expressed in most of human tissues with high expression in testis, ovary, placenta, and heart. Like GNPI, the recombinant GNPI2 has been proved to have the enzymatic activity to catalyze the conversion of GNP to fructose-6-phosphate. Our results indicated that GNPI2 is a novel protein with definite function as a GNPI. J. Cell. Biochem. 88: 932–940, 2003.

Key words: glucosamine-6-phosphate isomerase; functional genomics; novel molecule; gene cloning; dendritic cell

The enzyme, glucosamine-6-phosphate isomerase (2-amino-2-deoxy-D-glucose-6-phosphate ketol isomerase, GNPI) or glucosamine-6-phosphate deaminase (GNPDA) (EC 5.3.1.10), catalyzes the conversion of GNP to fructose-6-phosphate and ammonia in several microorganisms [Leloir and Cardini, 1956; Benson and Friedman, 1970] and mammals [Comb and Roseman, 1958]. It is the last step of the short metabolic pathway of *N*-acetyl-D-glucosamine-6-phosphate (GlcNAc6p). The reaction catalyzed by GNPI is an aldo–keto isomerization (Amadori rearrangement) coupled with an amination/deamination [Comb and Roseman, 1958]. The enzyme is involved in both directions of catabolic and anabolic pathways of hexosamine in bacteria, and is supposed to be an alternative route for the biosynthesis of amino sugars in some species [Comb and Roseman, 1958; Lara-Lemus et al., 1992]. But the latter way is believed impossible in the physiological conditions because of the extremely high concentration of ammonia required [Calcogno et al., 1984]. By partially purifying the enzyme from *Escherichea coli*, Midelfort and Rose [1977] studied the chemical mechanism of the reaction it catalyzed. Calcogno et al. [1984] described the kinetic properties of the enzyme. The *E. coli GNPDA* gene, *nagb*, was cloned [Rogers et al., 1988], and was proved to be a part of *nagenagbacd* operons [Plumbridge, 1989]. The transcriptional control of gene expression [Peri et al., 1990; Plumbridge, 1990, 1996; Plumbridge and Kolb, 1998] and the allosteric functional structure of the protein [Altamirano et al., 1992;

Grant sponsor: National Nature Science Foundation of China; Grant number: 30121002; Grant sponsor: National Key Basic Research Program of China; Grant number: 2001CB510002; Grant sponsor: National High Biotechnology Development Program of China; Grant number: 2002BA711A01.

*Correspondence to: Xuetao Cao, PhD, MD, Institute of Immunology, Second Military Medical University, 800 Xiangyin Road, Shanghai 200433, People's Republic of China. E-mail: caoxt@public3.sta.net.cn

Received 24 September 2002; Accepted 1 November 2002

DOI 10.1002/jcb.10444

Montero-Moran et al., 1998; Horjales et al., 1999; Lara-Gonzalez et al., 2000] have been studied intensively. In addition to *E. coli*, *GNPI* gene has been molecularly cloned from *Candida albican* [Natarajan and Datta, 1993], *Giardia lamblia* [Van Keulen et al., 1998], mouse [Amireault and Dube, 2000], and human [Nomura et al., 1994; Shevchenko et al., 1998].

Oocytes of mammals show rapid oscillations in the cytoplasmic calcium concentration, which is thought as a trigger for oocyte activation and embryonic development [Kline and Kline, 1992]. A protein (Oscillin) with high similarity to bacterial GNPI has been cloned and purified from hamster sperm, which appeared to be capable of inducing calcium oscillation in eggs at fertilization [Parrington et al., 1996]. Oscillin was supposed to be an important factor in starting embryonic development. However, a homolog from hamster [Wolosker et al., 1998] and human [Wolny et al., 1999] sperm failed to release calcium when injected into the mammalian eggs.

By large-scaled random sequencing of cDNA library of human dendritic cells (DC), we have identified several novel human genes with biological functions [Cao et al., 2000; Li et al., 2001; Zhang et al., 2001]. In this study, we report the identification, chromosomal localization, and functional analysis of a novel full-length cDNA. The novel gene encodes a protein with high homology to human GNPI/Oscillin. The recombinant novel protein has been demonstrated to have the enzymatic activity of GNPI. So the novel molecule is named as GNPI2.

MATERIALS AND METHODS

Isolation of GNPI2 cDNA

The full-length cDNA of GNPI2 was isolated from cDNA library of human DC by large-scale randomly sequencing. Briefly, peripheral monocytes were treated with granulocyte/macrophage colony stimulating factor (GM-CSF) and interleukin (IL-4) (Sigma, St. Louis, MO) for 7 days. The nonadherent cells were harvested as DC, which were enriched by CD1a-medicated positive selection. Plasmid cDNA library of pSPORT vector was constructed using the Superscript Plasmid System for cDNA synthesis and plasmid cloning (Life Technologies, Grand Island, NY) according to the manufacturer's instructions. From large-scale DNA sequencing of the library [Zhang et al., 2001], expressed sequence tags (EST) were created and compared with EMBL using BLAST in the Genetics Computer Group program package (Madision, WI). Based on this database of EST, we found a full-length cDNA clone SBBI52, which encodes a protein bearing high homology with human GNPI. The novel gene was named as GNPI2 and has been deposited in GenBank under the accession no. AF 247786.

Northern Blot Analysis of GNPI2 mRNA Expression

We used the cDNA containing the fragment of 400 bp GNPI2 cDNA restricted by *H*3/*Bgl*2 and the cDNA containing the fragment of 448 bp human GNPI cDNA restricted by *Eco*RI/*Bam*HI as templates for synthesis of probes. Filters containing human poly(A)$^+$ RNA from various tissues and cells (Clontech Laboratories, Inc., Palo Alto, CA) were hybridized with ^{32}P labeled cDNA probes in ExpressHyb hybridization solution (Clontech Laboratories) according to the manufacturer's instructions. Then, the filters were washed at 50°C for 20 min in 0.1 × SSC and 0.1% SDS, and followed by autoradiography. The filters were reprobed with a human β-actin cDNA probe.

Construction of GNPI2 Expression Vector

We amplified the GNPI2 cDNA containing the full-length encoding region except stop codon by PCR using the primers of 5′-GGAATTCCGCCATCATGAGGCTTGTAATTCTTGATAAC-3′ and 5′-GGGGTACCGTTTCCATCTTTCATACTGAATAG-3′. An *Eco*RI and a *Kpn*I restriction site were introduced into the cDNA. We inserted GNPI2 cDNA into pcDNA3.1 (Invitrogen, San Diego, CA) expression vector, under the control of CMV promoter/enhancer, and inframe fused at its 3′ end with the cDNA encoding a myc epitope and a polyhistidine tag. GNPI2 expression vector and its mock control were largely prepared with the Endofree Plasmid Maxi kit (Qiagen, Chatsworth, CA).

Eukaryotic Expression and Purification of Recombinant GNPI2

COS7 cells were cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Inc.) supplemented with penicillin, streptomycin, and 10% fetal bovine serum

(FBS) (Life Technologies, Inc.). When cells was 80% confluent in 100-mm dishes, they were transfected with GNPI2 expression vector or the mock control with LipofectAMINE reagent (Life Technologies, Inc.) according to the manufacturer's instructions. At 72 h after transfection, COS7 cells were washed with phosphate-buffered saline (PBS) three times and lysed for 30 min on ice with 1 ml cold lysis buffer (20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, and 1 mM Na_3VO_4, 1 μg/ml) plus 1 mM PMSF. Then the lysate was mixed vigorously by vortex for 5 min, and centrifuged at 4°C for 20 min (13,000 round per min). We subjected the cytosolic fractions to affinity chromatography using HiTrap Chelating 1 ml columns (Pharmacia Biotech, Piscataway, NJ), which had been chelated with copper ions and was pre-equilibrated with start buffer (50 mM PB, pH 7.0, 0.1 M NaCl). After washing with five column volumes of start buffer, the column was eluted with three column volumes of linear gradient elution buffer (0–50 mM PB, pH 7.0, 0.1 M NaCl, 0.1 M imidazole). The fraction containing GNPI2 protein of the elution buffer was collected for the detection of the enzymatic activity. Fifteen percent sodium dodecyl sulfate–polyacrylamide gels (SDS–PAGE) were presented to identify the purity of the protein. The protein concentration of the elution was determined by BCA-200 protein assay (Pierce, Illinois, USA).

Western Blot Analysis of GNPI2 Protein

The crude extract of COS7 cells transfected with GNPI2 expression vector or its mock control, purified recombinant GNPI2 protein, and its flow through (stored at 4°C) were separated on 15% SDS–PAGE, electrophoretically transferred into nitrocellulose membranes (Amersham, Arlington Heights, IL) at 100 V for 1 h. The membranes were blocked in 5% nonfat dry milk in TBST buffer (25 mM Tris-HCl, pH 8.0, 125 mM NaCl, and 0.1% Tween 20) for 3 h at room temperature, incubated with HRP-labeled anti-myc antibody (Invitrogen) diluted in blocking buffer (1:5,000 dilution) for 1 h at room temperature, and washed with TBST for 10 min × three times and TBS for 10 min once. HRP was detected using enhanced chemiluminescence according to the manufacturer's instructions (Santa Cruz Biotechnology, Santa Cruz, CA).

Enzymatic Assay of GNPI2

We evaluated the enzymatic activity of the recombinant GNPI2 spectrophotometrically using the resorcinol method to detect fructose-6-phosphate as described before [Davise and Gander, 1967]. All kinetic studies were kept at 30°C. Total volume of the reaction was 200 μl containing 75 mM Tris-HCl (pH 7.7), 0.85 mM GlcNAc6P, and 20 mM GNP. The reaction mixture without GNP served as a control. The reaction was initiated by adding 20 μl of purified recombinant GNPI2 protein (0.54 mg/ml), and incubated for various times indicated. At the end of reaction, 1.8 ml 11.6 N HCl and 0.05% resorcinol in absolute ethanol were added. After 8 min incubation at 77°C, the reaction tubes were put on ice immediately and the absorbance at 420 nm were measured using a spectrophotometer (UV-2401 PC) (Pharmacia Biotech, Inc.) after the reaction mixture reached to room temperature. There is a linear relationship between the absorbance at 420 nm and fructose concentration. So we used the absorbance at 420 nm per mg protein to present the enzymatic activity.

RESULTS

Cloning and Sequence Analysis of GNPI2 cDNA

By randomly large-scaled sequencing, we identified a 1,939 bp full-length cDNA clone of a novel protein designated as GNPI2 from human dendritic cell cDNA library. Sequence analysis shows that it contains an open reading frame (ORF) of 831 bp, which encodes a 276 amino acid protein with a calculated molecular weight of 31 kDa (Fig. 1). There are a 5′-untanslated region of 157 bp without in-frame stop codon and a 3′-untranslated region of 951 bp that ended with two consensus polyadenylation signal sequence (AATAA) followed by a run of adenines. Its amino acid sequence (Fig. 2) shares high homology with human GNPI (87% identity and 94% positive similarity) and hamster Oscillin (87% identity and 94% positive similarity). The major difference between this novel protein and human GNPI or hamster Oscillin is that the novel protein is 13 amino acids shorter than the human and the hamster protein at the 3′-end. So, it was designated as GNPI2. No signal peptide or transmembrane domain has been found, with

Fig. 1. Nucleotide and deduced amino acid sequence of human glucosamine-6-phosphate isomerase2 (GNPI2). The conserved GNPI signature is underlined. The cDNA sequence of human GNPI2 has been deposited in GenBank under the accession no. AF 247786.

which GNPI2 is predicted to be an intracellular protein. There is also no glycosylation site found by plot–structure analysis. Motif analysis indicates that *GNPI2* has the conserved *GNPI* signature (L, I, V, M)X3GX(L,I,T)X(L,I,V,M) XG(L,I,V,M)GX (D,E,I) X{3}GX(I)X(L)X(V)X-G(I)GX(D) H as under line (Fig. 1). The exon–intron organization is obtained by aligning the genomic sequence with *GNPI2* cDNA sequence. The new gene consists of seven exons and six introns. Using bioinformatics acquisition from Human Genome Blast, the novel gene is

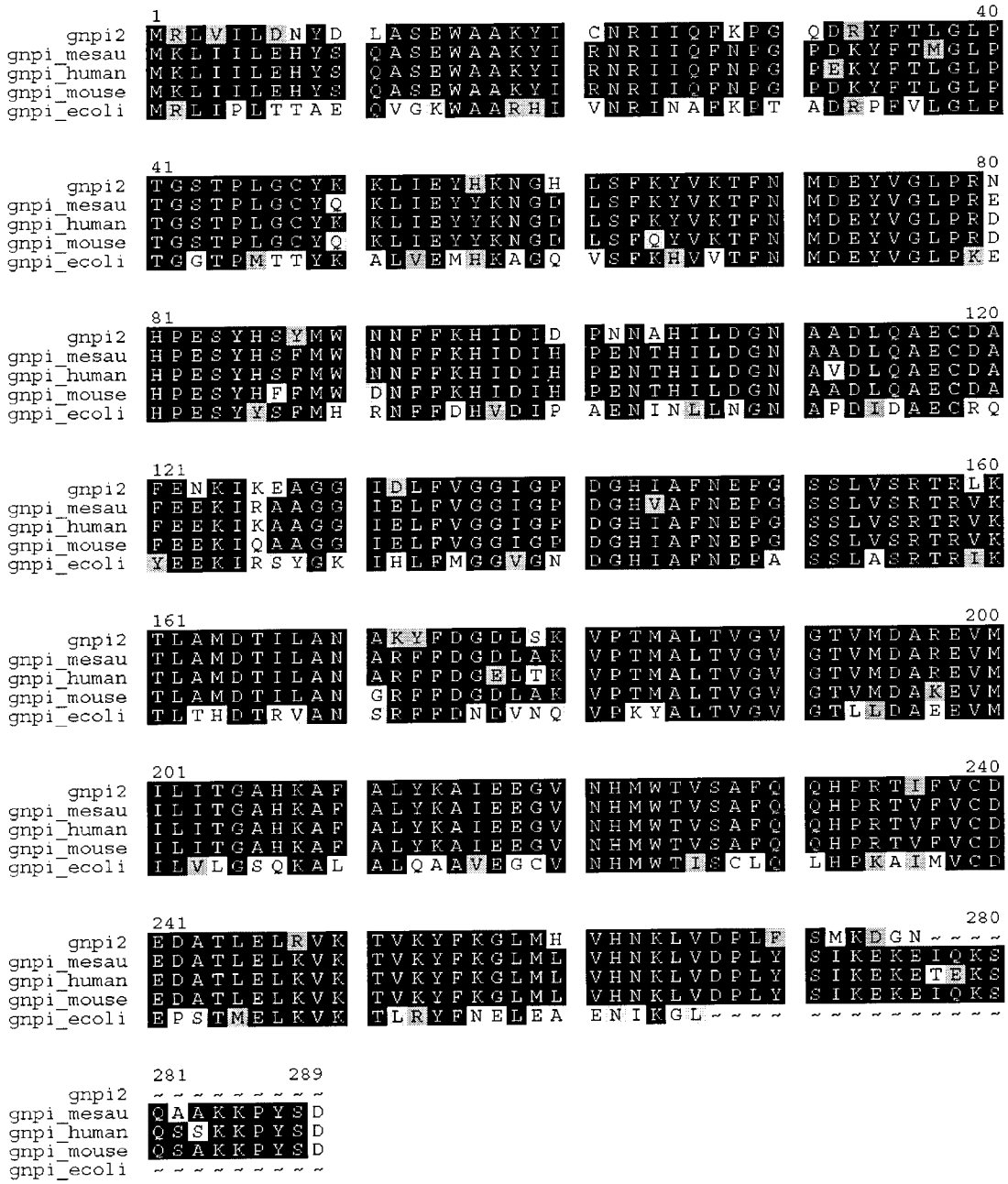

Fig. 2. Alignment of deduced amino acid sequence of GNPI2 with GNPI of mesau, human, mouse, and *Escherichea coli*. The homologous region was shown on a black background.

mapped to chromosome 4 (contig NT 022978.2). The novel gene has been deposited in GenBank under the accession no. AF 247786.

Tissue Expression Pattern of GNPI2 and GNPI mRNA

Northern blot analysis revealed a constitutive expression of ~2.4 kb of *GNPI2* transcripts in most normal tissues with high expression in testis, ovary, placenta, and heart. Moderate level of expression was detected in spleen, prostate, small intestine, colon, kidney, and pancreas. The extremely low densities occur in the thymus, PBL, brain, lung, liver, and skeletal muscles. We also observed *GNPI2* expression in most cancer cell lines examined (Fig. 3A) with high level in Molt4 (lymphoblastic leukemia Molt4) and Raji (Burkitt's lymphoma Raji) cells. Only in testis and placenta were two different

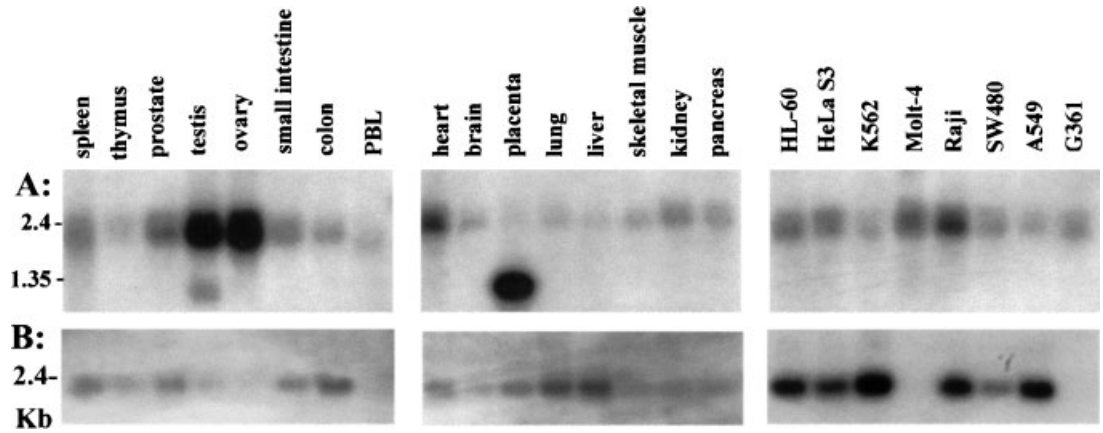

Fig. 3. Distribution of GNPI2 (**A**) and GNPI (**B**) mRNA in human tissues and cell lines. Poly $(A)^+$ RNA from the indicated tissues and cell lines were hybridized with ^{32}P-labeled GNPI2 cDNA probe (A) or GNPI (AJ00231) cDNA probe (B). The RNA markers are indicated in the left.

transcripts of *GNPI2* observed with the sizes of ~2.4 and 1.2 kb. In testis, the dominant transcript was the larger one, but in placenta, it seemed to be the smaller one. The expression pattern of *GNPI2* is different from that of human *GNPI* (Fig. 3B), whose high expression was seen in kidney and skeletal muscle except testis, ovary, and heart. In the cancer cell lines examined, Raji (Burkitt's lymphoma Raji) and promyelocytic leukemia HL-60 cells did not express *GNPI* mRNA.

Eukaryotic Expression of GNPI2/Myc–His Fusion Protein

In order to obtain the recombinant GNPI2 protein to analyze its function, we constructed a myc epitope-polyhistidine tag-linked GNPI2 expression vector. *GNPI2* was transiently expressed in COS7 cells and purified with cupric-imino-diacetic acid mediated high performance affinity chromatography. A ~33 kDa Myc–Histidine fusion protein has been detected by Western blot in crude extract of the transfected COS7 cells and the eluted fraction of the affinity chromatography using a HRP-labeled anti-myc antibody, but not in mock transfected COS7 cells or the flow through of the affinity chromatography (Fig. 4). The molecular weight of the protein was in agreement with that calculated from the deduced amino acid sequence of *GNPI2* cDNA. The recombinant GNPI2 protein was purified and its purity was about 95%, as analyzed by SDS–PAGE (data not shown). The concentration of the purified protein was 0.54 mg/ml.

Enzymatic Activity of Recombinant GNPI2

By a colorimetric reaction, we monitored the enzymatic activity of the recombinant GNPI2. We used GNP as a substrate and measured the evolution of fructose-6-phosphate. *N*-acetyl-glucosamine-6-phosphate was used as activator in the enzymatic reaction. The chemical assay used for the determination of fructose was based on the estimation of a chromophoric substance formed by the reaction of resorcinol and hydroxymethylfurfural. There was a linear relationship between the absorbance of the chromophore at 420 nm and the fructose concentration produced in the enzymatic reaction. So the absorbance per milligram protein was

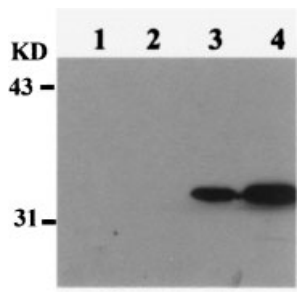

Fig. 4. Western blotting analysis of recombinant GNPI2. The immunoblot was probed with HRP-labeled anti-myc antibody. A 33 kDa recombinant protein which was in agreement with the deduced molecular weight of GNPI2 has been detected in the crude extract of COS7 cells transfected with pcDNA3.1/GNPI2/Myc–Histidin-linked eukaryotic expression vector (**lane 3**) and in the eluted fraction of HiTrap Chelating columns (**lane 4**), but not in the crude extract of COS7 cells transfected with mock control vector (**lane 1**) or the flow through of the affinity chromatography (**lane 2**).

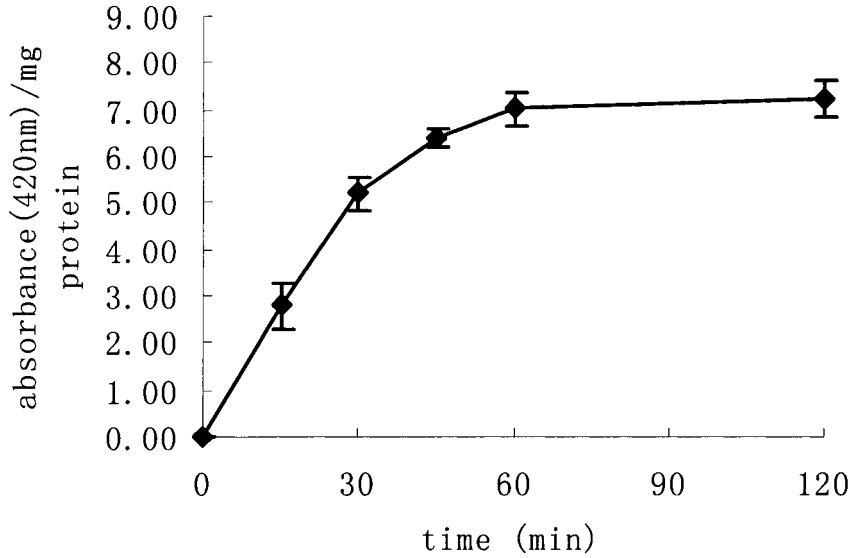

Fig. 5. Enzymatic assay of recombinant GNPI2. The enzymatic activity was assayed at 30°C at various time intervals in a medium containing 75 mM Tris-HCl (pH 7.7), 0.85 mM *N*-acetyl-D-glucosamin-6-phosphate (GlcNAc6P), 20 mM glucosamine-6-phosphate (GNP), and 20 µl of recombinant GNPI2 (0.54 mg/ml). Then the medium was added with 11.6 N HCl and resorcinol. The absorbace per milligram protein of the products in 420 nm was proportional to the amount of fructose generated during reaction and was used to indicate the enzymatic activity of GNPI2. The enzymatic activity was measured in triplicate.

used to express the enzymatic activity of the recombinant GNPI2 (Fig. 5). Product formation was increased linearly for about 30 min, and then slowed down. Like GNPI, the recombinant GNPI2 could catalyze the conversion of GNP to fructose-6-phosphate.

DISCUSSION

The physiological functions and tissue expression pattern of GNPI were rarely studied before Oscillin was reported. In an investigation of hexosamine catabolism in the human malaria parasite, Weidanz et al. [1995] first demonstrated the presence of GNPDA in normal human erythrocytes. Their results suggested that hexosamine catabolism is not a major source of energy in the erythrocyte because of the low level of enzymatic activity. They believed that the presence of the enzyme is just for catalyzing the substrate generated in small quantities in the erythrocytes. GNPI was cloned and purified only after the Oscillin was reported, but most studies were limited in testis.

At fertilization, the sperm initiates a sequence of events that leads to oocyte activation and embryonic development. Oocytes of mammals show rapid oscillations in the cytoplasmic calcium concentration, which was thought as a trigger for oocyte activation and embryonic development [Swann and Ozil, 1994]. There are two hypotheses to explain the changes in oocyte $[Ca^{2+}]$ at fertilization. One hypothesis suggests that there should be a sperm receptor on the oocyte membrane and the activation of such a receptor generate a cascade of reaction to start the Ca^{2+} oscillations [Schultz and Kopf, 1995]. The second hypothesis supposes that the sperm may release a soluble factor into the oocyte cytoplasm at fertilization to induce Ca^{2+} oscillations [Swann, 1994; Dale et al., 1996]. Oscillin was thought to be a candidate of such a factor [Parrington et al., 1996]. Oscillin shows 53% amino acid sequence identity with the bacterial GNPI, and it was sperm specific localizing to the equatorial region. When Oscillin was microinjected into mouse eggs, Ca^{2+} oscillation was induced identical to those occurring at fertilization. Although Parrington et al. [1996] believed that it was the 33 kDa soluble sperm-derived protein, Oscillin, induced the Ca^{2+} oscillation in mouse eggs, the exact nature and role of this protein is still under discussion. It was reported that a recombinant rat homolog of hamster Oscillin, which shared 98.5% homology with human GNPI, presented a strong enzymatic activity of GNPI but could not produce Ca^{2+} oscillations when injected into the mouse oocytes [Wolosker et al., 1998]. Moreover, by Western and Northern blot analyses they reported that Oscillin was ubiquitously expressed in rat tissues, especially those with high-energy

requirements such as kidney, small intestine, brain, and motile sperm. So the author proposed that Oscillin might provide a source of energy from the catabolism of hexosamines in glycoproteins, glycolipids, and sialic acid-containing macromolecules. The human homolog of Oscillin was identified [Shevchenko et al., 1998] and was also shown to have the GNPI activity but no Ca^{2+} oscillogen activity [Wolny et al., 1999]. Nakamura et al. [2000] also found that the human and mouse orthologous genes of GNPI/Oscillin were ubiquitously expressed in all tissues examined. Their results suggested that Oscillin is a housekeeping gene conserved throughout evolution and could not be the sperm-specific factor responsible for the calcium oscillations

In this study, we identified a novel human homolog of GNPI/Oscillin, GNPI2, from human dendritic cell cDNA library. GNPI2 shows 87% identity and 94% positive similarity with human GNPI and hamster Oscillin. By Northern blot analysis, we found that GNPI2 is expressed ubiquitously in all human tissues and cancer cell lines with high expression in testis, ovary, placenta, and heart. But its expression pattern is different from that of GNPI or Oscillin. The distribution of *GNPI2* was not restricted to tissues for energy supplement. Only a moderate level of *GNPI2* gene expression was found in kidney and small intestine. In addition, its high expression in ovary not fertilized suggests that GNPI2 might not play a key role in the activation of oocytes. The discovery of a novel member of GNPI family is helpful for us to understand the metabolic pathway of GlcNAc6p. And the wide expression of GNPI2 suggests its important role in cellular metabolism.

Our results indicated that the recombinant GNPI2 protein has the typical catalytic activity of the human [Wolny et al., 1999], hamster [Wolosker et al., 1998], and bacterial [Calcogno et al., 1984] GNPI to catalyze the conversion of GNP to fructose-6-phosphate. We proposed that GNPI2 might be an isoenzyme of GNPI that has different molecular structure from GNPI, catalyzes similar enzymatic reaction in different cell types from GNPI, and plays a different role during the cell lives. Further experiments will be performed to compare the enzymatic activity between GNPI and GNPI2.

In conclusion, we have identified a novel protein, GNPI2, from human DC. It shares high homology with human GNPI/Oscillin, and contains a conserved signature of GNPI. The novel gene consists of seven exons and six introns and is mapped to chromosome 4. By functional analysis, we proved that the recombinant GNPI2 has the enzymatic activity of catalyzing the conversion of GNP to fructose-6-phosphate.

ACKNOWLEDGMENTS

We thank Mrs. Mei Jin, Mr. Daoming Zhang, and Mrs. Xuebin Wu for their technical assistances.

REFERENCES

Altamirano MM, Plumbridge JA, Calcagno ML. 1992. Identification of two cysteine residues forming a pair of vicinal thiols in glucosamine-6-phosphate deaminase from *E. coli* and a study of their functional role by site-directed mutagenesis. Biochemistry 31:1153–1158.

Amireault P, Dube F. 2000. Cloning, sequencing, and expression analysis of mouse glucosamine-6-phosphate deaminase (GNPDA/Oscillin). Mol Reprod Dev 56:424–435.

Benson RL, Friedman S. 1970. Allosteric control of glucosamine phosphate isomerase from the adult housefly and its role in the synthesis of glucosamine-6-phosphate. J Biol Chem 245:2219–2228.

Calcogno M, Campos PJ, Mulliert G, Suastegui J. 1984. Purification, molecular, and kinetic properties of glucosamine phosphate isomerase (deaminase) from *E. coli*. Biochim Biophys Acta 787:165–173.

Cao X, Zhang W, Wan T, He L, Chen T, Yuan Z, Ma S, Yu Y, Chen G. 2000. Molecular cloning and characterization of a novel CXC chemokine macrophage inflammatory protein-2γ chemoattractant for human neutrophils and dendritic cells. J Immunol 165:2588–2595.

Comb DG, Roseman S. 1958. Glucosamine metabolism IV. Glucosamine-6-phosphate. J Biol Chem 232:807–827.

Dale B, Fortunato A, Monfrecola V, Tosti E. 1996. A soluble sperm factor gates Ca^{2+}-activated K^{+} channels in human oocytes. J Assist Reprod Genet 13:573–577.

Davise JS, Gander JE. 1967. A revaluation of the role procedure for the determination of fructose. Anal Biochem 19:72–79.

Horjales E, Altamirano MM, Calcagno ML, Garratt RC, Oliva G. 1999. The allosteric transition of glucosamine-6-phosphate deaminase: The structure of the T state at 2.3 A resolution. Structure Fold Des 7:527–537.

Kline D, Kline JT. 1992. Repetitive calcium transients and the role of calcium in exocytosis and cell cyle activation in the mouse egg. Dev Biol 149:80–89.

Lara-Gonzalez S, Dixon HB, Mendoza-Hernandez G, Altamirano MM, Calcagno ML. 2000. On the role of the N-terminal group in the allosteric function of glucosamine-6-phosphate deaminase from *Escherichea coli*. J Mol Biol 301:219–227.

Lara-Lemus R, Libreros-Minotta CA, Altamirano MM, Calcagno ML. 1992. Purification and characterization of glucosamine-6-phosphate deaminase from dog kidney cortex. Arch Biochem Biophys 297:213–220.

Leloir LF, Cardini CE. 1956. Enzymes acting on glucosamine phosphate. Biochim Biophys Acta 20:33–42.

Li N, Zhang W, Wan T, Zhang J, Chen T, Yu Y, Wang J, Cao X. 2001. Cloning and characterization of siglec-10, a novel sialic acid binding member of the Ig superfamily, from human dendritic cells. J Biol Chem 276(30):28106–28112.

Midelfort CF, Rose IA. 1977. Studies on the mechanism of *Escherichia coli* glucoamine-6-phosphate isomerase. Biochemistry 16:1590–1596.

Montero-Moran GM, Horjales E, Calcagno ML, Altamirano MM. 1998. Tyr254 hydroxyl group acts as a two-way switch mechanism in the coupling of heterotropic and homotropic effects in *E. coli* glucosamine-6-phosphate deaminase. Biochemistry 37:7844–7849.

Nakamura Y, Miura K, Fujino Y, Iwao H, Ogita S, Yamanaka S. 2000. Evolution, structure, and expression of GNPI/Oscillin orthologous gene. Genomics 68:179–186.

Natarajan K, Datta A. 1993. Molecular cloning and analysis of the NAG1 cDNA coding for glucosamine-6-phosphate deaminase form *Candida albicans*. J Biol Chem 268:9206–9214.

Nomura N, Nagase T, Miyjiama N, Sazuka T, Tanaka A, Sato S, Seki N, Kawarabayasi Y, Ishikawa K, Tabata S. 1994. Prediction of the coding sequences of unidentified human genes.II. The coding sequenced of 40 new genes (KIAA0041–KIAA0080) deduced by analysis of cDNA clones from human cell line KG-1. DNA Res 1:223–229.

Parrington J, Swann K, Shevchenko VI, Sesay AK, Lai FA. 1996. Calcium oscillations in mammalian eggs triggered by a soluble sperm protein. Nature 379:364–368.

Peri KG, Goldie H, Waygood EB. 1990. Cloning and characterization of the *N*-acetylglucosamine operon of *E. coli*. Biochem Cell Biol 68:123–137.

Plumbridge JA. 1989. Sequence of the *neg*bacd operon in *E. coli*. K12 and pattern of transcription within the *nag* regulon. Mol Microbiol 3:505–515.

Plumbridge JA. 1990. Induction of the *nag* regulon of *E. coli* by *N*-acetylglucosamine and glucosamine: Role of the cyclic ampcatabolitic activator protein complex in expression of the regulon. J Bacterial 172:2728–2735.

Plumbridge JA. 1996. How to archive constitutive expression of a gene within an inducible operon: The example of the *nag*c gene of *E. coli*. J Bacterial 178:2629–2636.

Plumbridge JA, Kolb A. 1998. DNA bending and expression of the divergent nagE-B operons. Nucleic Acids Res 26: 1254–1260.

Rogers MJ, Ohgi T, Plumbridge J, Soll D. 1988. Nucleotide sequences of the *E. coli* nagE and nagB genes: The structural genes for the *N*-acetylglucosamine transport protein of the bacterial phosphoenolpyruvate: Sugar phosphotransferase system and for glucosamine 6-phosphate deaminase. Gene 62:197–207.

Schultz RM, Kopf GS. 1995. Molecular basis of mammalian egg activation. Curr Top Dev Biol 30:21–62.

Shevchenko V, Hogben M, Ekong R, Parrington J, Lai FA. 1998. The human glucosamine-6-phosphate deaminase gene: cDNA cloning and expression, genomic organization, and chromosomal localization. Gene 216:31–38.

Swann K. 1994. Ca^{2+} oscillstions and sensitization of Ca^{2+} relase in unfertilized mouse eggs injected with a sperm factor. Cell Calcium 15:331–339.

Swann K, Ozil JP. 1994. Dynamics of the calcium signal that triggers mammalian egg activation. Int Rev Cytol 152:183–222.

Van Keulen H, Steimle PA, Bulik RK, Jarroll EL. 1998. Cloning of two putative *Giardia lamblia* glucosamine-6-phosphate isomerase genes only one of which is transcriptionally activated during encystment. J Eukaryot Microbiol 45:637–642.

Weidanz JA, Campbell P, Delucas LJ, Jin J, Moore D, Roden L, Yu H, Heilmann E, Vezza AC. 1995. Glucosamine-6-phosphate deaminase in normal human erythrocytes. Br J Haematol 91:72–79.

Wolny YM, Fissore RA, Wu H, Reis MM, Colombero LT, Ergün B, Rosenwaks Z, Palermo GD. 1999. Human glucosamine-6-phosphate isomerase, a homologue of hamster oscilline, does not appear to be involved in Ca^{2+} release in mammalian oocytes. Mol Reprod Dev 52:277–287.

Wolosker H, Kline D, Bian Y, Blackshaw S, Cameron AM, Fralich TJ, Schnaar RL, Snyder SH. 1998. Molecularly cloned mammalian glucosamine-6-phosphate deaminase localizes to transporting epithelium and lacks oscillin activity. FASEB J 12:91–99.

Zhang W, Wan T, Li N, Yuan Z, He L, Zhu X, Yu M, Cao X. 2001. Genetic approach to insight into the immunobiology of human dendritic cells and identification of CD84-H1, a novel CD84 homologue. Clin Cancer Res 7(3 Suppl):822s–829s.