Cloning and expression of human mitochondrial deoxyguanosine kinase cDNA

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Abstract Mammalian mitochondrial deoxyguanosine kinase (dGK) is responsible for phosphorylation of purine deoxyribonucleosides in the mitochondrial matrix. Using a RT-PCR-generated probe, based on amino acid sequence information from proteolytic fragments of purified bovine dGK, we have cloned a cDNA from a human brain cDNA library that encodes a 30 kDa protein. The deduced amino acid sequence of this protein included the sequence of all six peptides isolated and sequenced from purified dGK. Expression and purification of recombinant protein from induced *Escherichia coli* extracts revealed that it catalyses efficient phosphorylation of dGuo, arabinosyl guanine, dAdo, 2-chloro-2′-deoxyadenosine and dIno similar to purified dGK. Northern blot analysis demonstrated one dominant positive mRNA of 1.35 kb and it was found in several tissues at similar levels. The coding sequence of dGK showed 46% identity to the coding sequence of cytosolic deoxycytidine kinase, and conserved sequence motifs among the known deoxynucleoside kinase were identified.

Key words: Mitochondria; DNA precursor synthesis; Deoxynucleoside kinase; Sequence; Conserved motif; Chemotherapy

1. Introduction

Mammalian deoxyguanosine kinase (dGK, NTP:deoxyguanosine 5′-phosphotransferase, EC 2.7.1. 113) is a nuclear coded enzyme localised to the mitochondria (mt), catalysing the phosphorylation of purine deoxynucleosides and their analogs, using a nucleoside triphosphate as phosphate donor. dGK activity is found in most tissues and the enzyme has been purified from skin, thymus, placenta, liver, brain and leukemic cells [1–8]. The active form of dGK is a dimer of 28–29 kDa subunits and dGuo, dAdo, dIno and several cytostatic analogs, e.g. arabinosyl guanine (AraG) and 2-chloro-2′-deoxyadenosine (CdA) can serve as substrates [1,8]. The cytosolic enzyme deoxycytidine kinase (dCK) has an overlapping substrate specificity with that of dGK but can in addition phosphorylate several pyrimidine nucleosides. There have been considerable difficulties in distinguishing the dGK and dCK activities in extracts from different tissues [1,9]. In proliferating cells and lymphoid tissues dCK is found at much higher levels than dGK, but in many resting cells such as nerve cells and muscle cells dGK is the only purine deoxynucleoside phosphorylating enzyme present [1]. It is still unclear if the activity of dGK contributes to the pharmacological effects of cytostatic and antiviral purine analogs.

One form of severe combined immunodeficiency in man is associated with purine nucleoside phosphorylase (PNP) deficiency [10]. Increased salvage of purine deoxynucleosides, leading to accumulation of toxic levels of dGTP in lymphocytes, is the assumed mechanism of the disease [10]. Snyder et al. [11] have developed a mutant mouse strain lacking PNP activity and they observed that in all tissues from the mutant mice there was also a reduction of the dGK activity. This latter deficiency most likely protected the PNP mutants from the toxicity of dGuo, demonstrating a central role for dGK in the pathogenesis of PNP deficiency.

It is important to evaluate nucleoside analogs used in chemotherapy for their effects on mtDNA synthesis, since it is known that several nucleoside analogs are selective inhibitors of mtDNA polymerase. Treatment with the anti-HIV drugs, 3′-azido-2′-deoxythymidine, 2′-3′-dideoxycytidine and 2′-3′-dideoxyinosine, leads to decreased mtDNA synthesis which ultimately gives myophaties and neurophaties as serious side effects [12]. The accumulation of defect mtDNA has been observed in many tissues in degenerative disorders, as well as in normal aging, and discussed as an important contributing factor in these processes [13]. A decreased supply of mtDNA precursors may be one of the causes of mtDNA deletions.

A complete purification protocol for dGK has been developed starting from bovine brain [8] and we have used information from peptide sequences determined from this material to clone the cDNA for human dGK. The resulting cDNA encodes a 30 kDa protein with extensive homology to the coding sequence of human dCK [9]. The cloning of dGK may enable the evaluation of its role in mitochondrial DNA precursor synthesis, in inherited immunodeficiency disease and in chemotherapy with purine nucleoside analogs.

2. Materials and methods

2.1. Materials

Radiolabelled nucleotides, [α-^{35}S]dATP (1000 Ci/mmol), [γ-^{32}P]ATP (3000 Ci/mmol) and [α-^{32}P]dCTP (3000 Ci/mmol) were obtained from Amersham Corp. 9-β-D-[8-^{3}H]arabinofuranosylguanine (9 Ci/mmol) was from Moravek Biochemicals Inc. CA. IPTG (isopropyl-β-thiogalactopyranoside) and restriction enzymes were from Promega. All other materials and reagents were of the highest quality available.

2.2. Purification of bovine dGK

About 1 kg of calf brain was homogenised and proteins extracted as described [8]. The crude extract was treated with streptomycin sulphate and then subjected to DEAE chromatography, whereby the dGK-containing fractions (assayed with [^{3}H]AraG as substrate) were identified. These fractions were diluted and applied to a hydroxylapatite column, proteins were eluted with a KCl gradient as described [8].

In the last purification step we exchanged the 3′-dGMP-Sepharose

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by an ATP-agarose chromatography as follows: 150 ml of the dGK pool from hydroxyl apatite chromatography was applied to a 5 ml ATP-agarose column (C-8 coupled with a 9 carbon linker from Sigma) which was equilibrated with buffer B (10 mM potassium phosphate, pH 7.6, containing 5 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol and 15% glycerol). After washing the column with 100 ml of buffer B, dGK was eluted with 5 mM ATP in buffer B. Fractions were collected and assayed for dGK activity and fractions that showed dGK activity were precipitated and analysed by SDS-PAGE as described [8].

2.3. Sequence determination of proteolytic fragments

The 28 kDa protein band was excised and subjected to proteolytic cleavage, essentially as described [14]. In brief, the gel piece was washed with a mixture of digestion buffer and acetonitrile and then completely dried under a stream of nitrogen. During rehydration with buffer, modified trypsin (Promega) or endoproteinase LysC (WAKO) was added. Rehydration was continued until the gel piece had swollen to original shape. After overnight incubation at 30°C, the supernatant was saved and combined with extracts from the gel piece. Generated peptides were then isolated by reverse-phase liquid chromatography, using a SMART System (Pharmacia Biotech AB, Uppsala, Sweden). Selected peptides were sequence determined on an Applied Biosystems Model 470A instrument, following the manufacturer's instruction.

2.4. PCR amplification of a dGK cDNA fragment

All dGK peptide sequences were used to search the GenBank data base with the BLAST program. One of the peptide sequence (VQLEPFPEK) matched a sequence from the EST (expressed sequence tag) data base with the accession no. R70551 (a 424 bp fragment). Based on this information two oligonucleotide primers were synthesized, (sense primer: 5′-CAGAATGGCACGTAGCTACAGAACCT; antisense primer: 5′-TTTGCAAAGATATACCTGTCACTGT), and used in a RT-PCR reaction to amplify a dGK-specific DNA fragment of 278 bp, starting with human brain cDNA (prepared by using the Maraton™ cDNA amplification kit from Clontech Laboratories, Inc.). PCRs were performed in a total volume of 50 μl with 200 nM of each primer, 200 μM dNTPs, 2.5 mM $MgCl_2$ in 1× polymerase buffer and 2.5 U of Expend™ High Fidelity enzyme (Boehringer Mannheim). The reaction mixture was denatured at 94°C for 1 min and followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 3 min. The 278 bp PCR product was purified from the agarose gel and subcloned into the PCR™ vector (Invitrogen Corporation) and sequenced.

2.5. Isolation of cDNA clones

A human brain cDNA library (Stratagene, La Jolla, CA), constructed in the Lambda ZAP® vector, was screened with the 278 bp fragment as probe, labelled with ^{32}P (Random Primed DNA Labeling Kit, Boehringer Mannheim). Hybridization was carried out at 42°C in 6× SSC, 5× Denhardt's solution, 0.1% SDS and 50% formamide for 20 h, filters were washed at 42°C in 2× SSC, 0.1% SDS and autoradiographed. A total of 6 positive λ phages were isolated. The cDNA inserts were in vivo excised using the Exassist™/SOLR™ system (Stratagene) in Bluescript (sk−) phagemid vector, following the protocol provided by the supplier. Both strands were sequenced by the Sanger dideoxynucleotide method (Sequenase kit, United States Biochemicals) using synthetic oligonucleotide primers.

2.6. Northern blot analysis

Human Multi-Tissue Blot (Clontech) were used to determine dGK mRNA levels in different tissues. Blots were probed with full-length dGK cDNA (^{32}P labelled as described above) in 5× SSPE, 10× Denhardt's solution, 200 μg/ml freshly denatured salmon sperm DNA, 2% SDS and 50% formamide at 42°C for 20 h, washed in 0.2× SSC, 0.1% SDS twice at 42°C and autoradiographed.

2.7. Expression and characterization of recombinant dGK protein

Two oligonucleotides, which contained the 5′-flanking restriction sites *Nco*I or *Bam*HI, were used to PCR amplify the dGK coding sequence. The resulting fragment was digested with *Nco*I and *Bam*HI and subcloned into the pET-9d expression vector (Novagen, Madison). This vector provides a His-tag and a thrombin cleavage site fused to the N-terminal of the expressed protein. The recombinant dGK construct was transformed into pLysS BL21(DE3) cells [15]. Induction was performed for 2 h in the presence of 1 mM IPTG at 37°C. The bacteria were lyzed in 5 vol. of 50 mM Tris-HCl, pH 7.6, 50 mM NaCl, 1 mM EDTA, 2 mM dithiothreitol, 0.5% Triton X-100, and 140 μM phenylmethylsulfonyl fluoride by freezing and thawing. The lysate was centrifuged at 35 000×*g* for 90 min. dGK was purified by metal affinity (TALON, Clontech) column chromatography essentially as described in the manual and dGK activity was eluted with 0.25 M imidazole and 0.5 M NaCl. Part of these fractions were precipitated and analyzed by SDS-PAGE and the activity with several deoxynucleosides determined.

2.8. Enzyme assays

dGK activity was determined by using [^{3}H]araG as substrate as described previously [8]. The phosphoryl transfer assay was performed with 100 μM [γ-^{32}P]ATP (10 mCi/ml), 50 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 100 mM KCl, 10 mM dithiothreitol, 0.5 mg/ml bovine serum albumin, purified dGK, and 10 μM or 200 μM of nucleoside in a total volume of 50 μl. The phosphorylated products were separated by thin layer chromatography and quantitated as described [8].

3. Results

Earlier studies have shown that brain is a good source for the purification of mitochondrial deoxynucleoside kinases [1,8]. Because of the difficulty to obtain human brain material we used calf brain as starting material. After homogenisation and streptomycin sulphate precipitation, proteins were purified by DEAE-chromatography, followed by hydroxyl apatite chromatography and affinity chromatography [8].

In the present dGK purification the dGMP-Sepharose step was exchanged by an ATP-agarose step. However, several additional proteins bind to the ATP-agarose, but dGK is the only protein in the molecular weight range around 28 kDa. The overall recovery of dGK protein is approximately 20%, i.e. 500 μg from 1 kg of brain.

Affinity purified dGK preparations were precipitated, reduced, carboxymethylated and subjected to SDS gel electrophoresis. The 28 kDa protein band was excised and treated with trypsin or endoproteinase LysC and the resulting peptides separated by narrow-bore reverse-phase liquid chromatography.

Six peptide sequences were determined and when the EST sequence data base was searched with the BLAST program a positive match was obtained with one of the peptides (VQLEPFPEK). The EST sequence was 424 bp long and, based on this information, two primers were synthesized

Table 1
Phosphorylation of nucleosides by recombinant human dGK and bovine brain dGK

Substrate	Concentration	Bovine dGK	Human dGK*
	(μM)	(nmol/min/mg protein)	
dGuo	10	3.2	4.3
	200	3.9	4.5
dAdo	10	0.5	0.5
	200	10.1	4.8
dIno	10	9.9	8.3
	200	13.3	8.3
dCyd	200	0.6	1.2
CdA	10	3.7	9.5
	200	23.7	13.7
araG	10	1.3	2.9
	200	4.0	6.9

The phosphoryl transfer assay was performed as described in Section 2 and the values are from one typical experiment.
*Recombinant human dGK.

that were used to generate a dGK RT-PCR fragment of 278 nucleotides. This was used as probe for screening of a human brain cDNA library.

The full-length cDNA sequence of human mitochondrial deoxyguanosine kinase is shown in Fig. 1. All the peptide sequences from the bovine dGK protein could be identified. The cDNA clone includes 11 bp 5′-untranslated region, a 780 bp open reading frame that encodes a protein of 260 amino acids with a calculated molecular weight of 30 kDa, and 234 bp of 3′-untranslated sequence containing a polyadenylation signal at position 993 followed by a poly(A) tail.

The full-length dGK cDNA was used to probe Northern blots containing mRNA from different human tissues. As shown in Fig. 2, a major band of 1.35 kb was observed in all lanes with similar intensity, except in those from colon, kidney and peripheral blood leukocyte where it appeared to be reduced. A shorter transcript (≈1 kb) was seen in the mRNA from most tissues. The shortest transcript found in pancreas, lung and heart are the same, but appear to differ from the shortest transcript in the kidney and placenta. Testis showed the highest expression of the 1.35 kb mRNA and there was no indication of a 1 kb band. This pattern of expression is clearly different from that of human dCK, which showed the highest level of a 2.8 kb mRNA in spleen and thymus [18].

Insertion of the human dGK coding sequence in the pET-9d vector gave a 40-fold increased dGK activity in crude extracts. Purification of recombinant dGK from BL21(DE3) cells by metal affinity chromatography demonstrated a major protein band of approximately 32 kDa in SDS-PAGE (Fig. 3). The N-terminal His-tag and thrombin cleavage site sequences account for about 2–3 kDa of the total molecular weight of the fusion protein. The purified recombinant dGK

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CCTTCAGTTCCATGGCCAAGAGCCCACTCGAGGGCGTTTCCTCCTCCAGAGGCCTGCACGCGGGGCGCGG 70
          M  A  K  S  P  L  E  G  V  S  S  S  R  G  L  H  A  G  C  G  20

GCCCCGAAGGCTCTCCATCGAAGGCAACATTGCTGTGGGAAAGTCCACGTTTGTGAAGTTACTCACGAAA 140
 P  R  R  L  S  I  E  G  N  I  A  V  G  K  S  T  F  V  K  L  L  T  K  43

ACTTACCCAGAATGGCACGTAGCTACAGAACCTGTAGCAACATGGCAGAATATCCAGGCTGCTGGCACCC 210
 T  Y  P  E  W  H  V  A  T  E  P  V  A  T  W  Q  N  I  Q  A  A  G  T   66

AAAAAGCCTGCACTGCCCAAAGTCTTGGAAACTTGCTGGATATGATGTACCGGGAGCCAGCACGATGGTC 280
 Q  K  A  C  T  A  Q  S  L  G  N  L  L  D  M  M  Y  R  E  P  A  R  W  S  90

CTACACATTCCAGACATTTTCCTTTTTGAGCCGCCTGAAAGTACAGCTGGAGCCCTTCCCTGAGAAACTC 350
 Y  T  F  Q  T  F  S  F  L  S  R  L  K  V  Q  L  E  P  F  P  E  K  L  103

TTACAGCCCAGGAAGCCAGTACAGATCTTTGAGAGGTCTGTGTACAGTGACAGGTATATCTTTGCAAAGA 420
 L  Q  A  R  K  P  V  Q  I  F  E  R  S  V  Y  S  D  R  Y  I  F  A  K  126

ATCTTTTTGAAAATGATTCCCTCAGTGACATCGAGTGGCATATCTATCAGGACTGGCATTCTTTTCTCCT 490
 N  L  F  E  N  D  S  L  S  D  I  E  W  H  I  Y  Q  D  W  H  S  F  L  L 150

GTGGGAGTTTGCCAGCCGGATCACATTACATGGCTTCATCTACCTCCAGGCTTCTCCCCAGGTTTGTTTG 560
 W  E  F  A  S  R  I  T  L  H  G  F  I  Y  L  Q  A  S  P  Q  V  C  L  173

AAGAGACTGTACCAGAGGGCCAGGGAGGAGGAGGAAGGAATTGAGCTGGCCTATCTAGAGCAGCTGCATG 630
 K  R  L  Y  Q  R  A  R  E  E  E  E  G  I  E  L  A  Y  L  E  Q  L  H  196

GCCAACACGAAGCCTGGCTTATTCACAAGACAACGAAGCCTCCACTTTGAGGCTCTGATGAACATTCCAGT 700
 G  Q  H  E  A  W  L  I  H  K  T  T  K  L  H  F  E  A  L  M  N  I  P  V 220

GCTGGTGTTGGATGTCAATGATGATTTTTCTGAGGAAGTAACCAAACAAGAAGACCTCATGAGAGAGGTA 770
 L  V  L  D  V  N  D  D  F  S  E  E  V  T  K  Q  E  D  L  M  R  E  V  253

AACACCTTTGTAAAGAATCTGTAACCAATACCATGAAGTTCAGGCTGTGATCTGGGCTCCCTGACTTTCT 840
 N  T  F  V  K  N  L                                                  260

GAAGCTAGAAAAATGTTGTGTCTCCCAACCACCTTTCCATCCNNAGCCCCTCTCATCCCTGGAGCACTCT 910
GCCGCTCAAGAGCTGGTTTGTTAATTATTGTTAGACTTTGCCATTGTTGCCATTGTTTTCTTTGTACCT 980
GAAGCATTTTGAAAATAAAGTTTACTTAAGTTAAAAAAAAAAAAA
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Fig. 1. cDNA and deduced amino acid sequence of human dGK. The initiator ATG and termination signal TAA as well as a possible polyadenylation signal are in bold letters. The peptide sequences determined from proteolytic digests of bovine dGK are underlined. This sequence will appear in the EMBL, GenBank and DDBJ nucleotide sequence databases under the accession number X97386.

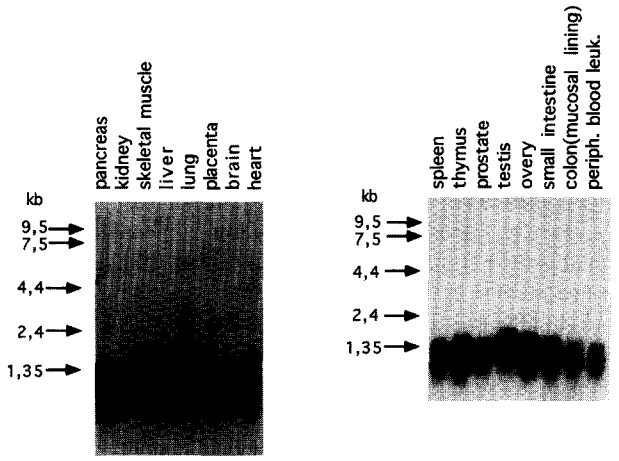

Fig. 2. Northern blot analysis of dGK mRNA levels in human tissues, using the multiple-tissue Northern blots from Clontech with 2 μg of mRNA in each lane. The estimated size of the major dGK mRNA was 1.35 kb.

had a specific activity of 5 nmol/min per mg protein, using AraG as substrate, equivalent to the activity of purified bovine dGK [8]. Purified recombinant dGK as well as bovine dGK showed similar activity with dGuo, dAdo, dIno, AraG and CdA in the phosphoryl transfer assay (Table 1). Some activity was also observed with high concentration of dCyd with both enzyme preparations.

4. Discussion

The cloning of mammalian dGK cDNA was achieved by using a peptide sequence from the bovine enzyme to identify an EST cDNA sequence. A PCR-generated dGK probe was used for the screening of a human brain cDNA library and a 1 kb cDNA, coding for a protein of 260 amino acids, was isolated. All six peptide sequences obtained from bovine dGK (a total of 41 amino acids) were identified in the deduced (260 amino acids) human protein and thus human and bovine dGK show a high degree of homology.

Northern blot analysis with the dGK cDNA as probe showed positive hybridisation with a major and two minor mRNA transcripts that were found in similar abundancy in most tissues in the multiple Northern blots. dGK cDNA contains in addition to the 780 bp coding region a 234 bp 3′-untranslated region including a poly(A) tail. The 5′-untranslated sequence described here is only 11 nucleotides and we do not know if the missing 300 nucleotides in the cDNA compared to the major mRNA species is due to an extended 5′-sequence or to the existence of an additional 3′-polyadenylation signal. The overall pattern of dGK mRNA expression is in accordance with the enzyme activity levels measured earlier [1,8].

The expression of human dGK cDNA, cloned into the pET vector system of Studier et al. [15] led to production of a 32 kDa protein which showed high enzyme activity with purine deoxynucleosides similar to that of purified bovine dGK. In SDS-PAGE analysis of recombinant dGK and purified dGK, the latter showed a lower apparent molecular mass, most likely due in part to proteolysis during the mitochondrial import process [16,17].

When the dGK sequence was used in a BLAST search

against the GenBank nucleotide sequence data base the best matches were to human [9], mouse [18] and rat [19] deoxycytidine kinase sequences. The similarity in the deduced protein sequence were overall 46% and in some regions as high as 70%. Two *Bacillus subtilis* proteins (25.4 and 24.1 kDa, respectively) [20], one Fowl pox virus 25.9 kDa polypeptide [21] as well as a ictalurid herpes virus 1 [22] and a saimiriine herpes virus 1 thymidine kinase [23] are all part of a group of sequences that show considerably lower but still significant similarities to human dGK. The only other dGK protein sequence present in the GenBank is that of *Lactobacillus acidophilus* [24]. In this case the *dGK* gene is arranged in tandem with the *deoxyadenosine kinase* gene to which it shows 65% identity. The *Lactobacillus* gene encodes for a 26 and a 25 kDa polypetide, respectively, which form an active heterodimer with dAK and dGK activity [24]. The identity between the *Lactobacillus* and the mammalian dGK is only 13%, but some interesting regions of conserved amino acids sequence can be observed (Fig. 4).

In the very N-terminal part of the protein there is little homology even between the dGK and the dCK sequences, which is due to the presence of a mitochondrial leader sequence in case of dGK. There are several basic residues in this region in dGK, including double arginine residues which most likely serve as the site of proteolysis upon entry of the protein into the mitochondrial matrix [16,17]. In common with most other phosphotransferases mentioned above dGK contain a glycine-rich region near the N-terminal, with the characteristics of an ATP-binding site [25]. The location of the glycine loop in the mammalian proteolysed dGK is almost identical to that of the unmodified *Lactobacillus* enzyme. A second region of conserved amino acids is the so-called DRS

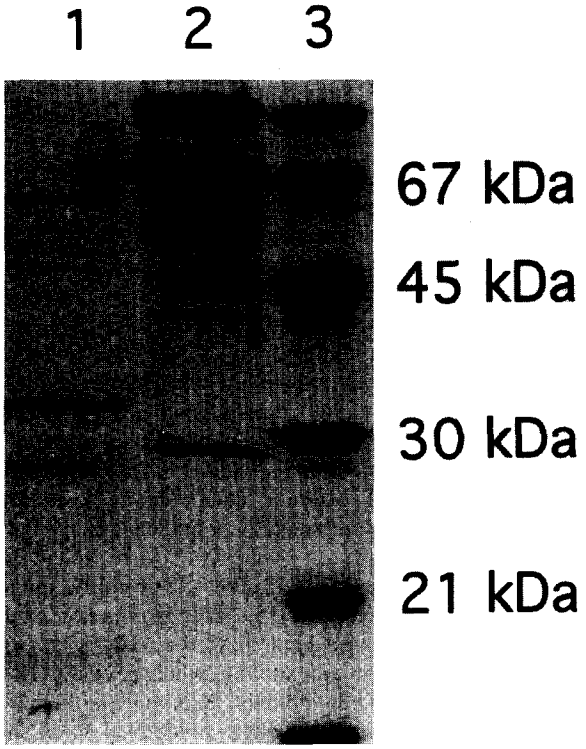

Fig. 3. SDS-PAGE of purified recombinant human dGK (lane 1), purified bovine dGK (lane 2), and molecular weight standards (lane 3).

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                                                  *  *  *  *****
dGKhum MAKSPLEGVSSSRGLHAGRGPRRLSIEGNIAVGKSTFVKL 40
dCKhum MATPPKRSCPSFSASSEGTRIKKISIEGNIAAGKSTFVNI 40
dGKlac MTV--------------------IVLSGPIGAGKSSLTGI 20
                               (Glycine-loop)

dGKhum LTKTYPEWHVATEPVATWQNIQAAGTQ---KACTAQSLGN 77
dCKhum LKQLCEDWEVVPEPVARWCNVQSTQDEFEELTMSQKNGGN 80
dGKlac LSK-----YLGTNPF------------YESV-----DDNP 38

dGKhum LLDMMYREPARWSYTFQTFSFLSRLKVQLEPFPEKLLQAR 117
dCKhum VLQMMYEKPERWSFTFQTYACLSRIRAQLASLNGKLKDAE 120
dGKlac VLPLFYENPKKYAFLLQVYFLNTRFR----SIKSALTDDN 74
               ********
dGKhum KPVQIFERSVYSDRYIFAKNLFENDSLSDIEWHIYQDWHS 157
dCKhum KPVLFFERSVYSDRYIFASNLYESECMNETEWTIYQDWHD 160
dGKlac N---VLDRSIYEDALFFQMNADIGRATPEEVDTYYELLHN 111
             (DRS motif)                 ***   *  *  **
dGKhum FLLWEFAS--RITLHGFIYLQASPQVCLKRLYQRAREEEE 195
dCKhum WMNNQFGQ--SLELDGIIYLQATPETCLHRIYLRGRNEEQ 198
dGKlac -MMSELDRMPKKNPDLLVHIDVSYDTMLKRIQKRGRNYEQ 150
                                  (Arginine-rich motif)
dGKhum GIELAYLEQLHGQHEAWLIHKTTKLHFEALMNIPVLVLDV 235
dCKhum GIPLEYLEKLHYKHESWLLHRTLKTNFDYLQEVPILTLDV 238
dGKlac ---LSYDPTLE-DYYKRLL-RYYKPWYAKYDYSPKMTIDG 185

dGKhum ND-DFSEEVTKQEDLMREVNTFVKNL.              261
dCKhum NE-DFKD---KYESLVEKVKEFLSTL-------------E 261
dGKlac DKLDFMASEEDRQEVLNQIVAKLKEMGKLEDDWKPNLVKE 225
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Fig. 4. Multiple alignment of human dGK with human dCK [9] and *L. acidophilus* dGK [24]. Sequence comparisons were performed with the DNASTAR program. Basic amino acids presumably involved in mitochondrial localization signal for dGK are underlined. Residues marked with * indicates regions of conservation with other members of the deoxynucleoside kinases [18–27].

motif which is observed in many viral thymidine kinases [26,27] and it appears to be an ERS or possibly a DRY motif in the dGK and dCK sequences (Fig. 4).

A third region with a set of conserved arginines located towards the C-terminal end of the proteins can also be identified in all these sequences and this motif is presumably also involved in phosphate donor binding [24]. Herpes simplex 1 thymidine kinase, which is responsible for the selectivity of the successful antiviral treatment with acyclovir, is known to show sequence similarities with human dCK [26,27] and contains these three sequence motifs. The recent determination of the 3D structure of the Herpes simplex 1 kinase [28,29] may enable the clarification of important structure–function relationships in the deoxynucleoside kinase family.

The physiological role of mammalian dGK is not known but it is likely that the enzyme is responsible for the supply of deoxyribonucleotides for mitochondrial DNA synthesis and in cells lacking de novo synthesis of deoxyribonucleotides this may be the only pathway leading to the production of purine deoxynucleotides. The cloning and sequencing of dGK cDNA will help to clarify the function of the enzyme in mitochondrial DNA precursor synthesis, in pharmacological treatments with purine deoxynucleoside analogs as well as in situations of altered purine nucleoside catabolism such as in PNP deficiency.

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