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HUMAN HEXOKINASE II : SEQUENCE AND HOMOLOGY TO OTHER HEXOKINASES*

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SUMMARY The amino acid sequence of human hexokinase II was deduced from the sequence of cDNA clones isolated from a skeletal muscle library. An open reading frame of 2751 bases encodes a protein of 917 amino acids. The deduced amino acid sequence has 94% identity with rat hexokinase II but only 72% identity with human hexokinase type I. In addition to hexokinase II clones, the human skeletal muscle cDNA library contained at least an equal number of clones of hexokinase I, the isoform reported to be typically found in kidney and brain. Genetic variation in hexokinase II could underlie insulin resistance in peripheral tissues and cause non-insulin-dependent diabetes mellitus. The availability of this sequence would facilitate investigating the role of mutations in the HKII gene in the etiology of this disease.

The four mammalian hexokinases (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1.) catalyze the conversion of glucose to glucose-6-phosphate, the first step of intracellular glucose metabolism (1). Hexokinases I-III have a molecular weight of approximately 100,000, have a high affinity for glucose and are subject to a allosteric inhibition by glucose-6-phosphate (Glc-6-P). In contrast, hexokinase IV, also known as glucokinase, has a molecular weight of 50,000, a lower affinity for glucose, and is not feedback inhibited by physiological concentrations of Glc-6-P (2). The extensive sequence homology between the N- and C-terminal halves of hexokinases I-III suggests that the mammalian hexokinases evolved from an ancestral enzyme similar to glucokinase or the yeast hexokinase by gene duplication and tandem fusion (3 - 5). Type I hexokinase has been cloned from rat brain (6), bovine heart muscle (7) and from human kidney (8) and mouse hepatoma cells (9). Rat hexokinase II has been cloned from skeletal muscle and adipose tissue libraries (5, 10).

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Abbreviations: Glc-6-P, glucose 6-phosphate, NIDDM, non-insulin-dependent diabetes mellitus.

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Hexokinase II is involved in the increased glucose uptake and utilization by adipose and skeletal muscle in response to insulin (11-13). Transcription of the hexokinase II gene in rat adipose and skeletal muscle is induced by insulin (5, 14). Non-insulin-dependent diabetes mellitus (NIDDM) is characterized by decreased glucose uptake and utilization by these tissues, and by relatively low basal levels of Glc-6-P (15, 16). Therefore, defects in HKII synthesis or activity may be implicated in the etiology of NIDDM. As a first step towards testing this hypothesis, we have isolated and sequenced HKII cDNA clones from a human skeletal library and deduced the complete amino acid sequence of the enzyme.

MATERIALS AND METHODS

A human (15 year old female Caucasian) skeletal muscle cDNA library (in bacteriophage λgt 11) was purchased from Clontech Laboratories, Inc. (Catalog number HL 1124b, Palo Alto, CA). The full length rat hexokinase II cDNA probe (10) was kindly provided by Dr. John E. Wilson, Michigan State University. The human skeletal muscle cDNA library was screened for hexokinase II clones according to the protocol provided by the manufacturer. The probe was labeled by random priming using [^{32}P]dCTP to a specific activity of approximately 10^9 cpm/μg. The inserts in hybridizing clones were amplified by the polymerase chain reaction (17) using the following primers that are complementary to sequences flanking the Eco RI cloning site of λgt 11:

Forward 5' GGTGGCGACGACTCCTGGAGCC 3'
Reverse 5' GACACCAGACCAACTGGTAATG 3'.

Thirty five cycles of 30 seconds of denaturation at 92°C, followed by 1 minute of annealing at 64°C, and 1-3 min. of extension at 72°C were employed using the Perkin-Elmer Cetus GeneAmp kit. Phage lysates (boiled) were used as templates for amplification reactions. The amplified inserts were directly sequenced as described (18) using the above two primers plus internal hexokinase II primers. Multiple alignment of hexokinase nucleotide and amino acid sequences were made using the GENALIGN program of IntelliGenetics, Inc. (Mountain View, CA).

RESULTS

Isolation of hexokinase cDNA clones. Forty two of the 3.5×10^5 phages in the human skeletal muscle cDNA library hybridized to the rat hexokinase II cDNA probe. The inserts in twenty of these clones were characterized by amplification and direct sequencing. The sequences of ten of these clones were identical to those found in human hexokinase I cDNA isolated from a kidney cDNA library (8). Seven of the 20 clones were found to have a high degree of nucleotide sequence homology with the rat hexokinase II cDNA sequence (10). These seven clones were used to determine the sequence of the coding region and parts of the untranslated regions of human hexokinase II cDNA. Based on the size of inserts in these clones, the human hexokinase II mRNA is predicted to be at least 4.8 kb in length.

Sequence of the human hexokinase II and homology to other hexokinases. The sequence of the entire coding region (both strands) and 117 and 273 nucleotides of the 5' and 3' untranslated regions, respectively is given in Figure 1. The sequence revealed an open reading frame of 2751 bases which encodes a protein of 917 amino acids. The calculated molecular weight of 102,365 closely resembles that deduced for rat hexokinase II (102,550)(5, 10) and human hexokinase I (102,519) (8). At the nucleotide level, the human and rat hexokinase II cDNA sequences are 87.2% identical, whereas human hexokinases I and II share 72.5% sequence identity. In the alignment shown in Fig. 2, the human and rat hexokinase II isozymes exhibit 94% sequence identity and 97% similarity with rat hexokinase II but only 72% identity and 87 % similarity with human hexokinase I.

As was observed for the rat hexokinases I, II, and III and human hexokinase I isozymes (8, 6,10, 20), there is striking homology between the N-terminal and C-terminal halves in human hexokinases I and II which are both homologous to the yeast hexokinase. Alignment of the two halves of hexokinases I and II indicates, respectively, 52% and 54% identity with a fusion point at amino acid residue 463, and that the C-terminal half lacks the first 15 N-terminal residues. It has been suggested that these N-terminal segments are important in anchoring these enzymes to the mitochondrial membrane (8, 21).

DISCUSSION

A significant finding in this study was that, in contrast to the extremely high ratio of hexokinase II to hexokinase I mRNA in rat skeletal muscle (5), clones encoding these two isoforms were observed to be equally represented in human skeletal muscle. This may have an important physiological implication since the synthesis of hexokinase II only is responsive to insulin action.

The striking sequence similarity between the N-terminal and C-terminal halves of hexokinase types I-III support the hypothesis advanced by several investigators that these isozymes evolved from hexokinase IV and yeast hexokinase-like enzymes (which have approximately half the molecular weight) by a process involving gene duplication followed by tandem ligation. The N-terminal half of rat brain hexokinase I contains the allosteric regulatory site for Glc-6-P (22), whereas the C-terminal half contains the catalytic site(23, 24). However, a recombinant C-terminal half of hexokinase I, expressed in *E. coli*, was shown to be both catalytically active and sensitive to inhibition by Glc-6-P (25). Putative amino acid residues involved in binding of glucose and of ATP have been deduced from the X-ray structure of yeast hexokinase and from comparison of sequences among the hexokinases (9, 20, 26-29). Residues corresponding to Ser-158, Asn-210, Glu-269, and Glu-302 (glucose binding domain) and Gly-X-Gly-X-X-Ala at 459-464

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TCCCCGCTCCCGGCCCGGCAGCCGAGCCCCAGCACAAAGCAGTCGGACCGCGCCGCCCGCCTCCCCTCTGCGTCTCCGCCTCGGTTTCCCAACTCTGCGCCGTCGGGCTGCGGCAGG  117

ATG ATT GCC TCG CAT CTG CTT GCC TAC TTC TTC TCG GAG CTC AAC CAT GAC CAA GTG CAG AAG GTT GAC CAG TAT CTC TAC CAC ATG CGC  207
Met Ile Ala Ser His Leu Leu Ala Tyr Phe Phe Ser Glu Leu Asn His Asp Gln Val Gln Lys Val Asp Gln Tyr Leu Tyr His Met Arg   30

CTC TCT GAT GAG ACC CTC TTG GAG ATC TCT AAG CGG TTC CGC AAG GAG ATG GAG AAA GGG CTT GGA GCC ACC ACT CAC CCT ACT GCA GCA  297
Leu Ser Asp Glu Thr Leu Leu Glu Ile Ser Lys Arg Phe Arg Lys Glu Met Glu Lys Gly Leu Gly Ala Thr Thr His Pro Thr Ala Ala   60

GTG AAG ATG CTG CCC ACC TTT GTG AGG TCC ACT CCA GAT GGG ACA GAA CAC GGA GAG TTC CTG GCT CTG GAT CTT GGA GGG ACC AAC TTC  387
Val Lys Met Leu Pro Thr Phe Val Arg Ser Thr Pro Asp Gly Thr Glu His Gly Glu Phe Leu Ala Leu Asp Leu Gly Gly Thr Asn Phe   90

CGT GTG CTT TGG GTG AAA GTA ACG GAC AAT GGG CTC CAG AAG GTG GAA ATG GAG AAT CAG ATC TAT GCC ATC CCT GAG GAC ATC ATG CGA  477
Arg Val Leu Trp Val Lys Val Thr Asp Asn Gly Leu Gln Lys Val Glu Met Glu Asn Gln Ile Tyr Ala Ile Pro Glu Asp Ile Met Arg  120

GGC AGT GGC ACC CAG CTG TTT GAC CAC ATT GCC GAA TGC CTG GCT AAC TTC ATG GAT AAG CTA CAA ATC AAA GAC AAG AAG CTC CCA CTG  567
Gly Ser Gly Thr Gln Leu Phe Asp His Ile Ala Glu Cys Leu Ala Asn Phe Met Asp Lys Leu Gln Ile Lys Asp Lys Lys Leu Pro Leu  150

GGT TTT ACC TTC TCG TTC CCC TGC CAC CAG ACT AAA CTA GAC GAG AGT TTC CTG GTC TCA TGG ACC AAG GGA TTC AAG TCC AGT GGA GTG  657
Gly Phe Thr Phe Ser Phe Pro Cys His Gln Thr Lys Leu Asp Glu Ser Phe Leu Val Ser Trp Thr Lys Gly Phe Lys Ser Ser Gly Val  180

GAA GGC AGA GAC GTT GTG GCT CTG ATC CGG AAG GCC ATC CAG AGG AGA GGG GAC TTT GAT ATC GAC ATT GTG GCT GTG GTG AAT GAC ACA  747
Glu Gly Arg Asp Val Val Ala Leu Ile Arg Lys Ala Ile Gln Arg Arg Gly Asp Phe Asp Ile Val Ala Val Val Asn Asp Thr  210

GTT GGG ACC ATG ATG ACC TGT GGT TAT GAT GAC CAC AAC TGT GAG ATT GGT CTC ATT GTG GGC ACG GGC AGC AAC GCC TGC TAC ATG GAA  838
Val Gly Thr Met Met Thr Cys Gly Tyr Asp Asp His Asn Cys Glu Ile Gly Leu Ile Val Gly Thr Gly Ser Asn Ala Cys Tyr Met Glu  240

GAG ATG CGC CAC ATC GAC ATG GTG GAA GGC GAT GAG GGG CGG ATG TGT ATC AAT ATG GAG TGG GGG GCC TTC GGG GAC GAT GGC TCG CTC  927
Glu Met Arg His Ile Asp Met Val Glu Gly Asp Glu Gly Arg Met Cys Ile Asn Met Glu Trp Gly Ala Phe Gly Asp Asp Gly Ser Leu  270

AAC GAC ATT CGC ACT GAG TTT GAC CAG GAG ATT GAC ATG GGC TCA CTG AAC CCG GGA AAG CAA CTG TTT GAG AAG ATG ATC AGT GGG ATG 1017
Asn Asp Ile Arg Thr Glu Phe Asp Gln Glu Ile Asp Met Gly Ser Leu Asn Pro Gly Lys Gln Leu Phe Glu Lys Met Ile Ser Gly Met  300

TAC ATG GGG GAG CTG GTG AGG CTT ATC CTG GTG AAG ATG GCC AAG GAG GAG CTG CTC TTT GGG GGG AAG CTC AGC CCA GAG CTT CTC AAC 1107
Tyr Met Gly Glu Leu Val Arg Leu Ile Leu Val Lys Met Ala Lys Glu Glu Leu Leu Phe Gly Gly Lys Leu Ser Pro Glu Leu Leu Asn  330

ACC GGT CGC TTT GAG ACC AAA GAC ATC TCA GAC ATT GAA GGG GAG AAG GAT GGC ATC CGG AAG GCC CGT GAG GTC CTG ATG CGG TTG GGC 1197
Thr Gly Arg Phe Glu Thr Lys Asp Ile Ser Asp Ile Glu Gly Glu Lys Asp Gly Ile Arg Lys Ala Arg Glu Val Leu Met Arg Leu Gly  360

CTG GAC CCG ACT CAG GAG GAC TGC GTG GCC ACT CAC CGG ATC TGC CAG ATC GTG TCC ACA CGC TCC GCC AGC CTG TGC GCA GCC ACC CTG 1287
Leu Asp Pro Thr Gln Glu Asp Cys Val Ala Thr His Arg Ile Cys Gln Ile Val Ser Thr Arg Ser Ala Ser Leu Cys Ala Ala Thr Leu  390

GCC GCC GTG CTG CAG CGC ATC AAG GAG AAC AAA GGC GAG GAG CGC GCT CGC TCT ACT ATT GGG GTC GAC GGT TCC GTC TAC AAG AAA CAC 1377
Ala Ala Val Leu Gln Arg Ile Lys Glu Asn Lys Gly Glu Glu Arg Ala Arg Ser Thr Ile Gly Val Asp Gly Ser Val Tyr Lys Lys His  420

CCC CAT TTT GCC AAG CGT CTA CAT AAG ACC GTG CGG CGG CTG GTG CCC GGC TGC GAT GTC CGC TTC CTC CGC TCC GAG GAT GGC AGT GGC 1467
Pro His Phe Ala Lys Arg Leu His Lys Thr Val Arg Arg Leu Val Pro Gly Cys Asp Val Arg Phe Leu Arg Ser Glu Asp Gly Ser Gly  450

AAA GGT GCA GCC ATG GTG ACA GCA GTG GCT TAC CGG CTG GCC GAT CAA CAC CGT GCC CGC CAG AAG ACA TTA GAG CAT CTG CAG CTG AGC 1557
Lys Gly Ala Ala Met Val Thr Ala Val Ala Tyr Arg Leu Ala Asp Gln His Arg Ala Arg Gln Lys Thr Leu Glu His Leu Gln Leu Ser  480

CAT GAC CAG CTG CTG GAG GTC AAG AGG AGG ATG AAG GTA GAA ATG GAG CGA GGT CTG AGC AAG GAG ACT CAT GCC AGT GCC CCC GTC AAG 1647
His Asp Gln Leu Leu Glu Val Lys Arg Arg Met Lys Val Glu Met Glu Arg Gly Leu Ser Lys Glu Thr His Ala Ser Ala Pro Val Lys  510

ATG CTG CCC ACC TAC GTG TGT GCT ACC CCG GAC GGC ACA GAG AAA GGG GAC TTC TTG GCC TTG GAC CTT GGA GGA ACA AAT TTC CGG GTC 1737
Met Leu Pro Thr Tyr Val Cys Ala Thr Pro Asp Gly Thr Glu Lys Gly Asp Phe Leu Ala Leu Asp Leu Gly Gly Thr Asn Phe Arg Val  540

CTG CTG GTC CGT GTT CGG AAT GGG AAG TGG GGT GGA GTG GAG ATG CAC AAC AAG ATC TAC GCC ATC CCG CAG GAG GTC ATG CAC GGC ACC 1827
Leu Leu Val Arg Val Arg Asn Gly Lys Trp Gly Gly Val Glu Met His Asn Lys Ile Tyr Ala Ile Pro Gln Glu Val Met His Gly Thr  570

GGG GAC GAG CTC TTT GAC CAC ATT GTC CAG TGC ATC GCG GAC TTC CTC GAG TAC ATG GGC ATG AAG GGC GTG TCC CTG CCT CTG GGT TTT 1917
Gly Asp Glu Leu Phe Asp His Ile Val Gln Cys Ile Ala Asp Phe Leu Glu Tyr Met Gly Met Lys Gly Val Ser Leu Pro Leu Gly Phe  600

ACC TTC TCC TTC CCC TGC CAG CAG AAC AGC CTG GAC GAG AGC ATC CTC CTC AAG TGG ACA AAA GGC TTC AAG GCA TCT GGC TGC GAG GGC 2007
Thr Phe Ser Phe Pro Cys Gln Gln Asn Ser Leu Asp Glu Ser Ile Leu Leu Lys Trp Thr Lys Gly Phe Lys Ala Ser Gly Cys Glu Gly  630

GAG GAC GTG GCG ACC CTG CTG AAG GAA GCG ATC CAC CGG CGA GAG GAG TTT GAC CTG GAT GTG GTT GCT GTG GTG AAC GAC ACA GTC GGA 2097
Glu Asp Val Ala Thr Leu Leu Lys Glu Ala Ile His Arg Arg Glu Glu Phe Asp Leu Asp Val Val Ala Val Val Asn Asp Thr Val GLY  660

ACT ATG ATG ACC TGT GGC TTT GAA GAC CCT CAC TGT GAA GTT GGC CTC ATT GTT GGC ACG GGC AGC AAT GCC TGC TAC ATG GAG GAG ATG 2187
Thr Met Met Thr Cys Gly Phe Glu Asp Pro His Cys Glu Val Gly Leu Ile Val Gly Thr Gly Ser Asn Ala Cys Tyr Met Glu Glu Met  690

CGC AAT GTG GAA CTG GTG GAA GGA GAA GAG GGG CGG ATG TGT GTG AAC ATG GAA TGG GGG GCA TTC GGG GAC AAT GGA TGC CTA GAT GAC 2277
Arg Asn Val Glu Leu Val Glu Gly Glu Glu Gly Arg Met Cys Val Asn Met Glu Trp Gly Ala Phe Gly Asp Asn Gly Cys Leu Asp Asp  720

TTC CGC ACA GAA TTT GAT GTG GCT GTG GAT GAG CTT TCA CTC AAC CCC GGC AAG CAG AGG TTC GAG AAA ATG ATC AGT GGA ATG TAC CTG 2367
Phe Arg Thr Glu Phe Asp Val Ala Val Asp Glu Leu Ser Leu Asn Pro Gly Lys Gln Arg Phe Glu Lys Met Ile Ser Gly Met Tyr Leu  750

GGT GAG ATT GTC CGT AAC ATT CTC ATC GAT TTC ACC AAG CGT GGA CTG CTC TTC CGA GGC CGC ATC TCA GAG CGG CTC AAG ACA AGG GGC 2457
Gly Glu Ile Val Arg Asn Ile Leu Ile Asp Phe Thr Lys Arg Gly Leu Leu Phe Arg Gly Arg Ile Ser Glu Arg Leu Lys Thr Arg Gly  780

ATC TTT GAA ACC AAG TTC TTG TCT CAG ATT GAG AGT GAC TGC CTG GCC CTG CTG CAA GTC CGA GCC ATC CTG CAA CAC TTA GGG CTT GAG 2557
Ile Phe Glu Thr Lys Phe Leu Ser Gln Ile Glu Ser Asp Cys Leu Ala Leu Leu Gln Val Arg Ala Ile Leu Gln His Leu Gly Leu Glu  810

AGC ACC TGT GAC GAC AGC ATC ATT GTT AAG GAG GTG TGC ACT GTG GTG GCC CGG CGG GCA GCC CAG CTC TGT GGC GCA GGC ATG GCC GCT 2647
Ser Thr Cys Asp Asp Ser Ile Ile Val Lys Glu Val Cys Thr Val Val Ala Arg Arg Ala Ala Gln Leu Cys Gly Ala Gly Met Ala Ala  840

GTG GTG GAC AGG ATA CGA GAA AAC CGT GGG CTG GAC GCT CTC AAA GTG ACA GTG GGT GTG GAT GGG ACC CTC TAC AAG CTA CAT CCT CAC 2737
Val Val Asp Arg Ile Arg Glu Asn Arg Gly Leu Asp Ala Leu Lys Val Thr Val Gly Val Asp Gly Thr Leu Tyr Lys Leu His Pro His  870

TTT GCC AAA GTC ATG CAT GAG ACA GTG AAG GAC CTG GCT CCG AAA TGT GAT GTG TCT TTC CTG CAG TCA GAG GAT GGC AGC GGG AAG GGG 2827
Phe Ala Lys Val Met His Glu Thr Val Lys Asp Leu Ala Pro Lys Cys Asp Val Ser Phe Leu Gln Ser Glu Asp Gly Ser Gly Lys Gly  900

GCG GCG CTC ATC ACT GCT GTG GCC TGC CGC ATC CGT GAG GCT GGA CAG CGA TAG AACCCCTGAAATCGGAAGGACTTCCTCTTTCTCTCCTTCTTCCCTGT 2928
Ala Ala Leu Ile Thr Ala Val Ala Cys Arg Ile Arg Glu Ala Gly Gln Arg  .                                                    917

TTTAAATTATAAGATGTCATCCCCTTGTGTCAGAGACAGACCCCTTGGCTTTTGCTTGGCAGAGAGGACCCCACTGGACTCCCTTTTGTCTCTGCTACTCATTGTAGAGCTTGGTGGCT 3047

GAGCTTGGCCCTATTAAGATAAATAGAGTTCCAAATAAGGATTTTGTTCACATGCATCATAACCATTCCCATTGGTTCTCCTAAAACATGAAAATTATCTCCCTTAG                3154
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Figure 1. Nucleotide sequence of the cDNA and deduced amino acid sequence of human skeletal muscle hexokinase II.

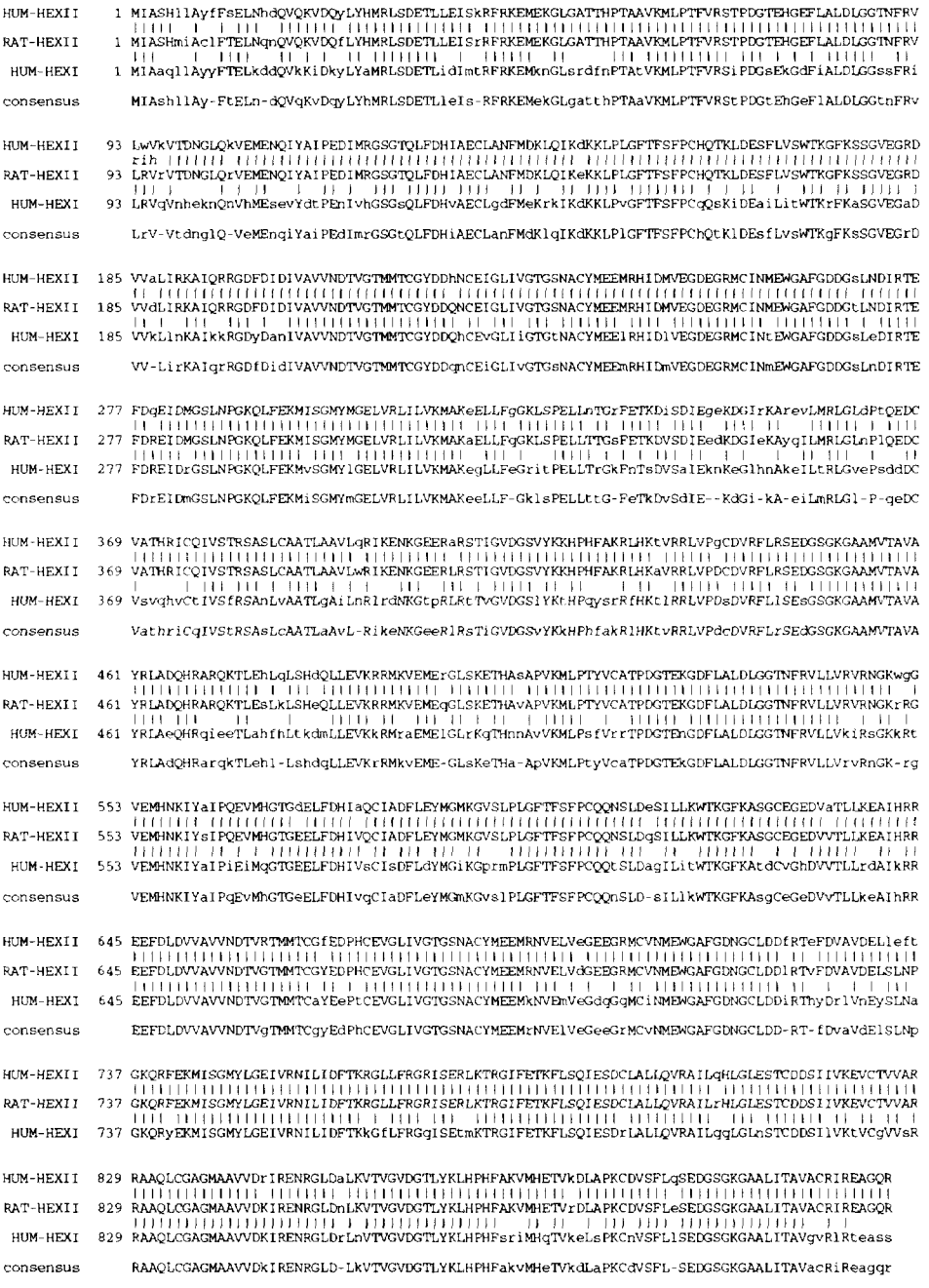

Figure 2. Comparison of the deduced amino acid sequences of human HKI, HKII, and rat HKII. Multiple alignment of human hexokinase II (HUM-HEXII), rat hexokinase II (RAT-HEXII), and human hexokinase I (HUM-HEXI). The vertical lines denote matches between pairs. The consensus sequence shows exact matches in uppercase letters and the consensus character in lowercase letters. Horizontal lines indicate no consensus.

(ATP binding domain) of the yeast hexokinase are all conserved in both halves of human and rat hexokinases I and II (residues 448-453 and 896-901). Botstein and colleagues (30) identified the following six missense mutations in the yeast

hexokinase II gene that significantly affected the function of this enzyme: Gly89Asp, Thr190Ile, Gly235Cys, Asn237His, Ser306Phe, and the double mutant Gln367Glu and Ile381Met. The residues corresponding to the first five of these positions are fully conserved in both halves of hexokinases I and II. The Gln residue at position 376 is conserved only in the corresponding position 365 of the N-terminal domain of hexokinase II, and the Ile residue is conserved only in the corresponding position 370 of the C-terminal domain of hexokinase II, suggesting that neither residue is important in catalysis.

Missense mutations in the human type IV hexokinase (glucokinase) gene have been identified in patients with an autosomal dominant form of NIDDM (31). All of these mutations (at residues 70, 175, 182, 203, 228, 256, 261, 279, 300, 299, 309, and 414) cause changes in catalytic properties. The residues in both halves of hexokinases I and II found at sites that correspond to all but two (279 and 175) of these mutated sites in hexokinase IV are conserved. Arg and Met are substituted for Glu at the position corresponding to residue 279 (hexokinase II 283) in only the C-terminal domains of hexokinases I and II, respectively. The residue 279 mutant allele encodes an enzyme with a higher K_m for glucose (but not ATP) and lower V_{max} (30). Thus, the apparent absence of catalytic activity in the N-terminal domain may be at least partially due to substitution at residue 279. Residue 179 in hexokinase I and hexokinase II corresponding to Gly 175 in hexokinase IV is conserved in both halves except for the presence of Asp in the C-terminal domain of hexokinase I. The Gly175Arg mutation in hexokinase IV causes a reduction in V_{max} and an increase in K_m for glucose(30). It appears, therefore, that the enzyme can tolerate substitution of an acidic but not a basic residue at this position.

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