Structure of the human lactate dehydrogenase B gene

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Human genomic clones containing parts of the lactate dehydrogenase B (LDH-B) gene (approx. 25 kb in length) were isolated and characterized. The protein-coding sequence of human LDH-B gene is interrupted by six introns at codons nos. 42–43, 82, 140, 198, 237 and 278–279, and the positions of these introns are homologous to those of LDH-A genes from man and mouse. The 5' non-coding region of human LDH-B gene is interrupted by an intron six nucleotide residues upstream of the ATG translation-initiation site, whereas those of human and mouse LDH-A genes are interrupted at 24 nucleotide residues 5' to the ATG initiation codon. As is the case of LDH-A genes from man and mouse, there is no intron in the 3' non-coding region of human LDH-B gene.

INTRODUCTION

In mammals, the lactate dehydrogenase A (LDH-A) (muscle), B (heart) and C (testis) polypeptide chains are encoded by three different genes originated from an ancestral gene during the course of evolution [1,2]. Human LDH-B gene is located at the short arm of chromosome no. 12, whereas human LDH-A and LDH-C genes are mapped on chromosome no. 11 [3,4]. The expression of these three LDH genes is developmentally regulated and tissue-specific [5]. In order to study the structural and evolutionary relationships and to elucidate the molecular mechanism(s) of the tissue-specific expression of these three LDH genes, we have investigated the protein structure and gene organization of mammalian

LDH isoenzymes A₄, B₄ and C₄. We have determined the primary structures of human and mouse LDH-A₄, human LDH-B₄, mouse and rat LDH-C₄ isoenzymes by structural analyses of proteins and/or DNAs [6–11]. We have also determined the exon organization of human and mouse LDH-A genes [12,13]. In the present paper, we describe the structural organization of human LDH-B gene.

MATERIALS AND METHODS

Isolation of genomic clones

The human genomic library was constructed from the *Mbo*I partially restricted leucocyte DNA fragments in-

Fig. 1. Exon-intron organization of human LDH-B gene along with restriction endonuclease map and nucleotide sequencing strategy

The seven protein-coding exons are numbered in Arabic numerals and shown by solid black blocks. Both 5' and 3' non-coding regions are stippled. The exact sizes of six introns interrupting the protein-coding sequence are not known. The restriction endonuclease maps of clones hB501, hB502 and hB503 were deduced from cleavage with *Eco*RI (R) and/or *Sal*I (located at both ends of genomic DNA inserts) and Southern-blot analysis with use of the human LDH-B cDNA probe. The isolated *Eco*RI-*Eco*RI and *Eco*RI-*Sal*I DNA fragments were further cleaved with *Xba*I (X), *Pst*I (P), *Sau*3A (S) or *Alu*I (A), and cloned into M13 mp10 or mp11 bacteriophages. The nucleotide sequences of the inserted DNA from the M13 bacteriophages exhibiting positive hybridization to the human LDH-B cDNA probe were determined by the dideoxy chain termination method. The nucleotide sequences of both strands of the putative LDH-B gene promoter, first two exons 0 and 1 and last exon 7 were determined. Although both strands of exons 2, 3, 4, 5 and 6 were not completely determined, their nucleotide sequences were found to be identical with that of LDH-B cDNA reported previously [11].

Abbreviation used: LDH, lactate dehydrogenase.

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These sequence data have been submitted to the EMBL/GenBank Data Libraries.

1 AGCTGAATGATTTATGCTAGGAGAGTTTGAGGCTTATGAATTCCTCTCAACAAACCCTTCTCTTAGTGATTCTTTACTCTGAATTTTTATTGACTTTAA
 101 CCAAATTTCTATTGCCACTTATTGTAATTGAAATAGGTTGCCTACTGTCTGTCTCCAATGGACTAGGAACACCTCAAAGGGCATAATCTTATATTTATG
 201 TTTTTTACCAATGCTTGGCACTGAGAATAAACTGAAGCAATAATTATAGAATTAGTGGTCATATACAAGAAATATGCATTTACAAAAAACAACCTTCAC
 301 ATACAGGAAGTTTGTGAGATGGCTATATTTGAAAGCATTTTAATTATAAGAAAAAGAACTGAAGCCAGACTCTTCAGAATTCCTCCAATTTATCATTCA
 401 TTCATTTATTCATCAATGCTATATTTAGGTACCAAGCACTGGCTGAGTGTGAAGACAGAATGAATGACAACATCCAGCTTTGGAGTCGATTAGGGACTA
 501 AAAAAGAAAGTGCATCTCACTTAGTGGTCCACATTTCCCTAGAGGGCAAAGCTGACCTTGAGACCAGGTGGAATTGTAGGGGAACCTCAGATGGCAGG
 601 GACAAAGCCTCATTGGTCTGTGAGGCAACGAGTAAGCAATCATTGCGTGTGTTGTTGAAACAGGTTGAAAAAATAGTCATTTTCATCAATGCAACTTT
 701 AAAACGGAGATGATCTAAATTCAGATGATTTTTTAAAGTCCTTTAAAGTTAACTGAAATGCAACATAGAAATTTATTTCTGGCCGGGCGCGGTGGCTC
 801 AGGCTGTAAATCCAGCACTTTGGGAGGCGAGGCGGGCGGATCAGAGGTGAGGATCGAGACCCTCTGGCTAACACGGTGAAACCCCTCTCTAC
 901 TAAAAATACAAAAATAGCCGGGCGAGGTGGCGGGCGCTGTAGTCCAGCTACTCGGAGGCTGAGGCAGGAGAATGTCGTGAGCCGGGAGGCGGAG
 1001 CTGTCAGTGAGCCGAGATCGCGCCACTGCACTCCAGCTGGGCGCAGAGTGAGACTCCGCTCAAAATAAAAAAAGAGGAAAGAAATTTATTTCTAT
 1101 TTGTACTAAATCTAATAGAACAGAGCGTTAAAGTCCAGAAGAGTCGGGATGGGCAAGTTTTATAATAACTTGGCTCCGCATTGAAGAGCCTGCGAG
 1201 ATGTGAGACCAGCTGAGAGCGGAAGCCTCTGGAAGGAGACCTCCTCATCTGGAGGGTGGGGAGGAGTGTGCACACTTGAGCCCTTGCTTGAAGGGG
 1301 ATTGAGCGAGCCGAGGGGAGGGTAGGCGGTACTCGGATTCCAATCACATGGAGAACTAAGAGGCTGCGGTGGTGTGGGGCCCGCCCTCTCTC
 1401 CCTCTTGAGAGCCGGCGCGGAGGAGCGACGACGCTGACTTTGTCTCTCCGACGACTGTACAGAGGCTCCAGAGCCTCTCTCTCTGGTAG exon 0
 1501 GTTTCGGCTCAGGACCTGAATCTGGCCACAGGCAAGCCTGATGGGACGTGCGGGTAGTACTTGTATGGGAGACGCGGGAAGGAGGAAGAAGCACA
 1601 TCTGTCTCCGCGGCTTTAGCTCTGAGCATCCGGCCGCGGGCCCTGACTTTGCCGCTCAGTTTGGGCGCAGCGGGGCGCTGCCCTGGCGGAGGAGCA
 1701 GAAGGCAGAGGGTAGCAGCTGCGGCTCAGCGGAGAGACTTGTGCAATTTGACGTAACCAGGCCCTACTTGTCTTGGTGGGTTCATGGCGCCACAT
 1801 CACCCCAAGCGCTGCTGGCGGAGTGGGACACGTGCCGAGTAGTGGGGAGGGGACGGAACAGGTGGCCTATGAAGGCTGGACGATCTTTACCT
 1901 CGAATATTTGAATATATTGCCAGGTAATAGAGAAATGCCCTGCACTCGTGGGATGAGGAGAAAGGCACCTAGATGACGACGCTTTGGCGCTGCAC
 2001 TTATTTTTACCGGCTCTGACGGTCTTCCAGGAGCCATGCGCCAGTTCTTTGTGTCAGGGCCTCTTTCTGGATGCTTAGGGGCTCGTTTACTTTACC
 2101 AGCCTTGGGTGCTCCGAGTAAGGAAGTCTAGACTTGTCCCACAGACCTTACTCGGGAGATCTCTAGCTTCTGCATGCCCTCGTGATCTGCTGTTCTT
 2201 TGCTGCCGTGCCCTGAGGAAAAGATGGTGTGCGCACCGCAGCAGTACAAGCTGAGTAAGGGCTGGGGAATGGGGGAGGGGAGGAATTCAAAAGC
 2301 AAAACCTGGATTTTTAAAGATTACGGTGAGAAAGATTCTGCAAAAGATCTTTGTTCTGAAATTAAGGCACCTCTTATTTCTAATGATTATATCC
 2401 ACAGCAGTGAGGAGACATAAGGGACCAAGACTCAGAGATTTTACCCTTGTGCTTCTTGTTCCTTACAGCCTGCACCTGATGTTGGCACAGGCTGTAT
 2501 ATCATGACGACCATTTTCTCTCATTTCTTAAACTCCTTTTAAAGAAAAATGAAGAAAAGCACTTCTCCAGAAATATCTGAAGAAAAATCTGGTTCG
 2601 AGCCTTTAAATTTGACTTCTGAAGTTTTCTAAATTAAGGCTATCAGAACACGAATCTGTATTATATAGCCACTGTTTCTTATTTTCTTTTCC
 2701 ACTGCTTCTGGGCCCCTGTATACCAAGGTCGTAAACAGACCATCTAACACAATAAACTTAAGTGCAGAACAACTATTATGTGTGACGTCCTTCAAAA
 2801 GAGATGCAGCATCTTCTCTGAGCCTGTTAAATGCAGGCTGTCTGAATTTATTTTCAATTTAAGGTCATCTATTGTGGAGGCACTTGACACAACATTGC
 2901 ATCTTCTCTTTAAGTCCACTAGGAACTGTAATGTGATCTGTAAGGGCAAAGAATCTCCTTTAATTTATCCTTACAGATGGGAGGAATATTTTT
 3001 TGAAGAAAACTATAAGATAAATAGTGAACACAGTGTCTCCAAATTCAGATGACTTTATTCACCTTTGAGTCTGCTAACTCTTACTTTACGTATA
 3101 CAGTTAATAGACTAGGCTTATTGCTTAGTCAATTGCAGATAGAATTAGAATTAATTAAGTGCAGTGAAGCTCTGGTACAGTTGAGACAGGTATGGTGACC
 3201 TCAGATGCGACATAAAGTAAAAACAAGTGAATCATGGACATTACAATTGAGTACTACAATTATAAGTAATAATTATGAAGAAAAGGCTTATTGCCAT
 3301 CTGTATTCTCTGTTCAAGAAGACCCCTGGTATATATTTAGTGTACTATAAATGCCATGGAATTGATGTTTATTTTCGATAAGTTTGTCTTTTTTCA
 3401 GTTATCTAGAAAACACATCTGGTTATCTGATGGCACAAGTACATTTGGTGTACTGTACAGATCGTTCATTTTCAACAGGTTTCGGTTTTGTTTTT
 3501 TTTACATTTTAGGGTCTTTAGTTTGGATGGTTAGACAGAACTGTTTCAGAGACTGATAGTCTCCTAATGTGATCCCTGAAACGCAATGTATTAAGT
 3601 GTCTATCTACTTTCAAGAAGCTTCTTAGGTGCCCTAAAGGAATTCAGTTGATATTTTGCAGACTTTGAATAAAAAATTATTCATTGCCATTATATTG
 3701 TCACTATGTATTAAGTATGCATTCTATGTCCAGTACGCTGTAAACACATTTTATGTGAGACAGAGTAGAAGAAATCAGTCCCTATCTTCAAGAAATGATAAC
 3801 CATTAGTAGTAGAAATATGTTTATAATTGGCCATTTATTTCTTTTATCCAAGCTTAAGTATGAGTCAATACTAGACCCAACTTAGTTTACATAGTTCC
 3901 ATATACATTTCTTAATAGAGGAAACAAACAAATAGCTAATTTAAACAAATGTTACAGGTTTTTAGCCAGAAATATTAACAACCTACTAAATCTCACAT
 4001 GGAATCATAGGCTGAAGGAAGAAATCCAATCTAATGGGCTTCATGAAAGTTTTACCTTAATGAATCTTCCATTAATGGGTCTCATGGTCTTTTTT
 4101 TCTTTTTTCTTTTGGAGCGAATCGCTCTGTTGCCAGGCTGGGGTGCAGTGGTGAGATCTAAGCTCACTGCAACCTCCACTTCTGGGTCAAGTGA
 4201 TTCTCTTCTCTTAGCCTCTGAGTAGTTAGGACTACAGGCTTGCAACCACTGCCCAGCTAATTTCTTTTTTATGAGAGACGGGGTTTACCATGTT
 4301 GGCCAGGATGGTCTCAATCTCTTGACCTCGTGATCCACCAGCCTCGCTCCCAAAGTGTGGGATTACAGGCATGAGCACCATGCCAGCCGGGTCTCA
 4401 TGGTTCTTTAAATATATTCTGCTTTCTAGAATATCATTAGGCTCATTTTAAAGTTGACTTCAGTTTGAATTTGCAACACTAACATTTAAGGATGATT
 4501 CTATTTGGATTTCATATGCAGATATATGTGAATGTTTTGATTCTTCTCTGCACTGCAAAATGGCAACTCTTAAGGAAAACTATTGCACCAAGTTGCG exon 1
 4601 MetAlaThrLeuLysGluLysLeuIleAlaProValAla
 GluGluGluAlaThrValProAsnAsnLysIleThrValValGlyValGlyGlnValGlyMetAlaCysAlaIleSerIleLeuGlyLys 42
AGTCTCTGGCTGATGAACCTGCTCTTGTGGATGTTTTGGAAGATAAGCTTAAAGGAGAAATGATG exon 2
 43 SerLeuAlaAspGluLeuAlaLeuValAspValLeuGluAspLysLeuLysGlyGluMetMet
 GATCTGCAGCATGGGAGCTTATTTCTTCCAGACCTAAAATGTGGCAGATAAAGT.....
 AspLeuGlnHisGlySerLeuPheLeuGlnThrProLysIleValAlaAspLysA 82
AGATTATTCTGTGACCGCAATTCT
 82 spTyrSerValThrAlaAsnSer

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AAGATTGTAGTGGTAACATGTCAGGAGTCCGTCAGCAAGAAGGGGAGAGTCGGCTCAATCTGGTCAGAGAAATGTTAATGTCTTCAAATTCATTATTCCTC exon 3
LysIleValValValThrAlaGlyValArgGlnGlnGluGlyGluSerArgLeuAsnLeuValGlnArgAsnValAsnValPheLysPheIleIleProG
AGATCGTCAAGTACAGTCTGATTGCATCATAATTGTGGTTTCAACCCAGGT.....
InIleValLysTyrSerProAspCysIleIleIleValValSerAsnProV 140
.....AGTGGACATTCTTACGTAT
140 aIAspIleLeuThrTyr
GTTACCTGGAACTAAGTGGATTACCCAAACACCGCGTATTGGAAGTGGATGTAATCTGGATTCTGCTAGATTTCGCTACCTTATGGTGAAAAAAGTTC exon 4
ValThrTrpLysLeuSerGlyLeuProLysHisArgValIleGlySerGlyCysAsnLeuAspSerAlaArgPheArgTyrLeuMetAlaGluLysLeuG
GCATTCATCCAGCAGCTGCCATGGATGGATTTTGGGGGAACATGGCGACTCAAGTGGT.....
IyIleHisProSerSerCysHisGlyTrpIleLeuGlyGluHisGlyAspSerSerV 198
...AGTGGCTGTGTGGATGGTGTGAATGTGGCAGGTGTTTCTCCAGGAATGAATCCAGAAATGGGAAGTACAATGATAGTGAAAATGGAAGGAA exon 5
198 aAlaValTrpSerGlyValAsnValAlaGlyValSerLeuGlnGluLeuAsnProGluMetGlyThrAspAsnAspSerGluAsnTrpLysGlu
GTGCATAAGATGGTGGTTGAAAGGT.....AGTG
ValHisLysMetValValGluSe 237
CCTATGAAGTCATCAAGCTAAAGGATATACCAACTGGGCTATTGGATTAAGTGTGGCTGATCTTATTGAATCCATGTTGAAAAATCTATCCAGGATTCA exon 6
IaTyrGluValIleLysLeuLysGlyTyrThrAsnTrpAlaIleGlyLeuSerValAlaAspLeuIleGluSerMetLeuLysAsnLeuSerArgIleHi
TCCCGTGTCAACAATGGTAAAGGT.....
sProValSerThrMetValLys 278
.....AGGGGATGTATGGCATTGAGAATGAAGTCTTCCT
279 GlyMetTyrGlyIleGluAsnGluValPheLe
GAGCCTTCCATGTATCCTCAATGCCCGGGATTAACAGCGTTATCAACAGAAGCTAAAGGATGATGAGGTTGCTCAGCTCAAGAAAGTGCAGATACC exon 7
uSerLeuProCysIleLeuAsnAlaArgGlyLeuThrSerValIleAsnGlnLysLeuLysAspAspGluValAlaGlnLeuLysLysSerAlaAspThr
CTGTGGGACATCCAGAAGGACCTAAAGACCTGTGACTAGTGAGCTCTAGGCTGTAGAAATTTAAAAACTACAATGTGATTAAGTTCGAGCCTTTAGTTTT
LeuTrpAspIleGlnLysAspLeuLysAspLeu 333
CATCCATGTACATGGATCACAGTTTGCTTTGATCTTCTTCAATATGTGAATTTGGGCTCACAGAATCAAAGCCTATGCTTGGTTTAATGCTTGCAATCTG
AGCTCTTGAACAAATAAAATTAATATTGTAGTGTGCTTCTGTTTCTGGGATTCTTCTTAAATGATTCTCTGAAAGATTTCAGTCAGTCTGCCAAGTCAT
TCTTACCACACGTCATAATGTCCTAAATTCACATCATCTACTCTCTCTGATGAGACAAAAATCTTTTTGCAACAGGTGCCACGGTTTG
TTGTTGTTGCTCAGATAATGATTATCTTTTAAGGTTATAACTGAAGAAGTTGAACGGATGTCAATGTATATGACATTACAAAGGAAGCCCGTTT
TAAAGGAAGAACCCTAAATGAAACTTTGTTAGCT

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Fig. 2. Nucleotide sequences of the putative promoter, protein-coding exons, 5' and 3' non-coding regions of the human LDH-B gene

The deduced acid sequences are shown below the seven coding exons, and the numbers of first and last codons in each exon are also given. The 5' and 3' non-coding sequences are underlined.

serted at *Bam*HI site of EMBL-3 lambda vector (Clontech). The human LDH-B cDNA of nearly full length excluding the poly(A) tail was labelled [14] with [α - 32 P]dCTP (Amersham) with the use of random primer (Boehringer-Mannheim), and was used as a probe to screen the genomic library by plaque hybridization as described previously [15]. The plaque DNAs bound to the plaque-screen-hybridization membrane (DuPont) were hybridized at 65 °C overnight, and the filters were washed as recommended by the supplier. Autoradiography of the filters was done with Kodak XAR-5 film and DuPont Cronex Lighting-plus intensifying screen at -70 °C. The positive plaques were subsequently purified to homogeneity through repeated rounds of screening.

Characterization of genomic clones

The DNAs purified from the genomic clones containing human LDH-B gene were analysed by restriction endonuclease mapping and Southern blotting, as described previously [15,16]. The DNA fragments isolated from the agarose gel were further cleaved and subcloned into M13 mp10 or mp11 bacteriophages [17]. The M13 bacteriophages exhibiting positive hybridization to a human LDH-B cDNA probe [11] were isolated, and the nucleotide sequences of the inserted DNA were determined by the dideoxy chain termination method with the sequencing protocol modified to use deoxyadenosine 5'-[α - 35 S]thio]triphosphate [18,19].

RESULTS AND DISCUSSION

Several human genomic clones exhibiting strong hybridization signals to the human LDH-B cDNA probe [11] were isolated and partially characterized by restriction endonuclease cleavage and Southern-blot analyses. Two positive clones, hB501 and hB502, containing DNA inserts of approx. 13 kb and 15 kb respectively, were tentatively identified to possess the LDH-B gene, since their genomic DNAs, but not LDH-B cDNA, were found to be cleaved by *Eco*RI at the presumptive introns (Fig. 1). The exons of LDH-B gene were identified by comparing nucleotide sequences of genomic clones with that of LDH-B cDNA [11]. Genomic clone hB501 contains protein-coding exons nos. 2, 3, 4, 5 and 6; clone hB502 possesses an additional exon 7 of C-terminal coding sequence and 3' non-coding region.

In order to isolate the genomic clone containing the promoter region, exon 0 and exon 1 of human LDH-B gene, the human LDH-B cDNA of the 5' non-coding region and the coding sequence for the N-terminal 56 residues was used as probe. Several positive genomic clones were identified and characterized. In addition to clones hB501 and hB502, a new genomic clone hB503, containing a DNA insert of approx. 21 kb, was isolated, and its *Eco*RI DNA fragments of 1.8 kb, 1.4 kb, 0.4 kb and 0.3 kb, as well as region containing exon 1, were sequenced and ordered (Fig. 1). This clone hB503 was

found to possess putative promoter, exon 0 (5' non-coding sequence) and exon 1.

The nucleotide sequences of the putative promoter, protein-coding region, 5' and 3' non-coding regions as well as the first intron are given in Fig. 2. The protein-coding sequence of human LDH-B gene is interrupted by six introns at codons nos. 42–43, 82, 140, 198, 237 and 278–279. The positions of these introns are homologous to those of the LDH-A genes from man and mouse [12,13], and correspond to random-coil regions or are near ends of secondary structures on the surface of monomeric LDH subunit. The 5' non-coding region of human LDH-B gene is interrupted by an intron at six nucleotide residues upstream of the ATG translation-initiation site. The human and mouse LDH-A genes are interrupted by an intron at 24 nucleotide residues 5' to the ATG initiation codon. As is the case of human and mouse LDH-A genes, the human LDH-B gene has no intron in the 3' non-coding sequence, and the poly(A)-addition site is located at 18 nucleotide residues downstream of the AATAAA signal [20]. The exon-intron junctions present in human LDH-B gene appear to follow the consensus splicing sequence of G-T at 5' donor site and A-G at 3' acceptor site [21,22]. The nucleotide sequence of eight exons determined from human genomic DNA clones was found to be identical with that of human LDH-B cDNA [11].

The determined sequence of 1418 nucleotide residues upstream of exon 0 present in genomic clone hB503 may contain the putative promoter of human LDH-B gene (approx. 25 kb in length), although the promoter activity and the exact transcription-initiation site remain to be determined. A preliminary analysis of the putative LDH-B promoter sequence indicates the absence of a regulatory sequence like the known promoter sequence of mouse LDH-A gene, which contains a cyclic AMP-responsive element [23]. These results are consistent with the observation that the expression of the gene for LDH-A (but not LDH-B) is induced by cyclic AMP [24,25]. The information on the structure of human LDH-A and LDH-B genes will facilitate the molecular characterization of genetic mutations affecting the expression of human LDH-A and LDH-B genes, including enzyme deficiencies [26].

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