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Research Article

A defect in the E1 beta subunit of the branched chain alpha-ketoacid dehydrogenase (BCKDH) complex is one cause of maple syrup urine disease (MSUD). In an attempt to elucidate the molecular basis of MSUD, we isolated and characterized a 1.35 kbp cDNA clone encoding the entire precursor of the E1 beta subunit of BCKDH complex from a human placental cDNA library. Nucleotide sequence analysis revealed that the isolated cDNA clone (lambda hBE1 beta-1) contained a 5'-untranslated sequence of four nucleotides, the translated sequence of 1,176 nucleotides and the 3'-untranslated sequence of 169 nucleotides. Comparison of the amino acid sequence predicted from the nucleotide sequence of the cDNA insert of the clone with the NH2-terminal amino acid sequence of the purified mature bovine BCKDH-E1 beta subunit showed that the cDNA insert encodes for a 342-amino acid subunit with a Mr = 37,585. The subunit is synthesized as the precursor with a leader sequence of 50 amino acids and is processed at the NH2 terminus. A search for protein homology revealed that the primary structure of human BCKDH-E1 beta was similar to the bovine BCKDH-E1 beta and to the E1 beta subunit of human pyruvate dehydrogenase complex, in all regions. The structures and functions of mammalian alpha-ketoacid dehydrogenase complexes are apparently highly conserved. Genomic DNA from lymphoblastoid cell lines derived from normal […]

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Maple Syrup Urine Disease

Complete Primary Structure of the $E_1\beta$ Subunit of Human Branched Chain α-Ketoacid Dehydrogenase Complex Deduced from the Nucleotide Sequence and a Gene Analysis of Patients with This Disease

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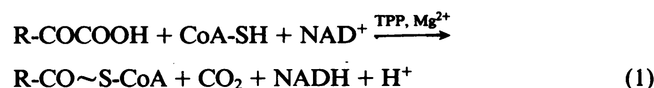
Abstract

A defect in the $E_1\beta$ subunit of the branched chain α-ketoacid dehydrogenase (BCKDH) complex is one cause of maple syrup urine disease (MSUD). In an attempt to elucidate the molecular basis of MSUD, we isolated and characterized a 1.35 kbp cDNA clone encoding the entire precursor of the $E_1\beta$ subunit of BCKDH complex from a human placental cDNA library. Nucleotide sequence analysis revealed that the isolated cDNA clone ($\lambda hBE_1\beta$-1) contained a 5′-untranslated sequence of four nucleotides, the translated sequence of 1,176 nucleotides and the 3′-untranslated sequence of 169 nucleotides. Comparison of the amino acid sequence predicted from the nucleotide sequence of the cDNA insert of the clone with the NH_2-terminal amino acid sequence of the purified mature bovine BCKDH-$E_1\beta$ subunit showed that the cDNA insert encodes for a 342-amino acid subunit with a M_r = 37,585. The subunit is synthesized as the precursor with a leader sequence of 50 amino acids and is processed at the NH_2 terminus. A search for protein homology revealed that the primary structure of human BCKDH-$E_1\beta$ was similar to the bovine BCKDH-$E_1\beta$ and to the $E_1\beta$ subunit of human pyruvate dehydrogenase complex, in all regions. The structures and functions of mammalian α-ketoacid dehydrogenase complexes are apparently highly conserved. Genomic DNA from lymphoblastoid cell lines derived from normal and five MSUD patients, in whom $E_1\beta$ was not detected by immunoblot analysis, gave the same restriction maps on Southern blot analysis. The gene has at least 80 kbp. (*J. Clin. Invest.* 1990. 86:242–247.) Key words: maple syrup urine disease • branched chain α-ketoacid dehydrogenase • complementary DNA • cloning • gene analysis

Introduction

Mammalian branched chain α-ketoacid dehydrogenase (BCKDH)[1] (EC 1,2,4,4) is a mitochondrial multienzyme complex catalyzing the oxidative decarboxylation of branched chain α-ketoacids derived from amino acids such as valine, leucine, and isoleucine (reaction [1]).



$$\text{R-COCOOH} + \text{CoA-SH} + \text{NAD}^+ \xrightarrow{\text{TPP, Mg}^{2+}} \text{R-CO}\sim\text{S-CoA} + CO_2 + \text{NADH} + \text{H}^+ \quad (1)$$

The BCKDH complex consists of three catalytic components: branched chain α-ketoacid decarboxylase (E_1), dihydrolipoyl transacylase (E_2), and dihydrolipoamide dehydrogenase (E_3). E_1 is further composed of two subunits, $E_1\alpha$ and $E_1\beta$ (1–3). E_1 and E_2 components are specific to BCKDH. On the other hand, the E_3 component is common among the three ketoacid dehydrogenase complexes, BCKDH, pyruvate dehydrogenase (PDH), and α-ketoglutarate dehydrogenase (4, 5). The BCKDH complex also contains two specific regulatory enzymes, a kinase (6–8) and a phosphatase (9, 10), responsible for regulation of the catalytic activity through phosphorylation and dephosphorylation.

$E_1\alpha$ is the catalytic subunit phosphorylated at two serine residues responsible for regulation of the catalytic activity by covalent modification (11–13). The function of $E_1\beta$ is unknown (5). E_2 catalyzes the transfer of the acyl group from the lipoyl moiety to coenzyme A and forms the structural core of the enzyme complex. To this E_1, E_3, kinase, and phosphatase are bound through noncovalent interactions (1, 10, 14).

Lack of BCKDH activity leads to maple syrup urine disease (MSUD), an autosomal recessive inborn error of metabolism (4). Several different phenotypes of MSUD have been elucidated on the basis of clinical features, as follows: classical, intermittent, intermediate, thiamine responsive type, $E_1\beta$ deficiency, E_2 deficiency, and E_3 deficiency (4). Etiology of MSUD is heterogenous, as mutations in different regions of any of the BCKDH proteins could lead to decreased functions of the entire complex.

We reported that a defect in the BCKDH-$E_1\beta$ subunit is one cause of MSUD (15, 16). The isolation and characterization of cDNAs encoding all or a part of the human BCKDH-$E_1\alpha$ (17, 18), BCKDH-E_2 component (19–22), and E_3 component (23, 24) have been reported but the primary structure of human BCKDH-$E_1\beta$ has not and the function of this subunit is not well understood. Molecular cloning of BCKDH-$E_1\beta$ had not been successful, despite repeated attempts. Most recently, we isolated a cDNA clone corresponding to bovine BCKDH-$E_1\beta$ (25). In an attempt to elucidate the molecular basis of the disease, we carried out cDNA cloning of the human BCKDH-$E_1\beta$. The nucleotide sequence and the primary structure of human BCKDH-$E_1\beta$ has to be determined to analyze the alterations seen in studies on MSUD patients. We report herein isolation and characterization of a 1.35-kbp cDNA clone that encodes the complete human BCKDH-$E_1\beta$ precursor. We also made a Southern blot analysis of the BCKDH-$E_1\beta$ gene in

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1. *Abbreviations used in this paper:* BCKDH, branched chain α-ketoacid dehydrogenase; MSUD, maple syrup urine disease; PDH, pyruvate dehydrogenase.

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Epstein-Barr virus transformed lymphoblastoid cell lines derived from five nonrelated MSUD patients. BCKDH-$E_1\beta$ was not detected by immunoblot analysis.

Methods

Materials. Restriction enzymes, pUC18 vector DNAs, 7-DEAZA Sequencing Kits, and random primer labeling kits were purchased from Takara Shuzo Co. (Kyoto, Japan). Nitrocellulose hybridization membranes were from Schleicher & Schuell, Inc. (Kassel, Federal Republic of Germany). [α-^{32}P]dCTP (specific activity, 3,000 Ci/mmol) were from Amersham Corp. (Arlington Heights, IL).

Isolation of cDNA. The human placental cDNA library constructed in λgt11 (26) was kindly provided by Dr. J. E. Sadler (Howard Hughes Medical Institute, Washington University School of Medicine). The 1.7-kbp cDNA clone (λb$E_1\beta$-2) for bovine BCKDH-$E_1\beta$ was isolated using the mixture of synthetic oligonucleotides (17 mer, 24 mixture) as a probe for a bovine liver cDNA library constructed in λgt11 (25). Approximately 6×10^5 recombinant phage plaques were screened from the human placental cDNA library using the 1.7-kbp cDNA clone (λb$E_1\beta$-2) as a probe. The insert was labeled with [α-^{32}P] dCTP (3,000 Ci/mmol) using the random primer labeling kit. Prehybridization, hybridization, and washing of nitrocellulose filters were as described (27). Hybridizing plaques detected by autoradiography were picked up from the mother agar plate. Successive screenings were carried out, using fewer and fewer plaques at each step until well-isolated phage plaques had been cloned.

Restriction endonuclease map and nucleotide sequence analysis. Recombinant phage DNA was prepared as described (28). Eco RI-excised cDNA inserts were subcloned into plasmid vector pUC18 and characterized by restriction endonuclease mapping. Restriction fragments were subcloned into pUC18 for sequencing. In addition, ordered serial deletions from the 5′ → 3′ end of both strands of the pUC18 insert were produced with exonuclease III/mung bean nuclease for sequencing (29). DNA sequencing was performed by the dideoxy chain termination method (30) using an alkali-denatured plasmid as the template (31).

Protein data base search. Homologous amino acid sequences were sought in the GenBank (Release 59.0)/EMBL (Release 18.0) protein data base on a VAX computor using the Wordsearch program (32) (Version 6.0, April 1989). The Segments program was used for the alignment procedure.

Cell lines and cell culture. Lymphoblastoid cell lines derived from disease-free control male, and MSUD patients were established by Epstein-Barr virus-infected transformation of peripheral blood B lymphocytes (33). Kinetic studies on BCKDH activities and immunoblot analysis of BCKDH proteins of these cell lines from MSUD patients (K.Y., Y.T., E.K., T.Ho., Y.O.) have been reported (16). Lymphoblastoid cells were grown in RPMI 1640 medium containing penicillin (100 IU/ml) and streptomycin (100 μg/ml) supplemented with 10% FCS in an incubator at 37°C. A subculture and a harvest were performed as described (15).

Southern blot analysis of genomic DNA. Total human genomic DNA was prepared from the lymphoblastoid cell lines, as described (34). Southern blotting was performed with 5.0–7.5 μg of total genomic DNA. DNA was cut by restriction enzymes Bam HI, Eco RI, and Hind III, and subjected to electrophoresis on an 0.8% agarose gel for 14–20 h at 40 V. Southern blot hybridization was principally carried out according to the description of Southern (35). The cDNA insert (λhB$E_1\beta$-1) was radiolabeled using a random primer labeling kit and [α-^{32}P] dCTP (3,000 Ci/mmol). The filters were prehybridized for 3 h in 50% Formamide, 5× SSC, 100 μg/ml salmon testis DNA, 50 mM sodium phosphate (pH 6.5), 0.1% SDS, and 10× Denhardt's solution (1× Denhardt's = 0.02% polyvinylpyrrolidone, 0.02% BSA, 0.02% Ficoll). Hybridization was for 24 h in prehybridization buffer containing the labeled cDNA insert. The filters were washed for 1 h at 56°C in 0.1× SSC and 0.1% SDS with two to three changes. Nitrocellulose membrane filters were exposed to film with intensifying screens at −76°C for 3–5 d.

Results and Discussion

To isolate the human BCKDH-$E_1\beta$ cDNA clone, we initially screened ~ 1×10^6 recombinant phage plaques of a human placental cDNA library constructed in λgt11 (26), with a specific rabbit antibody raised against bovine BCKDH-$E_1\beta$, and obtained five positive clones. All were found to be false positive by nucleotide sequencing.

We then isolated and characterized a cDNA clone coding for bovine BCKDH-$E_1\beta$ from a bovine liver cDNA library constructed in λgt11 by screening with a mixture of synthetic oligonucleotide probes corresponding to the COOH-terminal 5-residue sequence of the bovine BCKDH-$E_1\beta$ (25). Using this 1.7-kbp Eco RI fragment of bovine BCKDH-$E_1\beta$ cDNA insert (λb$E_1\beta$-2) as a probe, ~ 6×10^5 plaque-forming units were screened from a human placental cDNA library. Only one positive clone (λhB$E_1\beta$-1) was plaque purified to homogeneity through five successive rounds of screening, and the cDNA inserts were subcloned into the Eco RI site of pUC18 for further characterization. This recombinant phage clone insert had 1.35 kbp.

The restriction endonuclease map of a cDNA insert from the phage clone λhB$E_1\beta$-1 and sequencing strategy for the insert are shown in Fig. 1. The nucleotide sequence and deduced amino acid sequence are shown in Fig. 2. The λhB$E_1\beta$-1 insert is composed of 1,349 bp consisting of a 4-bp 5′-untranslated sequence, a 1,176-bp-long open reading frame, and a 169-bp 3′-untranslated sequence. The nucleotide sequence surrounding the putative initiator codon, GGGGAUGG, is rather different from the consensus sequence of CC$^{A}_{G}$CCAUGG (36). A polyadenylation signal of the type AATAAT (37) is found 7 bp upstream of a poly (A) tail.

The open reading frame could be translated into a 392–amino acid residue protein. Comparison of the amino acid sequence predicted from the nucleotide sequence of the clone cDNA insert with the NH_2-terminal amino acid sequence of purified bovine BCKDH-$E_1\beta$ determined by Edman degradation (25) revealed that the NH_2-terminal 50–amino acid residues of the putative precursor protein are missing the mature $E_1\beta$. BCKDH-$E_1\beta$ is a nuclear encoded mitochondrial protein, the precursor of which seems to contain a leader sequence of 50 amino acid residues. Comparison of the putative $E_1\beta$ leader sequence (negatively numbered amino acid residues in Fig. 2) with those of other mitochondrial proteins revealed a number of common features. The putative $E_1\beta$ leader sequence con-

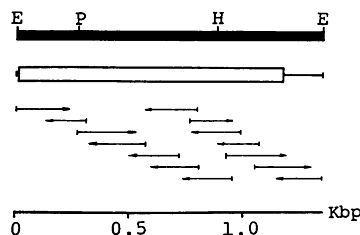

Figure 1. Restriction map and sequencing strategy for human BCKDH-$E_1\beta$ cDNA. The open box and the line depict coding and noncoding regions, respectively. Restriction sites are indicated above the λhB$E_1\beta$-1 insert (1,349 bp) at the top of the figure, with restriction enzymes used: Eco RI (*E*), Pst I (*P*), Hind III (*H*). Solid horizontal arrows indicate orientation and region of sequencing.

```
                                                                                      -4 GGGG

ATGGCGGTTGTAGCGGCGGCTGCCGGCTGGCTACTCAGGCTCAGGGCGGCAGGGGCTGAGGGGCACTGGCGTCGGCTTCCTGGCGCGGGG      90
MetAlaValValAlaAlaAlaAlaGlyTrpLeuLeuArgLeuArgAlaAlaGlyAlaGluGlyHisTrpArgArgLeuProGlyAlaGly
-50                            -40                            -30

CTGGCGCGGGGCTTTTTGCACCCCGCCGCGACTGTCGAGGATGCGGCCCAGAGGCGGCAGGTGGCTCATTTTACTTTCCAGCCAGATCCG     180
LeuAlaArgGlyPheLeuHisProAlaAlaThrValGluAspAlaAlaGlnArgArgGlnValAlaHisPheThrPheGlnProAspPro
-20                            -10                            1                            10

GAGCCCCGGGAGTACGGGCAAACTCAGAAAATGAATCTTTTCCAGTCTGTAACAAGTGCCTTGGATAACTCATTGGCCAAAGATCCTACT     270
GluProArgGluTyrGlyGlnThrGlnLysMetAsnLeuPheGlnSerValThrSerAlaLeuAspAsnSerLeuAlaLysAspProThr
                               20                             30                           40

GCAGTAATATTTGGTGAAGATGTTGCCTTTGGTGGAGTCTTTAGATGCACTGTTGGCTTGCGAGACAAATATGGAAAAGATAGAGTTTTT     360
AlaValIlePheGlyGluAspValAlaPheGlyGlyValPheArgCysThrValGlyLeuArgAspLysTyrGlyLysAspArgValPhe
                               50                             60                           70

AATACCCCATTGTGTGAACAAGGAATTGTTGGATTTGGAATCGGAATTGCGGTCACTGGAGCTACTGCCATTGCGGAAATTCAGTTTGCA     450
AsnThrProLeuCysGluGlnGlyIleValGlyPheGlyIleGlyIleAlaValThrGlyAlaThrAlaIleAlaGluIleGlnPheAla
                               80                             90                           100

GATTATATTTTCCCTGCATTTGATCAGATTGTTAATGAAGCTGCCAAGTATCGCTATCGCTCTGGGGATCTTTTTAACTGTGGAAGCCTC     540
AspTyrIlePheProAlaPheAspGlnIleValAsnGluAlaAlaLysTyrArgTyrArgSerGlyAspLeuPheAsnCysGlySerLeu
                               110                            120                          130

ACTATCCGGTCCCCTTGGGGCTGTGTTGGTCATGGGGCTCTCTATCATTCTCAGAGTCCTGAAGCATTTTTTGCCCATTGCCCAGGAATC     630
ThrIleArgSerProTrpGlyCysValGlyHisGlyAlaLeuTyrHisSerGlnSerProGluAlaPhePheAlaHisCysProGlyIle
                               140                            150                          160

AAGGTGGTTATACCCAGAAGCCCTTTCCAGGCCAAAGGACTTCTTTTGTCATGCATAGAGGATAAAAATCCTTGTATATTTTTTGAACCT     720
LysValValIleProArgSerProPheGlnAlaLysGlyLeuLeuLeuSerCysIleGluAspLysAsnProCysIlePhePheGluPro
                               170                            180                          190

AAAATACTTTACAGGGCAGCAGCGGAAGAAGTCCCTATAGAACCATACAACATCCCACTGTCCCAGGCCGAAGTCATACAGGAAGGGAGT     810
LysIleLeuTyrArgAlaAlaAlaGluGluValProIleGluProTyrAsnIleProLeuSerGlnAlaGluValIleGlnGluGlySer
                               200                            210                          220

GATGTTACTCTAGTTGCCTGGGGCACTCAGGTTCATGTGATCCGAGAGGTAGCTTCCATGGCAAAAGAAAAGCTTGGAGTGTCTTGTGAA     900
AspValThrLeuValAlaTrpGlyThrGlnValHisValIleArgGluValAlaSerMetAlaLysGluLysLeuGlyValSerCysGlu
                               230                            240                          250

GTCATTGATCTGAGGACTATAATACCTTGGGATGTGGACACAATTTGTAAGTCTGTGATCAAAACAGGGCGACTGCTAATCAGTCACGAG     990
ValIleAspLeuArgThrIleIleProTrpAspValAspThrIleCysLysSerValIleLysThrGlyArgLeuLeuIleSerHisGlu
                               260                            270                          280

GCTCCCTTGACAGGCGGCTTTGCATCGGAAATCAGCTCTACAGTTCAGGAGGAATGTTTCTTGAACCTAGAGGCTCCTATATCAAGAGTA    1080
AlaProLeuThrGlyGlyPheAlaSerGluIleSerSerThrValGlnGluGluCysPheLeuAsnLeuGluAlaProIleSerArgVal
                               290                            300                          310

TGTGGTTATGACACACCATTTCCTCACATTTTTGAACCATTCTACATCCCAGACAAATGGAAGTGTTATGATGCCCTTCGAAAAATGATC    1170
CysGlyTyrAspThrProPheProHisIlePheGluProPheTyrIleProAspLysTrpLysCysTyrAspAlaLeuArgLysMetIle
                               320                            330                          340

AACTATTGACCATATAGGTAGGTATGCATCTTGAGAAAGCTACTATGTGCCCCTGACATTAACGTACTGTTAACCAAGACACAGCAATCA    1260
AsnTyr***

TCAGTGTTTTGATGGTAACAAACTTTGATGGTAAAGTTGATAAAAGGCAACTTTCAGAAGAAAATAATGTGCTTTAAAAAAAAAA        1345
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Figure 2. Nucleotide sequence of the λhBE$_1$β-1 insert and deduced amino acid sequence of the human BCKDH-E$_1$β precursor. Numbers on the far right correspond to ordinate of the last nucleotide in each row. Nucleotides are numbered in the 5′ → 3′ direction, beginning with the first residue of the ATG triplet encoding the putative initiator methionine. Numbers below the amino acid sequence refer to residues beginning with the NH$_2$ terminus of the mature protein deduced from the purified bovine BCKDH-E$_1$β determined by Edman degradation (25). There is a double underline at the polydenylation signal of the type AATAAT (37).

tains periodically spaced basic amino acids rich in Leu and Arg and has few acidic residues (only one Asp at residue −7). These findings are compatible with those proposed for the leader sequence of mitochondrial targetting enzymes (38, 39). On the basis of these findings, the molecular mass of the E$_1$β precursor is estimated to be 43,130 and that of the mature E$_1$β is 37,585, in good agreement with the 37,000 estimated by immunoblot analysis (15, 16).

A protein homology search revealed that the primary structure of human BCKDH-E$_1$β is similar to the human PDH-E$_1$β (40), in all regions. Fig. 3 depicts the alignment of homologous regions of human BCKDH-E$_1$β, bovine BCKDH-E$_1$β, and human PDH-E$_1$β. 98% of the amino acid residues of human BCKDH-E$_1$β are identical to bovine BCKDH-E$_1$β and 33% of the amino acid residues of human BCKDH-E$_1$β are identical to the corresponding residues of

```
Bovine BCKDH   1  VAHFTFQPDPEPVEYGQTQKMNLFQAVTSALDNSLAKDPTAVIFGEDVA-FGGVFRCTVG  59
Human  BCKDH   1  VAHFTFQPDPEPREYGQTQKMNLFQSVTSALDNSLAKDPTAVIFGEDVA-FGGVFRCTVG  59
Human  PDH    24  --HWT---APAAVQ------VTVRDAINQGMDEELERDEKVFLLGEEVAQYDGAYKVSRG  72

Bovine BCKDH  60  LRDKYGKDRVFNTPLCEQGIVGFGIGIAVTGATAIAEIQFADYIFPAFDQIVNEAAKYRY 119
Human  BCKDH  60  LRDKYGKDRVFNTPLCEQGIVGFGIGIAVTGATAIAEIQFADYIFPAFDQIVNEAAKYRY 119
Human  PDH    73  LWKKYGDKRIIDTPISEMGFAGIAVGAAMAGLRPICEFMTFNFSMQAIDQVINSAAKTYY 132

Bovine BCKDH 120  RSGDLFNCGSLTIRSPWGCVGHGALYHSQSPQAFFAHCPGIKVVVPRSPFQAKGLLLSCI 179
Human  BCKDH 120  RSGDLFNCGSLTIRSPWGCVGHGALYHSQSPEAFFAHCPGIKVVVPRSPFQAKGLLLSCI 179
Human  PDH   133  MSGGL-QPVPIVFRGPNGASAGVAAQHSQCFAAWYGHCPGLKVVSPWNSEDAKGLIKSAI 191

Bovine BCKDH 180  EDKNPCIFFEPKILYRAAVE---QVPVEPYNIPLSQAEVIQEGSDVTLVAWGTQV-HVIR 235
Human  BCKDH 180  EDKNPCIFFEPKILYRAAAE---EVPIEPYNIPLSQAEVIQEGSDVTLVAWGTQV-HVIR 235
Human  PDH   192  RKNNPVVVLENELMYGVPFEFLRKLSQKILLIPIGKAKIERQGTHITVVSHSRPVGHCLE 251

Bovine BCKDH 236  EVDAMAQEKLGVSCEVIDLRTILPWDVDTVCKSVIKTGRLLVSHEAPLTG-GFASEISST 294
Human  BCKDH 236  EVDAMAQEKLGVSCEVIDLRTIIPWDVDTICKSVIKTGRLLISHEAPLTG-GFASEISST 294
Human  PDH   252  AAAVLSKE--GVECEVINMRTIRPMDMETIFASVMKTNH-LVTVEGGWPQFGVGAEICAR 308

Bovine BCKDH 295  VQEECFLN-LEAPISRVCGYDTPFPH--IFEPFYIPDKWKCYDALRKMINY 342
Human  BCKDH 295  VQEECFLN-LEAPISRVCGYDTPFPH--IFEPFYIPDKWKCYDALRKMINY 342
Human  PDH   309  INGSPAFNFLDAPAVRVTGADVPMPYAKILEDNSIPQVKDIIFAIKKTLNI 359
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Figure 3. Comparison of amino acid sequences of human BCKDH-$E_1\beta$ (present study), bovine BCKDH-$E_1\beta$ (25), and human PDH-$E_1\beta$ (40).

PDH-$E_1\beta$; this similarity increased to 82% if conservative amino acid substitutions are taken into account. It has been demonstrated that mammalian α-ketoacid dehydrogenase complexes such as PDH, BCKDH, and α-ketoglutarate dehydrogenase are functionally and structurally similar (4, 5). The amino acid sequence of the mammalian BCKDH-$E_1\alpha$ is highly homologous to that of mammalian PDH-$E_1\alpha$ (17); the same is true of BCKDH-E_2 (19–21). The present study showed that human BCKDH-$E_1\beta$ is also similarly homologous to human PDH-$E_1\beta$. Extensive homologies between BCKDH-

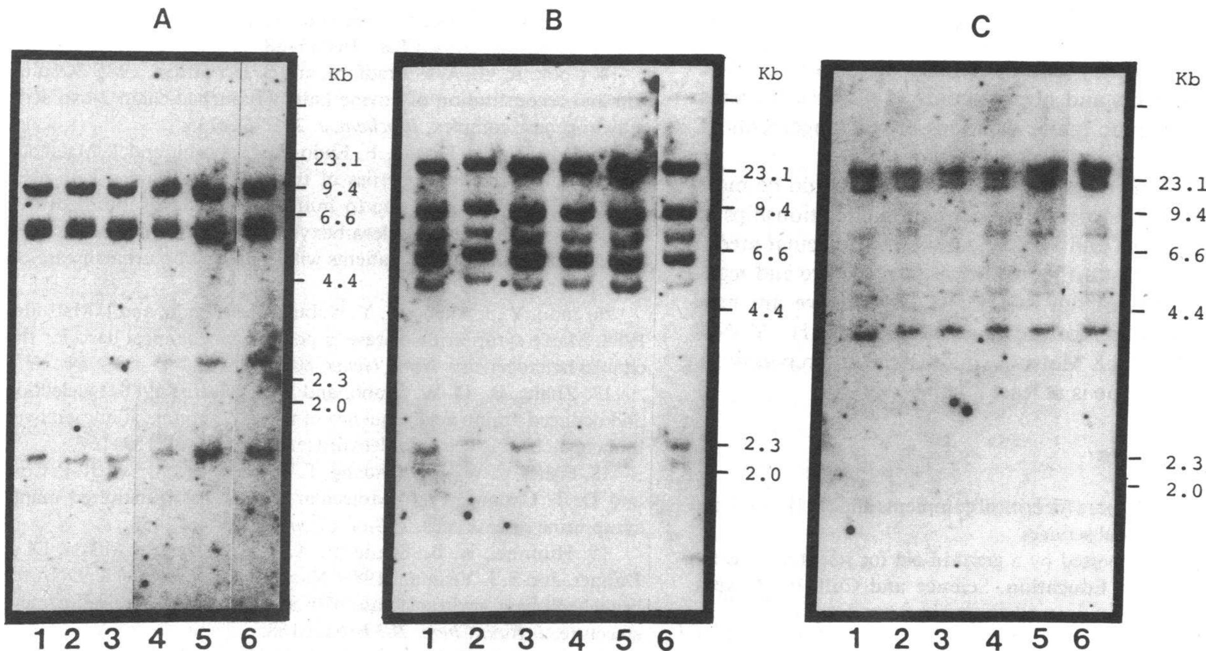

Figure 4. Southern blot analysis of genomic DNA from the MSUD patient in whom CRM for BCKDH-$E_1\beta$ are absent. (*A*) Eco RI–digested genomic DNA. Lane *1*, disease-free control cell line; lanes *2–6*, cell lines from MSUD patients (K.Y., Y.T., E.K., T.Ho., and Y.O.). Filters were probed with labeled human BCKDH-$E_1\beta$ cDNA ($\lambda hBE_1\beta$-1). (*B*) Hind III–digested DNA from individuals as in *A*, hybridized with labeled human BCKDH-$E_1\beta$ cDNA ($\lambda hBE_1\beta$-1). (*C*) Bam HI–digested DNA from individuals as in *A*, hybridized with labeled human BCKDH-$E_1\beta$ cDNA ($\lambda hBE_1\beta$-1). The BCKDH-$E_1\beta$ gene is estimated to be at least 80 kbp long as based on data obtained by cloning of the genomic DNA.

$E_1\beta$ and PDH-$E_1\beta$ throughout the primary structure suggest that the secondary structure, tertiary structure, and function are also similar. Furthermore BCKDH-$E_1\beta$ and PDH-$E_1\beta$ may possibly arise from a common ancestoral gene, although the function of $E_1\beta$ has yet to be determined (5). A highly conserved structure of the $E_1\beta$-subunit of 2-oxo acid dehydrogenases of mammals suggests that the $E_1\beta$-subunit of these enzymes no doubt plays important roles in enzyme activities.

A defect in the BCKDH complex results in MSUD (4). The etiology of this disease is heterogenous as mutations in different regions of any of the BCKDH proteins could lead to decreased functions of the entire complex. Recently, a defect of E_2 (15, 16, 18, 41, 42), a defect of $E_1\beta$ (15, 16, 18), mutation of $E_1\alpha$ (43), and loss of $E_1\alpha$ and $E_1\beta$ subunits (18) have been detected in MSUD patients.

We analyzed the biochemical basis for clinical heterogeneity in MSUD and noted a defect in $E_1\beta$ as one cause of the disease. In these cases, immunologically cross-reactive material corresponding to BCKDH-$E_1\beta$ was not detected. $E_1\beta$ is associated with $E_1\alpha$ by noncovalent binding and the E_1 subunit is attached to E_2. Thus, the absence of $E_1\beta$ theoretically could result from abnormalities in structures of $E_1\alpha$ or E_2. The absence of $E_1\beta$ might not indicate a genetic abnormality in the $E_1\beta$ gene. A detailed analysis of $E_1\beta$ deficiency suggested that the mutations might be heterogenous (15, 16). The $E_1\beta$ deficiency will have to be analyzed at the gene level.

To elucidate the molecular mechanism of $E_1\beta$ deficiency, we first analyzed the expression of $E_1\beta$ transcript in lymphoblastoid cell lines in which $E_1\beta$ was not detected by immunoblot analysis (15, 16). The $E_1\beta$ transcript was not clearly defined by Northern blot analysis using poly $(A)^+$ RNA (10–15 μg), even in normal control lymphoblastoid cell lines (data not shown). The quantity of the $E_1\beta$ transcript may be low or the transcript may readily degrade, a proposal based on our finding that we could obtain only one clone when screening the cDNA-library using the nucleotide probe ($\lambda bE_1\beta$-2), as described above.

We also analyzed by Southern blots the genomic DNA from MSUD patients and normal controls (Fig. 4). It seems likely that there are no major deletions or rearrangements of the BCKDH-$E_1\beta$ gene in these patients.

The cDNA clone of human BCKDH-$E_1\beta$ should be most useful for examining structural and functional relationships of the BCKDH complex and for elucidating the molecular mechanisms of MSUD. To further examine the structure and regulatory mechanisms of the BCKDH-$E_1\beta$ gene, we are now cloning genomic DNA for the gene (Mitsubuchi, H., Y. Nobukuni, F. Endo, and I. Matsuda, manuscript in preparation). The BCKDH-$E_1\beta$ gene is at least 80 kbp long.

Acknowledgments

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References

1. Pettit, F. H., S. J. Yeaman, and L. J. Reed. 1978. Purification and characterization of branched chain α-keto acid dehydrogenase complex of bovine kidney. *Proc. Natl. Acad. Sci. USA.* 75:4881–4885.

2. Danner, D. J., S. K. Lemmon, J. C. Besharse, and L. J. Elsas, II. 1979. Purification and characterization of branched chain α-ketoacid dehydrogenase from bovine liver mitochondria. *J. Biol. Chem.* 254:5522–5526.

3. Heffelfinger, S. C., E. T. Sewell, and D. J. Danner. 1983. Identification of specific subunits of highly purified bovine liver branched-chain ketoacid dehydrogenase. *Biochemistry.* 22:5519–5522.

4. Danner, D. J., and L. J. Elsas, II. 1989. Disorders of branched chain amino acid and keto acid metabolism. *In* The Metabolic Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill Information Services Company, New York. 671–692.

5. Yeaman, S. J. 1989. The 2-oxo acid dehydrogenase complexes: recent advances. *Biochem. J.* 257:625–632.

6. Odessey, R. 1982. Purification of rat kidney branched-chain oxo acid dehydrogenase complex with endogenous kinase activity. *Biochem. J.* 204:353–356.

7. Lau, K. S., H. R. Fatania, and P. J. Randle. 1982. Regulation of the branched chain 2-oxoacid dehydrogenase kinase reaction. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 144:57–62.

8. Paxton, R., and R. A. Harris. 1982. Isolation of rabbit liver branched chain α-ketoacid dehydrogenase and regulation by phosphorylation. *J. Biol. Chem.* 257:14433–14439.

9. Fatania, H. R., P. A. Patston, and P. J. Randle. 1983. Dephosphorylation and reactivation of phosphorylated purified ox-kidney branched-chain dehydrogenase complex by co-purified phosphatase. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 158:234–238.

10. Damuni, Z., M. L. Merryfield, J. S. Humphreys, and L. J. Reed. 1984. Purification and properties of branched-chain α-keto acid dehydrogenase phosphatase from bovine kidney. *Proc. Natl. Acad. Sci. USA.* 81:4335–4338.

11. Cook, K. G., A. P. Bradford, S. J. Yeaman, A. Aitken, I. M. Fearnley, and J. E. Walker. 1984. Regulation of bovine kidney branched-chain 2-oxoacid dehydrogenase complex by reversible phosphorylation. *Eur. J. Biochem.* 145:587–591.

12. Lau, K. S., C. E. Phillips, and P. J. Randle. 1983. Multi-site phosphorylation in ox-kidney branched-chain 2-oxoacid dehydrogenase complex. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 160:149–152.

13. Cook, K. G., R. Lawson, and S. J. Yeaman. 1983. Multi-site phosphorylation of branched-chain 2-oxoacid dehydrogenase complex within mitochondria isolated from rat liver, kidney and heart. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 164:85–88.

14. Cook, K. G., A. P. Bradford, and S. J. Yeaman. 1985. Resolution and reconstitution of bovine kidney branched-chain 2-oxo acid dehydrogenase complex. *Biochem. J.* 225:731–735.

15. Indo, Y., A. Kitano, F. Endo, I. Akaboshi, and I. Matsuda. 1987. Altered kinetic properties of the branched-chain α-keto acid dehydrogenase complex due to mutation of the β-subunit of the branched-chain α-keto acid decarboxylase (E_1) component in lymphoblastoid cells derived from patients with maple syrup urine disease. *J. Clin. Invest.* 80:63–70.

16. Indo, Y., I. Akaboshi, Y. Nobukuni, F. Endo, and I. Matsuda. 1988. Maple syrup urine disease: a possible biochemical basis for the clinical heterogeneity. *Hum. Genet.* 80:6–10.

17. Zhang, B., D. W. Crabb, and R. A. Harris. 1988. Nucleotide and deduced amino acid sequence of the $E1\alpha$ subunit of human liver branched-chain α-ketoacid dehydrogenase. *Gene.* 69:159–164.

18. Fisher, C. W., J. L. Chuang, T. A. Griffin, K. S. Lau, R. P. Cox, and D. T. Chuang. 1989. Molecular phenotypes in cultured maple syrup urine disease cells. *J. Biol. Chem.* 264:3448–3453.

19. Hummel, K. B., S. Litwer, A. P. Bradford, A. Aitken, D. J. Danner, and S. J. Yeaman. 1988. Nucleotide sequence of a cDNA for branched chain acyltransferase with analysis of the deduced protein structure. *J. Biol. Chem.* 263:6165–6168.

20. Lau, K. S., T. A. Griffin, C.-W. C. Hu, and D. T. Chuang. 1988. Conservation of primary structure in the lipoyl-bearing and dihydrolipoyl dehydrogenase binding domains of mammalian branched-chain α-keto acid dehydrogenase complex: molecular cloning of human and bovine transacylase (E2) cDNAs. *Biochemistry.* 27:1972–1981.

21. Danner, D. J., S. Litwer, W. J. Herring, and J. Pruckler. 1989.

Construction and nucleotide sequence of a cDNA encoding the full-length preprotein for human branched chain acyltransferase. *J. Biol. Chem.* 264:7742–7746.

22. Nobukuni, Y., H. Mitsubuchi, F. Endo, and I. Matsuda. 1989. Complete primary structure of the transacylase (E2b) subunit of the human branched chain α-keto acid dehydrogenase complex. *Biochem. Biophys. Res. Commun.* 161:1035–1041.

23. Otulakowski, G., and B. H. Robinson. 1987. Isolation and sequence determination of cDNA clones for porcine and human lipoamide dehydrogenase. *J. Biol. Chem.* 262:17313–17318.

24. Pons, G., C. Raefsky-Estrin, D. J. Carothers, R. A. Pepin, A. A. Javed, B. W. Jesse, M. K. Ganapathi, D. Samols, and M. S. Patel. 1988. Cloning and cDNA sequence of the dihydrolipoamide dehydrogenase component of human α-detoacid dehydrogease complexes. *Proc. Natl. Acad. Sci. USA.* 85:1422–1426.

25. Nobukuni, Y., H. Mitsubuchi, F. Endo, J. Asaka, R. Oyama, K. Titani, and I. Matsuda. 1990. Isolation and characterization of a complementary DNA clone coding for the $E_1\beta$ subunit of bovine branched-chain α-ketoacid dehydrogenase complex: complete amino acid sequence of the precursor protein and its proteolytic processing. *Biochemistry.* 29:1154–1160.

26. Ye, R. D., T.-C. Wun, and J. E. Sadler. 1987. cDNA cloning and expression in *Escherichia coli* of a plasminogen activator inhibitor from human placenta. *J. Biol. Chem.* 262:3718–3725.

27. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 312–328.

28. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 371–372.

29. Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene.* 28:351–359.

30. Sanger, F., S. Nicken, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

31. Hattori, M., and Y. Sakaki. 1986. Dideoxy sequencing method using denatured plasmid templates. *Anal. Biochem.* 152:232–238.

32. Wilbur, W. J., and D. J. Lipman. 1983. Rapid similarity searches of nucleic acid and protein data banks. *Proc. Natl. Acad. Sci. USA.* 80:726–730.

33. Matsuda, I., J. Yamamoto, N. Nagata, N. Ninomiya, I. Akaboshi, H. Otsuka, and T. Katsuki. 1977. Lysosomal enzyme activities in cultured lymphoid cell lines. *Clin. Chim. Acta.* 80:483–486.

34. Kunkel, L. M., K. D. Smith, S. H. Boyer, D. S. Borgaonkar, S. S. Wachtel, O. J. Miller, W. R. Breg, H. W. Jones, Jr., and J. M. Rary. 1977. Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. *Proc. Natl. Acad. Sci. USA.* 74:1245–1249.

35. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503–517.

36. Kozak, M. 1984. Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. *Nucleic Acids Res.* 12:857–872.

37. Birnstiel, M. L., M. Busslinger, and K. Strub. 1985. Transcription termination and 3′ processing: the end is in site! *Cell.* 41:349–359.

38. von Heijne, G. 1986. Mitochondrial targetting sequences may form amphiphilic helices. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:1335–1342.

39. Roise, D., and G. Schatz. 1988. Mitochondrial Presequences. *J. Biol. Chem.* 263:4509–4511.

40. Koike, K., S. Ohta, Y. Urata, Y. Kagawa, and M. Koike. 1988. Cloning and sequencing of cDNAs encoding α and β subunits of human pyruvate dehydrogenase. *Proc. Natl. Acad. Sci. USA.* 85:41–45.

41. Danner, D. J., N. Armstrong, S. C. Heffelfinger, E. T. Sewell, J. H. Priest, and L. J. Elsas. 1985. Absence of branched chain acyltransferase as a cause of maple syrup urine disease. *J. Clin. Invest.* 75:858–860.

42. Litwer, S., W. J. Herring, and D. J. Danner. 1989. Reversion of the maple syrup urine disease phenotype of impaired branched chain α-ketoacid dehydrogenase complex activity in fibroblasts from an affected child. *J. Biol. Chem.* 264:14597–14600.

43. Zhang, B., H. J. Edenberg, D. W. Crabb, and R. A. Harris. 1989. Evidence for both a regulatory mutation and a structural mutation in a family with maple syrup urine disease. *J. Clin. Invest.* 83:1425–1429.