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Molecular cloning of the mature E1b-β subunit of human branched-chain α-keto acid dehydrogenase complex

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We have isolated a cDNA encoding the E1b-β subunit of the human branched-chain α-keto acid dehydrogenase complex. The human E1b-β cDNA is 1401 base pairs in length. It encodes the entire mature E1b-β subunit consisting of 342 amino acid residues, and a mitochondrial targeting presequence of 31 residues. The calculated molecular mass of the mature human E1b-β subunit is 37851 Da, and the calculated isoelectric point is pH 5.18. A hydropathy plot shows that the human E1b-β subunit is highly hydrophobic. Northern blot analysis shows that the human E1b-β mRNA is approximately 1.4 kb in size. It is present at the normal level in fibroblasts from two unrelated maple syrup urine disease patients.

cDNA; Branched-chain E1b-β; Nucleotide sequence; Amino acid sequence; (Human liver)

1. INTRODUCTION

The mammalian branched-chain α-keto acid dehydrogenase complex (the branched-chain complex) catalyzes the oxidative decarboxylation of the α-keto acids derived from the transamination of the branched-chain amino acids leucine, isoleucine and valine. The multienzyme complex is associated with the inner-membrane of the mitochondrial matrix, and is both structurally and mechanistically analogous to pyruvate and α-ketoglutarate dehydrogenase complexes [1,2]. The mammalian branched-chain complex has 3 catalytic components: a branched-chain α-keto acid decarboxylase (E1b), a dihydrolipoyl transacylase (E2b) and a dihydrolipoyl dehydrogenase (E3). The enzyme complex also contains two regulatory enzymes, a specific kinase and a specific phosphatase that regulate the activity of the complex via a phosphorylation-dephosphorylation cycle [3]. Our laboratory has recently isolated cDNAs encoding the entire E1b-β subunits of both bovine and human branched-chain complexes. In this paper, we report for the first time the isolation and sequencing of the human E1b-β cDNA. The availability of this cDNA will facilitate investigations into the molecular basis of inborn errors involving the branched-chain complex, i.e. maple syrup urine disease [4]. The isolation and expression of the bovine E1b-β cDNA will be described elsewhere[1].

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Abbreviations: E1b, branched-chain α-keto acid decarboxylase; E1p, pyruvate dehydrogenase; E2b, dihydrolipoyl transacylase; E3, dihydrolipoyl dehydrogenase; SDS, sodium dodecyl sulfate; MSUD, maple syrup urine disease; bp, base pairs; kb, kilobase pairs

2. MATERIALS AND METHODS

2.1. *Screening of a λgt-11 library*

A bovine E1b-β cDNA isolated with the anti-E1b-β antibody[1] was radiolabeled by the random-priming method [5]. It was utilized as a probe to screen a λgt-11 library of human fetal liver cDNA (Clontech). Positive clones were plaque-purified and subcloned into the Bluescript SK$^-$ vector as described previously [6].

2.2. *Nucleotide sequencing*

The nucleotide sequencing of cDNA inserts was carried out by the dideoxynucleotide chain termination method [7] using T7 DNA polymerase. The templates used were double-stranded Bluescripts (pBSSK$^-$) containing the cDNA inserts. These double-stranded templates were sequenced in both directions. The primers used for sequencing included SK, KS, T3, T7, M13-20, the M13-reverse primer, and four 20-bp synthetic oligonucleotides specific for internal sequences of the cDNA insert.

2.3. *Peptide sequencing and CNBr digestion*

The sequences of the amino-terminal region and CNBr fragments of the bovine E1b-β subunit were determined by gas-phase microsequencing as described previously [8]. For CNBr digestion, the bovine E1b-β subunit (20 μg) isolated by electroelution from SDS-polyacrylamide gel was mixed with 50 μl of CNBr solution (5 mg in 1 ml of 70% formic acid). The mixture was incubated at 25°C in the dark for 48 h. The digest was electrophoresed in 15% SDS-polyacrylamide gel and transblotted onto a polyvinylidene difluoride membrane for peptide sequencing [8].

2.4. *Cell culture and Northern blot analysis*

Fibroblasts derived from MSUD patients (PK and Lo) [6] were cultured as described previously [6]. Total or poly(A)$^+$ RNA was prepared from normal fibroblasts, and Northern blot analysis was carried out using random-primed cDNA probes [6].

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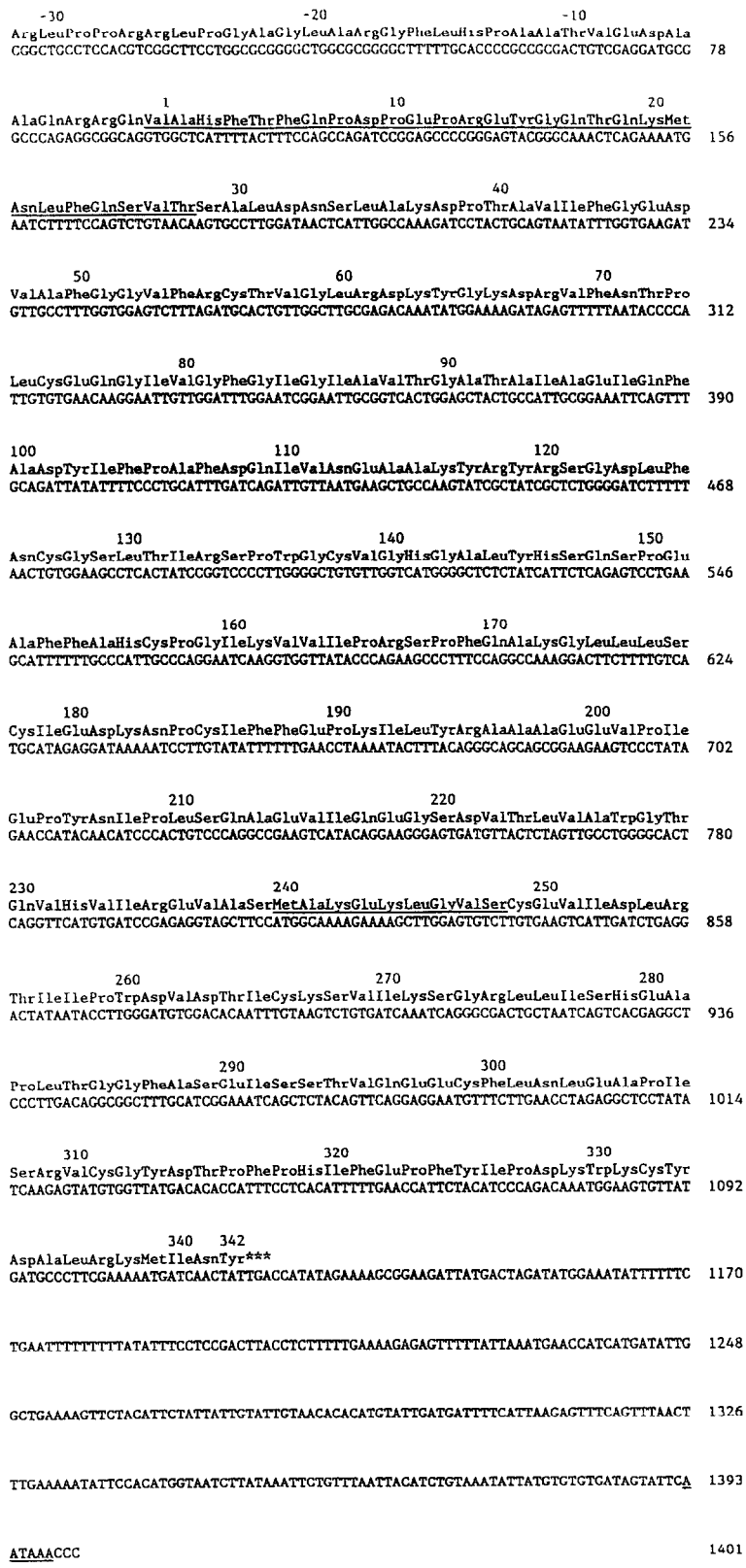

Fig.1. Nucleotide and deduced amino acid sequences of the human E1b-β cDNA (hE1β-1). The numbers on the far right correspond to ordinates of the last nucleotide in each row. The numbers above the sequence refer to the positions of amino acids relative to the amino-terminal Val residue (position 1) of the mature human E1b-β polypeptide. Amino acid sequences corresponding to the amino-terminal regions of the bovine E1b-β subunit (residues 1 to 28) and a CNBr fragment (residues 240 to 248) determined by peptide sequencing are underlined. The putative polyadenylation signal AATAAA (ordinates 1393 to 1398) is also underlined.

3. RESULTS AND DISCUSSION

The screening of the λgt-11 library (400 000 pfu) produced 3 human cDNA clones (hE1β-1, -2 and -4) that hybridized with the 1393 bp bovine E1b-β cDNA[1]. Nucleotide sequencing disclosed that the 3 human cDNA clones were identical. As shown in fig.1, the human E1b-β cDNA (hE1β-1) is 1401 bp in length. It

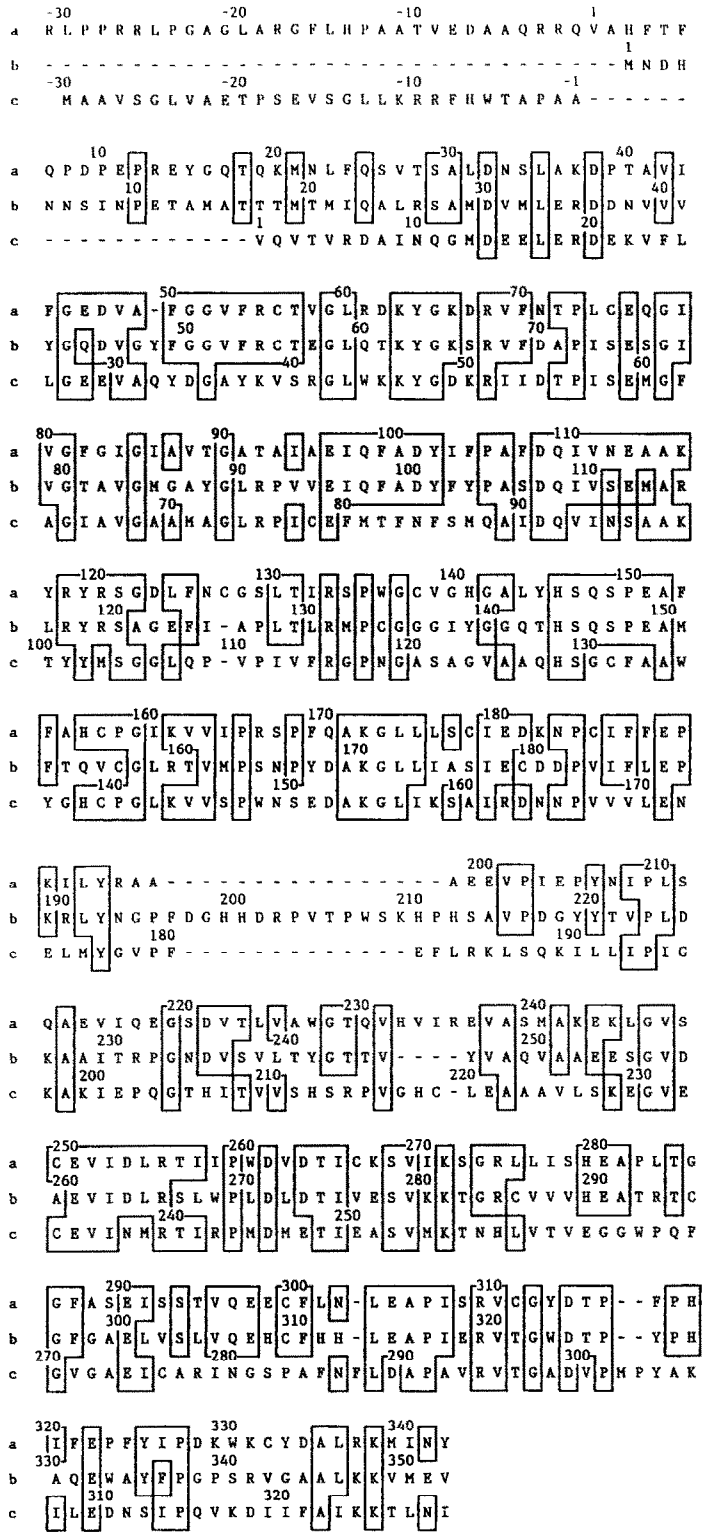

Fig.2. Comparison of the amino acid sequences of three E1β polypeptides. The deduced primary structures of human E1b-β (sequence a) (the present study), *Pseudomonas* E1b-β (sequence b) [11] and the related E1p subunit (sequence c) of the human pyruvate dehydrogenase complex [12] are aligned for maximal identity. The boxed residues are those which are identical between any two of the protein subunits.

encodes a mature peptide of 342 amino acids and a mitochondrial presequence of 31 residues. The insert contains a 282 bp 3′-untranslated region with no poly(A)$^+$ tail, although a potential polyadenylation signal AATAAA is present (underlined bases 1393 to 1398). The fidelity of the human E1b-β cDNA is established by the matching of the deduced human amino acid sequence with the determined bovine E1b-β sequence in the amino-terminal region (underlined residues 1 to 28) and in a CNBr fragment (underlined residues 240 to 248) (fig.1). The only substitutions are in positions 13, 26 and 242 which are Val, Ala and Gln respectively in the bovine E1b-β subunit. The calculated molecular mass is 37 851 Da and the isoelectric point is 5.18 for the human E1b-β subunit.

A hydropathy plot according to Kyte and Doolittle [9] shows that the entire human E1b-β chain is highly hydrophobic. Hydrophobicity (>1.3) is invariably associated with predicted α-helices or β-pleated sheets based on Chou and Fasman [10] (data not shown). It is tempting to suggest that the E1b-β subunit is buried inside the hydrophilic E1b-α subunit to form an $\alpha_2\beta_2$ structure. The relatively inaccessible topography may explain the poor antigenicity of E1b-β when the purified bovine E1b component is used as an antigen [8].

To assess possible sequence conservation, the deduced primary structures of human E1b-β, *Pseudomonas* E1b-β [11] and the human E1p-β of the pyruvate dehydrogenase complex [12] are aligned for maximal identity as shown in fig.2. There is significant sequence conservation throughout the entire stretches of the 3 polypeptides. The identity over a span of 342 amino acid residues is 46% between human E1b-β and *Pseudomonas* E1b-β, and 31% between human E1b-β and human E1p-β subunits. The lengths of the E1β subunits are also similar with 342, 329 and 352 residues for human E1b-β, *Pseudomonas* E1b-β and human E1p-β, respectively. The significant sequence conservation suggests that these functionally related E1β subunits will have similar secondary, tertiary, and quaternary structures. The results also support the view that the genes for the E1β subunits were evolved from a common ancestor.

MSUD is genetically heterogeneous [13] as the branched-chain complex is encoded by at least 6 structural genes. A mutation in any of these genes could result in the dysfunction of the branched-chain complex and consequently the MSUD phenotype. We have shown previously [6] that the E1b-α mRNA is present at the normal level in fibroblasts derived from a Mennonite MSUD patient (P.K.); however, both E1b-α and E1b-β subunits are markedly reduced as observed by Western blotting. In fibroblasts from a second unrelated MSUD patient Lo, the E1b-α mRNA was significantly reduced, and both the E1b-α and E1b-β subunits were nearly absent. Fig.3 shows that the E1b-β mRNA (1.4 kb) is present at normal levels in fibroblasts from P.K. (panel A) and Lo (panel B). With respect to P.K., the MSUD mutation might involve either the E1b-α or E1b-β gene. However, in Lo the mutation affects the E1b-α gene as demonstrated by a marked reduction in its level of mRNA. The presence of a normal level of E1b-β mRNA in Lo thus strengthens the previous suggestion that the failure to assemble into a stable $\alpha_2\beta_2$ structure results in degradation of the unpaired E1b-β subunits in the cell [6,14].

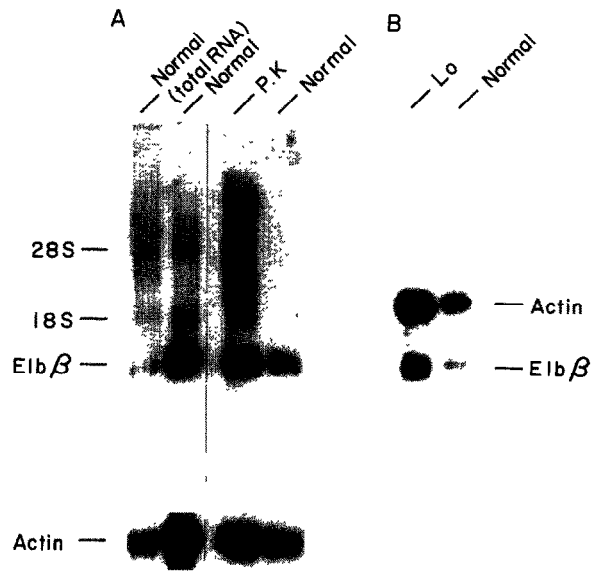

Fig.3. Northern blot analysis of the E1b-β mRNA in normal and MSUD fibroblasts. Total (indicated) or poly(A)$^+$ RNA prepared from normal or MSUD fibroblasts was subjected to Northern blotting with the hE1β-1 cDNA as a probe. P.K. is a Mennonite classical patient. Lo is a varient MSUD patient. The actin mRNA served as internal standards was probed either separately (panel A) or along with the E1b-β mRNA using a ^{32}P-labeled cDNA mixture (panel B). The lower level of normal E1b-β mRNA in panel B resulted from the lower amount of poly(A)$^+$ RNA applied. The size of human E1b-β mRNA (1.4 kb) was estimated using 28 S and 16 S rRNAs as standards.

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