

Nucleotide sequence of a cDNA for the dihydrolipoamide acetyltransferase component of human pyruvate dehydrogenase complex

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Deoxynucleotide sequencing of a cDNA for the dihydrolipoamide acetyltransferase (PDC-E₂) component of human pyruvate dehydrogenase complex (PDC) revealed an open reading frame of 1848 base pairs corresponding to a leader sequence of 54 amino acids and a mature protein of 561 amino acids (59551 Da). Both an amino-terminal lipoyl-bearing domain and a carboxy-terminal catalytic domain are present in the deduced amino acid sequence. The lipoyl-bearing domain contains two repeating units of 127 amino acids, each harboring one lipoic acid-binding lysine. Thus, mammalian PDC-E₂ differs as to the number of lipoic acid-binding sites from other dihydrolipoamide acyltransferases in both prokaryotic and eukaryotic organisms.

cdNA; Dihydrolipoamide acetyltransferase; Nucleotide sequence; Amino acid sequence; (Human liver)

1. INTRODUCTION

Dihydrolipoamide acetyltransferase (PDC-E₂, EC 2.3.1.12) is one of the six components of the mammalian pyruvate dehydrogenase complex (PDC) [1,2]. PDC-E₂ is encoded by a nuclear gene and is synthesized as a precursor with a leader sequence which is subsequently cleaved to generate the mature form in the mitochondria [3]. PDC-E₂ is made up of two heterologous domains: the carboxyterminal inner core-forming (catalytic) domain and the amino-terminal lipoyl-bearing domain, which are linked by a trypsin-sensitive hinge region [4]. Lipoic acid is covalently linked to a lysyl-residue of the lipoyl-bearing flexible do-

main thereby allowing the functional group of the lipoyl-lysine to interact with active sites residing in other components of the complex. Here, we report the deduced amino acid sequence of human PDC-E₂ including the core-forming and lipoyl-bearing domains. The deduced amino acid sequence establishes the presence of two lipoyl-binding sites in the lipoyl-bearing domain of human PDC-E₂. An analysis of the structural relationship of human PDC-E₂ as compared to the E₂ components of other α -ketoacid dehydrogenase complexes is also presented.

2. MATERIALS AND METHODS

2.1. Sequencing strategy

The isolation of seven λ recombinant phage containing cDNAs for human PDC-E₂ was previously reported [5]. These recombinants were digested with *EcoRI* or other restriction endonucleases and the resultant cDNA fragments were cloned into M13mp19. Overlapping directional deletion clones were generated starting with single-stranded M13mp19 template, RD20 primer (New England Biolabs), and T₄ DNA polymerase

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession no. Y00978

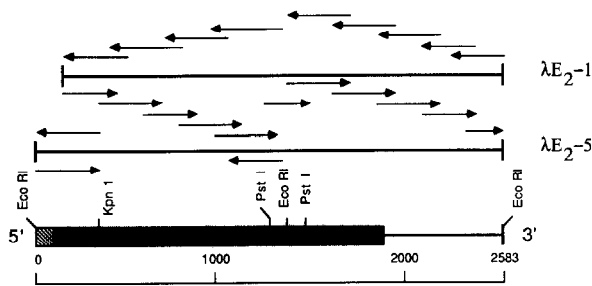


Fig.1. Partial restriction endonuclease map of PDC-E₂ cDNA clones derived from λE₂-1 and λE₂-5 and their sequencing strategy. Arrows indicate the position and direction of deletion fragments that were sequenced. The solid box and the hatched box represent the coding region of the mature PDC-E₂ and its leader sequence, respectively.

[6]. Deoxynucleotide sequencing of overlapping single-stranded M13mp19 clones was performed by the dideoxy chain-termination method [7] employing a modified T₇ DNA polymerase (Sequenase, US Biochemical).

2.2. Northern analysis

Total RNA from human heart and rat kidney was isolated and RNA blotting was performed as in [8], using a 1.4 kb EcoRI cDNA fragment of λE₂-1 labeled with [α-³²P]dCTP by the random priming method [9].

3. RESULTS AND DISCUSSION

A partial restriction endonuclease map of PDC-E₂ cDNA (2583 bp) and the DNA sequencing strategy are shown in fig.1. The deoxynucleotide sequence and deduced primary amino acid sequence from these clones are shown in fig.2. PDC-E₂ cDNA contained an open reading frame of 1848 base pairs which corresponded to a protein of 615 amino acids. The identity of these two clones (λE₂-1 and λE₂-5) as PDC-E₂ cDNA is established by the match of the deduced PDC-E₂ amino acid

Fig.2. Nucleotide sequence of PDC-E₂ cDNA and its deduced amino acid sequence. Nucleotides are numbered 5' to 3'. Amino acid residues (one-letter code) of the mature form and its leader sequence are separately numbered with the mature peptide beginning at +1. X indicates the stop codon. The two lipoyl-binding sites are underlined (solid line) and the lipoyl-binding lysine within each site is marked by an asterisk (*). The two repeating units in the lipoyl-binding domain are bracketed. The E₃ binding site (amino acid residues 272-303) is marked by a broken underline and a highly conserved stretch of amino acids near the carboxy-terminal is identified by a double broken underline. Several potential polyadenylation signals are overlined.

CGAGTGACTCGCGATCTGGCCCGCTCCCGCTCGTCCGCAACGCGTACTACAGGGTAT	60
R V T S R S G P A P A R R N S V T T G Y	(-35)
-54	
GGCGGGTCCGGCACTGTGGGCTGGACCCCACTTCTGGGCAACGCGCGGAACCCG	120
G G V R A L C G W T P S S G A T P R N R	(-15)
TTACTGCTGCAGCTTTGGGGTGGCCCGCGCTATTACAGTCTTCCCCCGCATCAG	180
L L L Q L L G S P G R R Y Y [S L P P H Q	(06)
-1 +1	
AAGTTCCATTGCCTTCTTCCCCCAATGCAGGCAGCCACCATAGCCCGTTGGAAA	240
K V P L P S L S P T M Q A G T I A R W K	(26)
AAAAAGAGGGGACAAAATCAATGAAGGTGACCTAATGCAGAGTTGAAACTGATAAA	300
K K E L G D K I N E G D L I A E V E T D K*	(46)
GCCACTGTGGATTGAGAGCCTGGAGGAGTGTATATGGCAAAGTACTTGTGTGAA	360
A T V G F E S L E E C Y M A K I L V A E	(66)
GGTACCAGGAGTGTCCCATCGGAGCATCTCTGTATCAGACTGGCAAGCTGGAGAT	420
G T R D V P I G A I I C I T I V 'G' K P E D	(86)
ATTGAGGCTTTAAAAATATACACTGTCTTCTCAGCAGCACTACCCCAAGCGGCC	480
I E A F K N Y T L D S S A A P T P Q A A	(106)
CCAGCACCAACCCCTGCTGCCACTGCTCGCCACTACACTTCTGCTCAGGCTCCTGCT	540
P A P T P A A T A S P P T P S A Q A P G	(126)
AGCTCATATCCCCCTCACATGCAGGTACTTCTTCTGCCCTCTCTCCACCATGACCATG	600
S [S Y P P H M Q V L L P A L S P T M T M	(146)
GCCACAGTTCAGAGATGGGAAAAAAGTGGGTGAGAAGCTAAGTGAAGGACTTACTG	660
G T V Q R W E K K V G E K L S E G D L L	(166)
CGAGAGTAGAACTGACAAGCCACTATAGTGTGAAAGTACAGGAAGAGGTTATCTG	720
A E I E T D K* A T I G F E V Q E E G Y L	(186)
GCAAAAATCTGGTCCCTGAGGCAAGAGATGTCCTCTAGGAACCCCACTCTGTATC	780
A K I L V P E G T R D V P L G T P L C I	(206)
ATTGTAGAAAAGAGGAGCATATCAGCATTTGCTGACTATAGGCCAACCCGAAGTAA	840
I V E K E A D I S A F A D Y R P T E V T	(246)
GATTTAAAACCACAAGTGCCACCCTACCCCAACCCCGGTGGCCGCTGTCTCCCACT	900
D L K P Q V P P P T P P V A A V P P T	(246)
CCCCAGCCTTAGCTCTACACCTTCAGCACCCTGCCAGCTACTCTCTGGACCAAAAG	960
F Q P L A P T P] S A P C P A T P A G P K	(266)
GGAAGGGTGTGTAGCCCTCTGCAAGAGTGGCAGTAGAGAAAGGATGATCTG	1020
G R V F V S P L A K K L A V E K G I D L	(286)
ACACAAGTAAAGGGACAGGACAGATGTTAGAAATCACAAGAGGATATCGACTCTTTT	1080
T Q V K G T G P D G R I T K K D I D S F	(306)

GTGCCGTAGAAAGTGTCTGCTCCGGCAGCTGTGTGCTCCACAGTCTCGGAATG	1140
V P S K V A P A P A A V P P T G P G M	(326)
GCACCAGTCTCAGGTGTCTTCACAGATATCCCAATCAGCAATTCGTCGGGTATT	1200
A P V P T G V F T D I P I S N I R R V I	(346)
GCACAGGATTAATGCAATCAAGCAAAACCATCTCATATTATTCATCTCATCGATG	1260
A Q R L M O S K Q T I P H Y Y L S I D V	(366)
AATATGGAGAAGTTTTGTTGGTACGGAAGAACTTAATAAGATATTAGAAGGAGAAGC	1320
N M G E V L L V R K E L N K I L E G R S	(386)
AAAATTTGCTCAATGACTTCATCATAAAAGCCTTCAGCTTTGCCATCTTTAAAAGTCC	1380
K I S V N D F I I K A S A L A C L K V P	(406)
GAAGCAAAATCTTCTTGGATGGACAGTTATAGACAAATCATGTGTGTATGTCAGT	1440
E A N S S W M D T V I R Q N H V V D V S	(426)
GTTGGGTCAGTACTCTGCAGGACTCATCACACTATTGGTAAATGACATATAAAA	1500
V A V S T P A G L I T P I V F N A H I K	(446)
GGATGGAAACCATTGCTAATGATGTTGTTCTTAGCAACCAAGCAAGAGGGTAAA	1560
V E T I A N D V V S L A T K A R E G K	(466)
CTACAGCCACATGAATCCAGGGTGGCCTTTTACGATCTCCAATTTAGGAATGTTTGA	1620
L Q P H E F Q G G T F T I S N L G M F G	(486)
ATTAAGAATTTCTGTCTATTATTAACCCACTCAAGCATGTATTTGGCAATGGTGTCT	1680
I K N F S A I I N P P Q A C I L A I G A	(506)
TCAGAGGATAAATGGTCCCTGCAGATAAAGAAAAGGTTGATGTGGCTAGCATGAT	1740
S E D K L V P A D N E K G P D V A S M M	(526)
TCTGTACTACTAGTTGATCACCAGGTTGGTGGATGGAGCAGTTGGAGCCAGTGGCTT	1800
S V T L S C D H R V V D G A V G A Q W L	(546)

GCTGAGTTTGAAGTACCTTGAAGAACCTATCCTATGTTGTTGTAACCTCAAGAA	1860
A E F R K Y L E K P I T M L X	(561)
TTTCTAAACTCCCAAGTCACTGATTCATCTTAAACAGATATTATATGTTATTTAA	1920
ACAGGGTGTCTTTTATTTAAACCACTATTTTTATTTAGTCGTCGTCAGATAGT	1980
TATTTATAAGGGCACTACTGAATTTTAAATGGCCGATACCCCAAATTTGTGCACA	2040
TTTAAATTCAGACCCAGATTTTGTCTCTGTACTCTTAAATGAGGACATGATGTGG	2100
CCTTGGCTAGCCCTTTGGTATAAGTACTTCCCTTAGGAAATGTACGATAGTGAATG	2160
TGTTCCCTAAAGACAAGTACATAAAGTGCACCTGATGAACCTTGAAGTTCGAAT	2220
TAAGTCCCTAAATGTCTCTTGTAGATGCAGGAAGAGAAATCAGAAATTTAAATCT	2280
CTTGGGGAAGGCTTGAATGAAAGCTTTACTTTAGAAATTTAGCCCTGGTTGAAATTT	2340
CCATTACATGATCTGGTTTATCATCGATGGGAGGGTAGAAAATCTCAAGGAAATTA	2400
TGAATTTTAAAGTCAGCATTTCTTAGACCTCTCAGCTGATGTTTATTTTCTATG	2460
AATCTGTACTCAAAATACACATTTGTTTAAATATATCCACCAAAATCTCAGTTAC	2520
ATCAAGTAGCTGTTTATATTAGATTTATCTCAAGTACCGGGCAATACCACTGTGTAGA	2580
ATT	2583

sequence with (i) a known sequence of 10 amino acids surrounding the lipoyl-binding lysine residue of bovine kidney PDC-E₂ [10], and (ii) 22 of 23 residues of the amino acid sequence at the amino-terminal of bovine heart PDC-E₂. [The amino-terminal amino acid sequence (SLPPHEKVPL-PSLSPTMQAGTIA) generated from trypsin digestion of the lipoyl-bearing domain of bovine heart PDC-E₂ (Thomas E. Roche, Kansas State University; personal communication).] The calculated molecular mass of the deduced amino acid sequence of the mature PDC-E₂ (561 amino acids) is 59551 Da. The molecular mass of the leader sequence of PDC-E₂ is estimated to be approx. 6000 Da. This is consistent with the previously reported mass based on mobility of the precursor PDC-E₂ on SDS-PAGE [3].

The deduced amino acid sequence of human PDC-E₂ shows the presence of the amino-terminal lipoyl-bearing domain and the carboxy-terminal catalytic domain, both of which have been demonstrated to be present in PDC-E₂ based on the proteolytic cleavage of mammalian PDC-E₂ [4] and the sequencing of *E. coli* PDC-E₂ [11]. Our data show that human PDC-E₂ contains two lipoyl-binding sites [amino acid residues 35–52 (site I) and 162–179 (site II) in figs 2,3] in the lipoyl-bearing domain. The second lipoyl-binding site (site II) shows approx. 83% homology (15 out of 18 residues) with the first (site I) and the differences that exist between the two lipoyl-binding sites are conservative. The amino acid sequence of site II of human PDC-E₂ is also identical to a lipoyl-binding site present in a deduced protein sequence of rat liver PDC-E₂ cDNA [12,13].

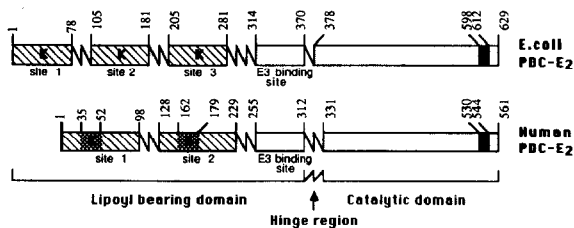


Fig.3. Comparison of the domain structures of *E. coli* and human liver PDC-E₂. The limits of these domains are approximate. K, the lipoyl-binding lysine; (▨) lipoyl-bearing repeating units; (▩) the highly conserved region surrounding the lipoyl-binding lysine residue; (□) E₃-binding site; (■) catalytic domain; (■) the conserved region in the catalytic domain; and (W) the alanine-proline rich region.

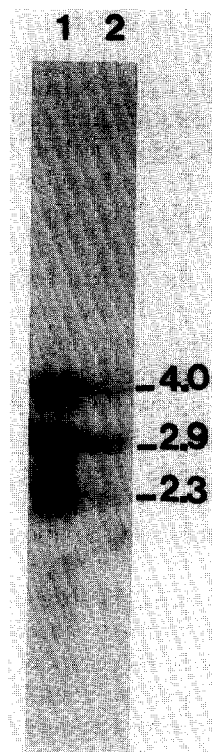


Fig.4. Northern blot analysis of total RNA isolated from human heart (25 µg; lane 1) and rat kidney (50 µg; lane 2). The size of the different mRNA species was determined by comparing their electrophoretic mobility to that of ribosomal RNA.

However, the deduced amino acid sequence of PDC-E₂ from rat liver did not extend far enough into the amino-terminus of the protein to include the lipoyl-binding site I.

An earlier study suggested that there was only one functional lipoic acid-binding site per molecule of PDC-E₂ based on radiolabeled acetylation of mammalian PDC [4]. Recently, Bradford et al. [14] were able to identify two different but closely related short peptides containing lipoyl-lysine residues. These investigators proposed that there are either two or more lipoyl-binding sites per mammalian PDC-E₂ polypeptide or possible microheterogeneity of bovine PDC-E₂ [14]. Our results (fig.2) demonstrate the presence of two lipoyl-binding sites per human PDC-E₂ polypeptide. This is in contrast to the E₂ component of mammalian branched-chain α -ketoacid dehydrogenase complex which contains only one lipoyl-binding site per polypeptide [13,15].

Mammalian PDC-E₂ differs from *E. coli* PDC-E₂ in that the latter contains three repeating units of approx. 100 amino acids in its lipoyl-bearing domain [10]. Each repeating unit surrounds a lipoyl-binding lysine and a region rich in alanine and proline residues at the carboxy-terminal of each repeating unit [10,11]. The repeating units in *E. coli* PDC-E₂ contain identical regions of 18 amino acids surrounding the critical lysine residue. The remaining amino acids in the repeating units show considerable homology (ranging from 69 to 80%), with many of the differences being conservative substitutions. In contrast, human PDC-E₂ includes two similar repeating units of 127 amino acids each containing a conserved stretch of 38 amino acids (amino acid residues 35–72 and 162–199; 79% homology) encompassing the lipoyl-binding lysine residue (figs 2,3).

A dihydrolipoamide dehydrogenase (E₃) binding site has been identified based on sequence homology among the E₂ components of the three α -ketoacid dehydrogenase complexes [13]. A sequence of 32 amino acids (from residues 272 to 303 in figs 2,3) which is highly homologous to the E₃-binding site [13], is also present in human PDC-E₂. Overall, there is approx. 90% homology in the E₃-binding site between human and rat liver PDC-E₂ [12] and 53% homology between human and *E. coli* PDC-E₂ [11]. Further comparison of the amino acid sequences of the catalytic domains of PDC-E₂ from human liver and *E. coli* revealed [11] a segment of 14 amino acids (residues 530–543 in figs 2,3) near the carboxy-terminal with approx. 79% homology:

Human liver PDC-E₂: ...L S C D H R V V D G A V G A ...
E. coli PDC-E₂: ...L S F D H R V I D G A D G A ...

Although the nucleotide sequence encoding this region in rat liver PDC-E₂ cDNA [12] is largely preserved, the deduced amino acid sequence of this segment of rat liver PDC-E₂ is apparently altered due to a shift in its reading frame. A histidine residue is conserved in both sequences and this

amino acid may serve as the active site residue for catalysis.

Northern blot analysis of RNA isolated from human heart and rat kidney showed the presence of three hybridizing species (fig.4). This could be either due to the presence of multiple polyadenylation signals in the 3' untranslated region of PDC-E₂, alternative splicing sites, or multiple transcription initiation sites.

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