

Short Sequence-Paper

The cDNA sequence of the flavoprotein subunit of human heart succinate dehydrogenase

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Abstract

We report the full-length cDNA sequence for the flavoprotein subunit of human heart succinate dehydrogenase (succinate: (acceptor) oxidoreductase EC 1.3.99.1). Identical sequence was obtained for part of the cDNA of the human placental flavoprotein, in contrast to a previously published sequence. The human sequence, like the bovine one, contains a cysteine triplet and at the active site there is an additional cysteine when compared with yeast or prokaryotes.

Key words: Succinate dehydrogenase; Flavoprotein; cDNA; Nucleotide sequence; (Human heart)

Succinate dehydrogenase (succinate: (acceptor) oxidoreductase EC 1.3.99.1) catalyses the oxidation of succinate to fumarate, feeding electrons directly into the respiratory chain. The enzyme consists of two hydrophilic subunits, a larger flavoprotein and a smaller iron-sulphur protein [1]. Mammalian succinate dehydrogenase is anchored to the inner mitochondrial membrane by two small hydrophobic polypeptides, the whole comprising complex II of the respiratory chain [2].

Respiratory chain disorders are important causes of human disease. Complex II deficiency has been reported in combination with other defects and in isolation [3–6]. Identification of mutations depends on knowing the human cDNA sequence of each subunit. cDNA sequences have been published for the mature iron-sulphur protein in human liver [7] and for the flavoprotein in human placenta [8]. However, the latter was incomplete and its validity has been questioned on

several grounds [9,10]. Here we report the full-length cDNA sequence for the flavoprotein precursor in human heart. It closely resembles the sequence in other eukaryotes but not the previously reported placental sequence. Significantly, a PCR fragment encoding the active site of human placental succinate dehydrogenase has the same sequence as the heart enzyme.

The flavoprotein cDNA was isolated by screening a human heart cDNA library constructed in bacteriophage λ ZAPII (Stratagene). DNA probes were derived from the bovine flavoprotein cDNA [9]. The 2.2 kb cDNA was digested with *Taq*I and *Sau*3A and subcloned in pBluescript SK-. All fragments were completely sequenced from both ends using the dideoxynucleotide chain termination method [11].

Fig. 1 shows the cDNA sequence for the flavoprotein from human heart. It is 2277 basepairs long and contains an open reading frame of 1992 nucleotides. The 5' and 3' untranslated regions are 24 and 261 nucleotides long, respectively. The poly(A) tail is not included, but 12–17 nucleotides from the end there is a consensus polyadenylation signal (AATAAA) [12].

Fig. 2 compares the deduced polypeptide sequence of 664 amino acids, with the bovine, yeast, *E. coli*, *B. subtilis* and putative placental sequences [9,10,13,14,8]. The published bovine sequence differs most in the first

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half of the leader peptide. Reinvestigation of this region of the bovine cDNA revealed two errors in the published sequence: the insertion of a cytosine at residue 43 and the omission of a cytosine between nucleotides 55 and 56. These frame shift alterations change 5 amino acids in the deduced sequence. It is the corrected bovine sequence that is shown in Fig. 2.

This is the first complete sequence to be published for the human flavoprotein subunit of succinate dehydrogenase. As shown in Table 1, it closely resembles the bovine sequence and has considerable homology to those of yeast and *E. coli*. It is less similar to the *B. subtilis* sequence or the partial sequence reported for the human placental flavoprotein [8]. Neither of the latter sequences contains the active site cysteine that confers sensitivity to thiol reagents [15,16]. However, we have previously shown that the placental enzyme is inhibited by *N*-ethylmaleimide [9]. To resolve this inconsistency the active site region was amplified by PCR from a human placental cDNA library using primers derived from the human heart cDNA sequence (nucleotides 981 to 996 forwards and 1101 to 1116 reverse). The PCR product had identical sequence to the human heart cDNA and included the reagent-sensitive cysteine. Our data suggest that the published placental sequence is incorrect and that there are no differences between the succinate dehydrogenases of human heart and placenta.

The bovine and yeast flavoproteins are translated as precursors with presequences required for targeting, which are removed inside mitochondria. In view of the similarity of the human precursor to the bovine one the

Table 1

Percentage identity of the human heart flavoprotein and other reported sequences

	Bovine ^a	Yeast ^b	<i>E. coli</i> ^c	<i>B. subtilis</i> ^d	Human placenta ^e
Entire polypeptide	93.2	63.5			
Mature protein	95.2	65.0	50.6	28.8	27.9

% identity is measured as $100 \times \text{matches} / (\text{matches} + \text{mismatches} + \text{gaps})$.

^a [9]; ^b [10]; ^c [13]; ^d [14]; ^e [8].

cleavage site is probably the same. This would give a human presequence of 43 amino acids and a mature protein of 621 residues. As in many imported mitochondrial proteins, arginine is found two residues upstream of the putative cleavage site, preceded by another basic amino acid. 8–13 residues upstream is a motif characteristic of presequences that are removed in two stages: RXFXXS where R is arginine, X is any amino acid, F is phenylalanine or another hydrophobic residue and S is serine, threonine or glycine [17]. This motif is also found in the yeast and bovine flavoprotein precursors. Compared with the N terminal parts of cytosolic proteins, mitochondrial presequences have an increased number of arginine, leucine and hydroxylated residues whereas acidic residues are decreased [18]. The human flavoprotein precursor conforms to this pattern with 14.0% arginine, 14.0% leucine, 16.3% serine and threonine and only 2.3% acidic residues.

Within the mature polypeptide there are 10 clusters of highly conserved residues (labelled A to J in Fig. 2). Functions have been ascribed to some of these. The FAD prosthetic group is covalently attached to the histidine in region B and regions A, G, H and I also contribute to the FAD binding fold [19]. Regions E and F contain residues involved in the active site, including the cysteine responsible for sensitivity to thiol-reactive agents. This cysteine lies in region E in *E. coli* and between regions E and F in yeast. The human and bovine flavoproteins have cysteine residues in both sites. The presence of two cysteines here may allow thiohemiacetal formation, explaining the tighter binding of inhibitory oxaloacetate in mammals as compared with prokaryotes [20,21].

The cysteine triplet at residues 146–148 is a most unusual motif. Its function here is unknown. It is present in the bovine flavoprotein but not in other species and has only been found in three other classes of protein: keratins, metallothioneins and mitochondrial alcohol dehydrogenase.

Mitochondrial DNA abnormalities have been reported in a number of respiratory chain disorders. As

1 GACTGCGCGG CGGCAACAGC AGACATGTGCG GGGGTCCGGG GCCTGTGCGG GCTGCTGAGC
61 GCTCGGCGCC TGGCGCTGGC CAAGGCGTGG CCAACAGTGT TSCAACAGG AACCCAGGT
121 TTCTACATCA CTGTTGATGG GAACAGAGGG GCATCTGCTA ATCCATTTCT
181 GCTCAGTATC CACTAGTGGG TCTGAATTTT GATCAGTGG TGGTAGGCGC TGGAGGGGCA
241 GGCTTCCGAG CTCATTCTGG CTTTCTCTGAG CGAGGGTTTA ATACAGCATG TGTATCCAA
301 CTGTTTCTTA CCAGTGCACA CACTGTTGCA GCGCAGGGAG GAATCAATGC TGTCTGGGG
361 AACATGGAGG AGGACAACAT GAGTGGCAT TTCTACGACA CCGTGAAGGG CTCGAGCTGG
421 CTGGGGGACC AGGATGCCAT CCACTACATG ACGGAGCAGG CCCCAGCGCG CTGGTCTGAG
481 CTAGAAAAAT ATGGCATGCC GTTTAGCAGA ACTGAAGATG GGAAGATTTA TCAGGTGCGA
541 TTTGTTGGAC AGAGCTGCAA GTTTGGAAGG GCGGGGCGAG CCCATCGGTTG CTGCTGTGTG
601 GCTGATCGGA CTGGCCACTC GCTATTGAC ACCTTATATG GACGGTCTCT GCGATATGAT
661 ACCAGCTATT TTTGAGAGTA TTTTGCCTTG GATCTCTCTA TGGAGAACGG GAGTGGCGGT
721 GGTGTCATCG CACTGTGCAT AGAGGACGGG TCCATCCATC GCATAAGAGC AAAGAACAAT
781 GTTGTGCGCA CAGGAGGCTA CCGGCGCACC TACTTCAGCT GCACGCTGCG CCACACCAGC
841 ACTGGCGACG GCACGCCCAT GATCACCAGG GCAGGCTTTC CTTGCCAGGA CCTAGAGTTT
901 GTTCAGTTCC ACCCCACAGG CATATATGGT GCTGGTTGTC TCATTACGGA AGGATGTCGT
961 GGAGAGGGAG GCATTCTCAT TAACAGTCAA GCGGAAAGGT TATAGATGTG ATACGCCCT
1021 GTGCGAAGAG ACCTGGCGTC TAGAGATGTG GTTCTCTGCT CGTACTCTCT GGAGATCCGA
1081 GAAGAGAGAG GCTGTGGGCC TGAGAAAGAT CACGTCTACC TGCAGCTGCA CCACCTACT
1201 GTGGAAGTCA CGAAGGAGCC GATCCCTGTC CTCCCAACCG TGCAATTATAA CTTGCGTGGC
1261 ATTCCCAACA ACTACAAGGG GCGAGTCTCT AGGCACGTGA ATGGCCAGGA TCAGATTGTG
1321 CCGGCGCTGT ACGCCCTGGG GGAGGCCGCG TGTGCTCTGG TACATGTGTC CAACCGCTC
1381 GGGGCAAACT CGCTCTTGGG CTTGCTTGGT TTTGTTGCGG CATGTGCCCT GAGCATCGAA
1441 GAGTCATGCA GCGCTGGAGA GCAATTAAAC CAATTAAGC CAAAGCTGCT GGAAGAATCT
1501 GTCATGAATC TTGCAAAATT GAGATTGCTT GATGGAAGCA TAAGAATCAT GGAAGTGGCA
1561 GTCAGCATGC AGAAGTCAAT GCAAAATGAT GCTCCGTGT TCCGTGTGGG AAGCGTGTG
1621 CAAGAAGGTT GTGGGAAATT CAGCAAGCTC TATGGAGACC TAAAGACACT GAAGCGTCT
1681 GACCGGGGAA TGGTCTGGAA CACAGACCTG GTGGAGACCC TGGAGCTGCA GAACCTGATG
1741 CTGTGTGCGC TGCAGACCAT CTACGAGGCA GAGGCGCGGA AGGAGTCAAG GCGCGCGCAT
1801 GCGAGGGAAG ACTACAAGGT GCGGATTTAT GAGTACGATT ACTCCAGGCC CATCCAGGGT
1861 CAACAGAGA AGCCCTTTGA GAGCACTGG AGGAAACACA CCGTAACTG ACACAACTTT GAACGAGCT
1921 GCACTCTGGA AGCTCACTCT GAAATATGCA CCGTAACTG ACACAACTTT GAACGAGCT
1981 GACTGTGCGA CCATCCCGCC AGCCATTGCG TCTACTGAT GTGTCATGAT GTGTCATGAT CATTAAGT
2041 CAGATACGCT TTTTGTAAAT ATGTATAATA GCTCATGAT GTGTCATGAT GTGTCATGAT CATTAAGT
2101 TTTCACTGCT TCTGACTCTT GAGTACATTT AAGGAGATTT AAGGAGATTT AAGGAGATTT
2161 GCGTGGGAGG TTGCGAGGAA CCGAGTGGCC AGGGAGCGTG GCACCTTACT TTTGCTCTGT
2221 CTTCATTCTT GTGAGATGAT AAAAATGGGC ACAGCTCTTA AATAAATAT AAATGAG

Fig. 1. Complete cDNA sequence for the flavoprotein precursor of human heart succinate dehydrogenase.

yet, no mutations have been found in the nuclear-encoded subunits of respiratory complexes. Complex II, being the simplest and entirely nuclear-encoded, may offer the best opportunity to identify such defects. The

human flavoprotein sequence published here is essential for these studies.

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Hum ht	MSGVRLSRLLSARRLALA-KAWPTVLQGTGRGF--HFTVDGNKRASAKVSDSISAQYFVVDHEFDVAVVGAGGAGLRAAFGLSEAGFNTACVTKL	50
Bov ht	MSGVAASRLWRARRLALCTKWSAAWQTGRSF--HFTVDGNKRSSAKVSDAISQYFVVDHEFDVAVVGAGGAGLRAAFGLSEAGFNTACVTKL	50
Yeast	MLSLKKSALSCLTLRLNRTFTSSALVRQTQGSVNGSASRSADGKYHIDHEYDCVVIGAGGAGLRAAFGLAEAGYKTPACISKL	56
E.coli	MKLPRVREFDAVVGAGGAGIARALQISQSGQTCALLSKV	39
B.subt	MSQSSIIIVGGGLAGLMATIKAAESGMAVKLSFIV	35
Hum ht	FPTRSHTVAAQGGINAALGNMEEDNW-RWHFYDVTVGSDWLGDDQDAIHYMTQAPAAVVELENYGMPPFSRTEDGKIYQRAFGGQSLKFKGKGQA	144
Bov ht	FPTRSHTVAAQGGINAALGNMEEDNW-RWHFYDVTVGSDWLGDDQDAIHYMTQAPASVVELENYGMPPFSRTEDGKIYQRAFGGQSLKFKGKGQA	144
Yeast	FPTRSHTVAAQGGINAALGNMHKDNW-KWHMYDVTVGSDWLGDDQDSIHYMTREAPKSIIELEHYGVPPFSRTENGKIYQRAFGGQTKYEGKGAQAY	150
E.coli	FPTRSHTVSAQGGITVALGNTHEDNW-EWHMYDVTVGSDYIGDDQDAIHYMTCKTGPAALEHMLPFSRLDDGRIYQRFPGGQSKNFG-GEQAA	132
B.subt	PVKRSHSVCAQGGINGAVNTKGEKDSPEWHEFDDTVYGGDFLANQPPVKAMCEAAPSIHLLDRMGVMFNRTPEGLLDFRRF-----G-GTQHH	122
Hum ht	RCCCVADRTGHSLLHTLYGRSLR-YDTSYFVEYFALDLMEN--GECRGVIALCIEDGSIHRIRAKNTV-VATGGYGRTYFSCSTSAHTSTGDDGT	234
Bov ht	RCCCVADRTGHSLLHTLYGRSLR-YDTSYFVEYFALDLMES--GECRGVIALCIEDGSIHRIRARNTV-IATGGYGRTYFSCSTSAHTSTGDDGT	234
Yeast	RTCAVADRTGHALHTLYGQALR-HDTHFFIEYFALDLLTHN--GEVGVIAVYQEDGTIHRFRAHKTII-IATGGYGRAYFSCSTSAHTCTGDGN	240
E.coli	RTAAADRTGHALLHTLYQNLK-NHTTIFSEWYALDLVNQ--DGAVVGCTALCIETGEVYVFKARATV-LATGGAGRIYQSTTNAHINTGDGV	223
B.subt	RTAYAGATTGQQLLYALDEQVRRYEVAGLVTKYEGWEFLGAVLDDDDRTCRGIVAQNLTNMQIESFRSDAVIMATGGPGIIFGKSTNSMINTGSAA	217
Hum pl	EFLGIVKDDDSARGIVAQNMTTAEIETFGSDAVIMATGGPGIIFGKTTNSMINTGSAA	59
Hum ht	AMITRAGLPQCDLEFVQFHTGIYAGC--LITEGCRGEGGILINSQGERFMERY-APVAK--DLASRDVVSRS-MTLEIREGRG-CGPEKDHVY	322
Bov ht	AMVTRAGLPQCDLEFVQFHTGIYAGC--LITEGCRGEGGILINSQGERFMERY-APVAK--DLASRDVVSRS-MTLEIREGRG-CGPEKDHVY	322
Yeast	AMVSRAGFPLQKDEFVQFHPGSIYSGC--LITEGARGEGGFLVNSEGERFMERY-APTAK--DLACRDVVSRA-ITMEIREGRG-VGKKKDHMY	328
E.coli	GMAIRAGVPQDMEMNQFHTGIAGAV--LVTEGCRGEGGILYNKHGERFMERY-APNAK--DLACRDVVSRA-ITMEIREGRGCDPWGPHAK	312
B.subt	SIVYQQGAYYANGFIQIHPTAIPGDDKLRLMESARGEGGRVWTKDGKWPYFLEEKYPAYGNLVRDIAITREIFDVCVNQKLGIN--ENMVY	310
Hum pl	SIVYQQGAYYANGFIQIHPTAIPGDDKLRLMESARGEGGRVWTKDGKWPYFLEEKYPDYGNLVRDIPDMPTREIFDVCINQKLGIN--ENMVY	152
Hum ht	LQLHHLPPQLATRLPGISETAMIFAGVDVTKEPIVPLTVHYNMGGIPTNYKGQVL--RHVNGQDQIVPGLYACGEAAACASVHGANRLGANSLL	415
Bov ht	LQLHHLPPAQLAMRLPGISETAMIFAGVDVTKEPIVPLTVHYNMGGIPTNYKGQVL--RHVNGQDQIVPGLYACGEAAACASVHGANRLGANSLL	415
Yeast	LQLSHLPPPEVLKERLPGISETAIFAGVDVTKEPIIPTVHYNMGGIPTKWNGEALTIDEETGEDKVIPLMACGEAAACVSHGANRLGANSLL	423
E.coli	LKLDHLGKEVLESRLPGILELSRTFAHVDVPKEPIPIPTCHYMMGGIPTKVTGQALTV-NEKGEDVVPGLFAVGEIACVSHGANRLGANSLL	406
B.subt	LDSLHKDPKELDIKLGIIIEIYEKFMGDDPRKLPKIFPAVHYSMGGL-----WVDYDQM-TNIPGLFAAGECD-YSMHGNGRLGANSLL	393
Hum pl	LDSLHKDPHELDVKLGIIIEIYEKFTGDDPRKVPKIFPAVHYSMGGL-----YVDYDQM-TNIKGLFAAGECD-FSQHGNGRLGANSLL	235
Hum ht	DLVVFGACALSIIESCRPGDKVPPIK--PNAGEESVMNLDKLRFDAGSIRTSELRLSMQKSMQNHAAVFRVGSVLQEGCGKISKLYGDL-----	503
Bov ht	DLVVFGACALSIIESCRPGDKVPPIK--PNAGEESVMNLDKLRFDAGSIRTSELRLSMQKSMQSHAAVFRVGSVLQEGCEKISSLYGDL-----	503
Yeast	DLVVFGRAVAHTVADTLQPLPHKPL--SDLGKESIANLKLRLNANGSRSTAEIRNMKQTMQKDVSVFRTQSSLDDEGVNRITAVEKTF-----	511
E.coli	DLVVFGRAAGHLQESIAEQGALRDAS--ESDVEASLDRLNRWNNNNGEDPVAIRKALQECMHNFSVFREGDAMAKLEQLKVIKIRERL-----	494
B.subt	SAIYGGMVAG--PNAVYVNGLESSAESDMSLFDHVKKEEEKWADIMSDGTENAYVLHKLGEWMTANVTVRHNDKLLKTDKIQELMER	485
Hum pl	SAIYGGTVAG--PNAIDYISNIDASYTMDSEIFEKRAKEQERFDKLLAMRGTENAYKLHRELGEIMTANVTVRHNEKLLKTDKIKIVELMKR	327
Hum ht	-KHLKTFDRGMVWNTDLVETLELQNLMLCALQTIYGAERKESRGHAREDYKVR-IDEYDYSKPIQGGQKKPFEEHWRKHTLSFVDVGTGKVT	596
Bov ht	-RHLKTFDRGMVWNTDLVETLELQNLMLCALQTIYGAERKESRGHAREDFKER-VDEYDYSKPIQGGQKKPFEEHWRKHTLSVVDIKTGKVT	596
Yeast	-DDVKTDRSMIWNSDLVETLELQNLMLTASQTAVSAANRKEESRGHAREDYKVR-DD-----EHWKHTLSWQKDVAAFPVTL	587
E.coli	-KNARLDDTSSEFNTQRVECELDNLMETAYATAVANFRTESRGHAREDFPDR-DDENWLCHSLYLPESSEMTSRVSNMPEKLRPAFPKIRT	587
B.subt	FKKININDTTKSNQGMFTROFSNMLQLARVITLGAYNRNESGAHYKPDYPERNDEWLKTTMAKHVSPEYAEFEYQDQVSLITPRKRDY	580
Hum pl	YEDIDMEDTQTSNQAVFFTRQLWNMLVLARVITIGAYNRNESAAYHKP	377
Hum ht	EYRPVIDKTLNEADCATIPPAIRSY	
Bov ht	EYRPVIDRTLNETCATVPPAIRSY	
Yeast	KYRRVIDHTLDEKECPSVPPTVRAV	
E.coli	Y	
B.subt	KKKVAK	

Fig. 2. Deduced amino acid sequence of the flavoprotein precursor of human heart succinate dehydrogenase compared with other reported sequences. The black triangle indicates the bovine presequence cleavage site and the probable cleavage site in the human flavoprotein precursor while the arrow indicates the cleavage site in the yeast precursor. Highly conserved regions are indicated by letters A–J and the cysteine triplet is marked *. In flavoproteins with presequences numbers relate to the mature protein. (Abbreviations: Hum ht: human heart; Bov: bovine; E.coli: *E. coli*; B. subt: *B. subtilis*; Hum pl: human placenta)

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