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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 λZAPII (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 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

Table 1
Percentage identity of the human heart flavoprotein and other reported sequences

	Bovine [a]	Yeast [b]	*E. coli* [c]	*B. subtilis* [d]	Human placenta [e]
Entire polypeptide	93.2	63.5			
Mature protein	95.2	65.0	50.6	28.8	27.9

% identitiy is measured as 100× matches ÷ (matches + mismatches + gaps).
[a] [9]; [b] [10]; [c] [13]; [d] [14]; [e] [8].

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   1 GACTGCGCGG CGGCAACAGC AGACATGTCG GGGGTCCGGG GCCTGTCGCG GCTGCTGAGC
  61 GCTCGGCGCC TGGCGCTGGC CAAGGCGTGG CCAACAGTGT TGCAAACAGG AACCCGAGGT
 121 TTTCACTTCA CTGTTGATGG GAACAAGAGG GCATCTGCTA AAGTTTCAGA TTCCATTTCT
 181 GCTCAGTATC CAGTAGTGGA TCATGAATTT GATGCAGTGG TGGTAGGCGC TGGAGGGGCA
 241 GGCTTGCGAG CTGCATTTGG CCTTTCTGAG GCAGGGTTTA ATACAGCATG TGTTACCAAG
 301 CTGTTTCCTA CCAGGTCACA CACTGTTGCA GCGCAGGGAG GAATCAATGC TGCTCTGGGG
 361 AACATGGAGG AGGACAACTG GAGGTGGCAT TTCTACGACA CCGTGAAGGG CTCCGACTGG
 421 CTGGGGGACC AGGATGCCAT CCACTACATG ACGGAGCAGG CCCCCGCCGC CGTGGTCGAG
 481 CTAGAAAATT ATGGCATGCC GTTTAGCAGA ACTGAAGATG GGAAGATTTA TCAGCGTGCA
 541 TTTGGTGGAC AGAGCCTCAA GTTTGGAAAG GGCGGGCAGG CCCATCGGTG CTGCTGTGTG
 601 GCTGATCGGA CTGGCCACTC GCTATTGCAC ACCTTATATG GACGGTCTCT GCGATATGAT
 661 ACCAGCTATT TTGTGGAGTA TTTTGCCTTG GATCTCCTGA TGGAGAACGG GGAGTGCCGT
 721 GGTGTCATCG CACTGTGCAT AGAGGACGGG TCCATCCATC GCATAAGAGC AAAGAACACT
 781 GTTGTTGCCA CAGGAGGCTA CGGGCGCACC TACTTCAGCT GCACGTCTGC CCACACCAGC
 841 ACTGGCGACG GCACGGCCAT GATCACCAGG GCAGGCCTTC CTTGCCAGGA CCTAGAGTTT
 901 GTTCAGTTCC ACCCCACAGG CATATATGGT GCTGGTTGTC TCATTACGGA AGGATGTCGT
 961 GGAGAGGGAG GCATTCTCAT TAACAGTCAA GGCGAAAGGT TTATGGAGCG ATACGCCCCT
1021 GTCGCGAAGG ACCTGGCGTC TAGAGATGTG GTGTCTCGGT CGATGACTCT GGAGATCCGA
1081 GAAGGAAGAG GCTGTGGCCC TGAGAAAGAT CACGTCTACC TGCAGCTGCA CCACCTACCT
1141 CCAGAGCAGC TGGCCACGCG CCTGCCTGGC ATTTCAGAGA CAGCCATGAT CTTCGCTGGC
1201 GTGGACGTCA CGAAGGAGCC GATCCCTGTC CTCCCCACCG TGCATTATAA CATGGGCGGC
1261 ATTCCCACCA ACTACAAGGG GCAGGTCCTG AGGCACGTGA ATGGCCAGGA TCAGATTGTG
1321 CCCGGCCTGT ACGCCTGTGG GGAGGCCGCC TGTGCCTCGG TACATGGTGC CAACCGCCTC
1381 GGGGCAAACT CGCTCTTGGA CCTGGTTGTC TTTGGTCGGG CATGTGCCCT GAGCATCGAA
1441 GAGTCATGCA GGCCTGGAGA TAAAGTCCCT CCAATTAAAC CAAACGCTGG GGAAGAATCT
1501 GTCATGAATC TTGACAAATT GAGATTTGCT GATGGAAGCA TAAGAACATC GGAACTGCGA
1561 CTCAGCATGC AGAAGTCAAT GCAAAATCAT GCTGCCGTGT TCCGTGTGGG AAGCGTGTTG
1621 CAAGAAGGTT GTGGGAAAAT CAGCAAGCTC TATGGAGACC TAAAGCACCT GAAGACGTTC
1681 GACCGGGGAA TGGTCTGGAA CACAGACCTG GTGGAGACCC TGGAGCTGCA GAACCTGATG
1741 CTGTGTGCGC TGCAGACCAT CTACGGAGCA GAGGCGCGGA AGGAGTCACG GGGCGCGCAT
1801 GCCAGGGAAG ACTACAAGGT GCGGATTGAT GAGTACGATT ACTCCAAGCC CATCCAGGGG
1861 CAACAGAAGA AGCCCTTTGA GGAGCACTGG AGGAAGCACA CCCTGTCCTT TGTGGACGTT
1921 GGCACTGGGA AGGTCACTCT GGAATATAGA CCCGTAATCG ACAAAACTTT GAACGAGGCT
1981 GACTGTGCCA CCATCCCGCC AGCCATTCGC TCCTACTGAT GAGACAAGAT GTGGTGATGA
2041 CAGAATCAGC TTTTGTAATT ATGTATAATA GCTCATGCAT GTGTCCATGT CATAACTGTC
2101 TTCATACGCT TCTGCACTCT GGGGAAGAAG GAGTACATTG AAGGGAGATT GGCACCTAGT
2161 GGCTGGGAGC TTGCCAGGAA CCCAGTGGCC AGGGAGCGTG GCACTTACCT TTGTCCCTTG
2221 CTTCATTCTT GTGAGATGAT AAAACTGGGC ACAGCTCTTA AATAAAATAT AAATGAG
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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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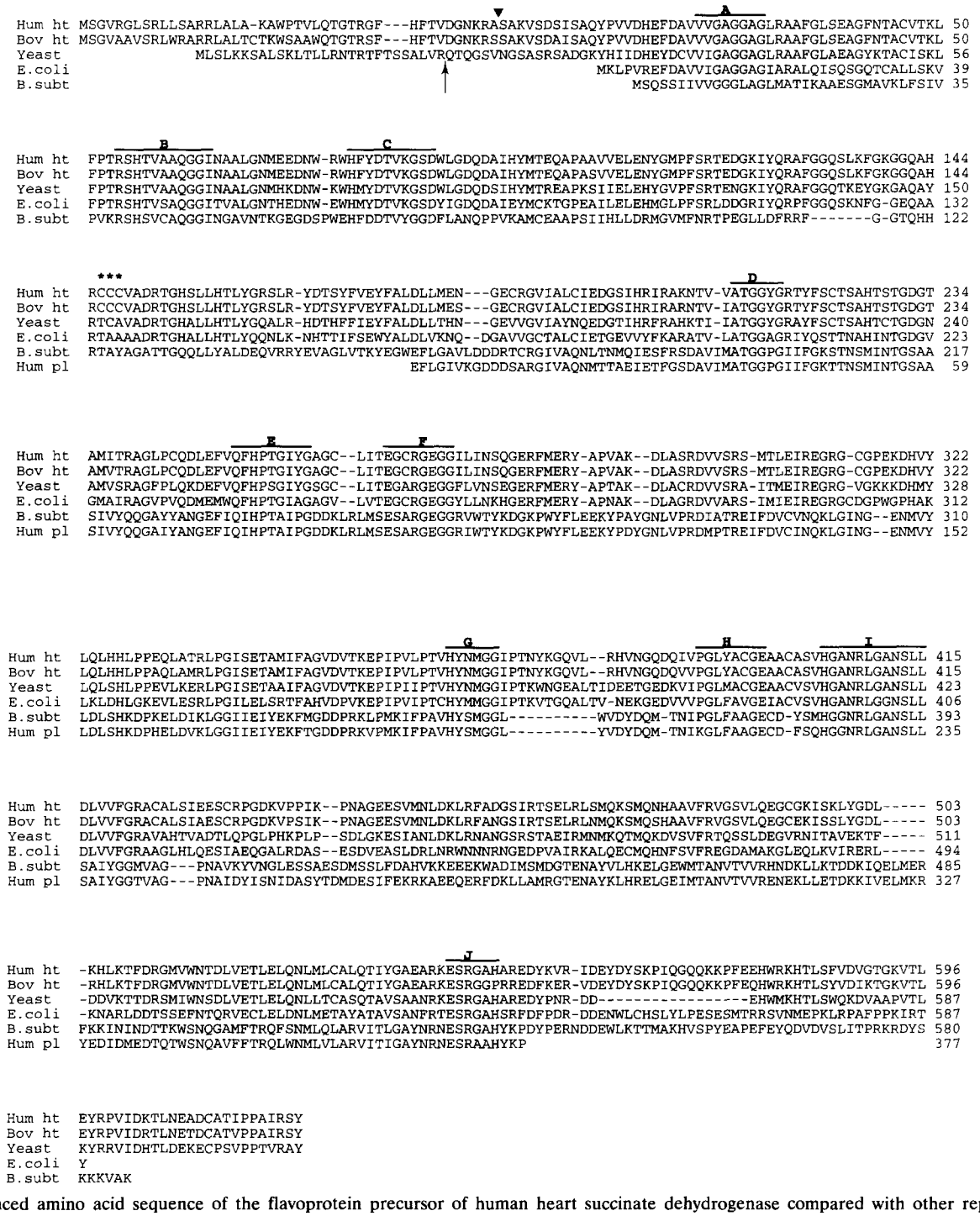

Fig. 2. Deduced amino acid sequence of the flavoprotein precursor of human heart succinate dehydrogenase compared with other reported sequences. The black triangle indicates the bovine presequence cleavage site and the probable cleavage site in the human flavoprotein precursor while the arrow indicates 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.col: *E. coli*; B. subt: *B. subtilis*; Hum pl: human placenta)

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