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HUMAN COMPLEX II(SUCCINATE-UBIQUINONE OXIDOREDUCTASE): cDNA CLONING OF IRON SULFUR(Ip) SUBUNIT OF LIVER MITOCHONDRIA[1]

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Complex II (succinate-ubiquinone oxidoreductase) is an important enzyme complex of both the tricarboxylic acid cycle and of the aerobic respiratory chains of mitochondria in eukaryotic cell and prokaryotic organisms. In this study, the amino acid sequence of iron sulfur-subunit in human liver mitochondria was deduced from cDNA which was isolated by immunoscreening a human liver λgt11 cDNA library. An isolated clone contains an open reading frame of 786 nucleotides and encodes a mature potein of 252 amino acids with a molecular weight of 28,804. The amino acid sequence was highly homologous with that of bovine heart (94.1%) which has been determined from the purified peptide and that of *Escherichia coli sdh B* product (50.8%). Striking sequence conservation was found around the three cysteine-rich clusters which have been thought to comprise the iron-sulfur centers of the enzyme. This is the first report on the cDNA sequence of mitochondrial complex II.

Complex II (succinate-ubiquinone oxidoreductase) catalyzes the oxidation of succinate to fumarate (succinate dehydrogenase) and transfers its reducing equivalents to ubiquinone (see Refs. 1 and 2 for reviews). Complex II also catalyzes the reduction of fumarate, which is the reverse reaction catalyzed by succinate dehydrogenase, in the respiratory chain of

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anaerobic bacteria (3). This activity has been observed not only in bacteria but also in mitochondria of facultative anaerobic animals such as *Ascaris suum*(4,5). Complex II is generally composed of four polypeptides. The largest flavoprotein subunit (Fp) contains covalently bound flavin and second largest subunit contains iron-sulfur (Ip). Two smaller hydrophobic membrane-anchoring peptides (cytochrome b subunit) seem to be essential for converting succinate dehydrogenase into succinate-ubiquinone oxidoreductase. Iron-sulfur clusters are essential prosthetic group for electron transfer in complex II, and three distinct types of iron-sulfur center are present in complex II: S-1; [2Fe-2S], S-2; [4Fe-4S], and S-3; [3Fe-4S](1). At least two centers, [2Fe-2S] and [3Fe-4S] are located in Ip subunit. Association of the [4Fe-4S] center to the Ip subunit has been suggested based on the amino acid sequence of bovine and bacterial Ips, including bacterial fumarate reductase, while there has been controversy on the subunit location of this center (6). Recently, we have found a relationship between the high fumarate reductase activity and novel redox properties of S-3 in mitochondrial complex II from *Ascaris* (7). Clearly, the Ip subunit is a key subunit in complex II and much more information is required to understand its structure, function and assembly. Complete amino acid sequences of complex II have been deduced from the DNA sequences of *sdh* genes in *Escherichia coli* (8,9) and *Bacillus subtilis* (10,11). However, no information on the primary structure of mitochondrial complex II has been available except for the sequence of bovine heart Ip, which was determined from the purified peptide (12), and the partial sequences of *Ascaris* third subunit (cyt bL) (5) and Ip subunit of several species (13). Further, no full length cDNA for the mature polypeptides of mitochondrial complex II has been reported.

This paper reports on the amino acid sequence of the Ip subunit in human liver mitochondria as deduced from the corresponding cDNA. We have chosen human Ip because well established human cell lines will be useful for the investigation on the mechanism of expression and assembly of the subunits as well as the biochemical analysis as described by Ohta *et al.* (14). The homology between mammalian and bacterial enzymes, especially focusing on the three cysteine-rich clusters of Ip which are thought to comprise the iron-sulfur centers, is discussed.

MATERIALS AND METHODS

Screening of cDNA library and sequencing

Human liver cDNA library in λgt11 (Clontech) was screened (15) with antibody against the Ip subunit of bovine heart complex II. The preparation of the antibodies cross-reacting with the fusion protein was carried out as described (16). cDNA insert of the positive clone was isolated and sequenced by using vector pUC118 and helper phage M13K07

(17). DNA sequencing was carried out on both strands by the dideoxy method (18) with appropriate primers: universal primer for M13 phage system and 17 mers at the sequence positions of 41-56, 268-284, 387-403, 447-463, 600-616 and 706-712 going in both directions. The sequence was checked also by using a DNA sequencer (Applied Biosystems Model 370A).

Other methods

Preparation of mitochondria and immunological analysis by Western Blotting were done as described previously (5). Complex II of human liver was identical in catalytic activity, subunit composition and has the epitopes common to bovine enzyme.

RESULTS

Molecular cloning of human liver Ip subunit cDNA

A human liver cDNA library was screened with polyclonal antibody raised against the Ip subunit of bovine heart complex II. From 4×10^5 recombinants, 14 positive clones were obtained and these clones were analyzed by immunoblot analysis. All of the antibodies prepared by the fusion proteins recognized a subunit corresponding to Ip with molecular weight of 27,000 in human liver mitochondria and isolated bovine complex II. The largest cDNA insert of ~1 Kb was sequenced. The DNA sequence and the deduced amino acid sequence of this clone are shown in Fig. 1.

The entire cDNA clone contains an open reading frame of 786 nucleotides from the 5' Eco RI site which encodes a protein of 262 amino acids. The open reading frame ends with a TAA terminator codon followed by 169 nucleotides in the 3' untranslated region. Fifteen nucleotides upstream of the poly d(A) tail, there is a consensus AATAAA polyadenylation signal (20). The amino terminus residue of human Ip is unknown. However, the close homology between human and bovine Ips (12) suggests that the amino terminal of the mature Ip subunit is the alanine which is 11th from amino terminal in this clone, and that the peptide with 10 amino acids from amino terminal is a part of mitochondrial presequence. The partial putative presequence of human Ip is rich in arginine and contains the hydroxylated amino acids serine and threonine. This is characteristic of cleavable amino terminal presequences which are essential for import of mitochondrial proteins encoded by nuclear DNA (21). Thus, the mature human Ip appears to have 252 amino acid residues, and this number is identical with that from bovine heart determined from the purified polypeptide (12). The molecular weight of mature human Ip is calculated to be 28,804, and this value is close to that estimated from immunoblotting (~27,000).

A comparison of the entire amino acid sequence of the human Ip subunit with that of bovine heart and *E. coli sdh* product is presented in Fig. 2. The Ip subunit of complex II is a highly conserved protein and

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                                        ↓
-10  Trp Arg Thr Cys Leu Gln Ala Ser Arg Gly Ala Gln Thr Ala Ala Ala Thr Ala Pro Arg   10
-30  TGG CGG ACG TGC CTG CAG GCC TCC CGA GGA GCC CAG ACA GCT GCA GCC ACA GCT CCC CGT   30

 11  Ile Lys Lys Phe Ala Ile Tyr Arg Trp Asp Pro Asp Lys Ala Gly Asp Lys Pro His Met   30
 31  ATC AAG AAA TTT GCC ATC TAT CGA TGG GAC CCA GAC AAG GCT GGA GAC AAA CCT CAT ATG   90

 31  Gln Thr Tyr Lys Val Asp Leu Asn Lys Cys Gly Pro Met Val Leu Asp Ala Leu Ile Lys   50
 91  CAG ACT TAT AAG GTT GAC CTT AAT AAA TGT GGC CCC ATG GTA TTG GAT GCT TTA ATC AAG  150

 51  Ile Lys Asn Glu Val Asp Ser Thr Leu Thr Phe Arg Arg Ser Cys Arg Glu Gly Ile Cys   70
151  ATT AAG AAT GAA GTT GAC TCT ACT TTG ACC TTC CGA AGA TCA TGC AGA GAA GGC ATC TGT  210

 71  Gly Ser Cys Ala Met Asn Ile Asn Gly Gly Asn Thr Leu Ala Cys Thr Arg Arg Ile Asp   90
211  GGC TCT TGT GCA ATG AAC ATC AAT GGA GGC AAC ACT CTA GCT TGC ACC CGA AGG ATT GAC  270

 91  Thr Asn Leu Asn Lys Val Ser Lys Ile Tyr Pro Leu Pro His Met Tyr Val Ile Lys Asp  110
271  ACC AAC CTC AAT AAG GTC TCA AAA ATC TAC CCT CTT CCA CAC ATG TAT GTG ATA AAG GAT  330

111  Leu Val Pro Asp Leu Ser Asn Phe Tyr Ala Gln Tyr Lys Ser Ile Glu Pro Tyr Leu Lys  130
331  CTT GTT CCC GAT TTG AGC AAC TTC TAT GCA CAG TAC AAA TCC ATT GAG CCT TAT TTG AAG  390

131  Lys Lys Asp Glu Ser Gln Glu Gly Lys Gln Gln Tyr Leu Gln Ser Ile Glu Glu Arg Glu  150
391  AAG AAG GAT GAA TCT CAG GAA GGC AAG CAG CAG TAT CTG CAG TCC ATA GAA GAG CGT GAG  450

151  Lys Leu Asp Gly Leu Tyr Glu Cys Ile Leu Cys Ala Cys Cys Ser Thr Ser Cys Pro Ser  170
451  AAA CTG GAC GGG CTC TAC GAG TGC ATT CTC TGT GCC TGC TGT AGC ACC AGC TGC CCC AGC  510

171  Tyr Trp Trp Asn Gly Asp Lys Tyr Leu Gly Pro Ala Val Leu Met Gln Ala Tyr Arg Trp  190
511  TAC TGG TGG AAC GGA GAC AAA TAT CTG GGG CCT GCA GTT CTT ATG CAG GCC TAT CGC TGG  570

191  Met Ile Asp Ser Arg Asp Asp Phe Thr Glu Glu Arg Leu Ala Lys Leu Gln Asp Pro Phe  210
571  ATG ATT GAC TCC AGA GAT GAC TTC ACA GAG GAG CGC CTG GCC AAG CTG CAG GAC CCA TTC  630

211  Ser Leu Tyr Arg Cys His Thr Ile Met Asn Cys Thr Arg Thr Cys Pro Lys Gly Leu Asn  230
631  TCT CTA TAC CGC TGC CAC ACC ATC ATG AAC TGC ACA AGG ACC TGT CCT AAG GGT CTG AAT  690

231  Pro Gly Lys Ala Ile Ala Glu Ile Lys Lys Met Met Ala Thr Tyr Lys Glu Lys Lys Ala  250
691  CCA GGG AAA GCT ATT GCA GAG ATC AAG AAA ATG ATG GCA ACC TAT AAG GAG AAG AAA GCT  750

251  Ser Val ***                ***     ***                                          252
751  TCA GTT TAA CTG TTT CCA TGC TAA ACA TGA TTT ATA ACC AGC TCA GAG CTG AAC ATA ATT  810

             ***                ***
811  TAT ATC TAA TTT GAG TTC CTT TAA AGA TCT TGG TTT TCC ATG AAT ACA GCA TGT ATA ATA  870


871  AAA ATT TTA AGA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA A    928
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Fig. 1 Nucleotide sequence of the cDNA for Ip subunit of human liver complex II. Numbers indicate the position of nucleotides and amino acid residues from the first amino acid residue of the putative mature peptide. Arrow indicates putative cleavage site of mitochondrial pre-sequence. The polyadenylation signal is underlined.

the homology of mature human liver Ip with bovine Ip was 94.1% and that of human Ip with 237 residues of *E, coli sdh B* product was 50.8%. The partial amino acid sequences of the Ip subunit from several species determined by the polymerase chain reaction (13) are also presented in Fig. 2. The striking homology of the human Ip is observed with the Ip subunits from lower eukaryotes, as well as those from mammalian sources. It is worth noting that there are two different amino acid residues in comparing Ip subunits from human liver and human fibroblast. This difference suggests that there are tissue specific isozymes of complex II in human mitochondria, although much more analysis is essential. Polarity

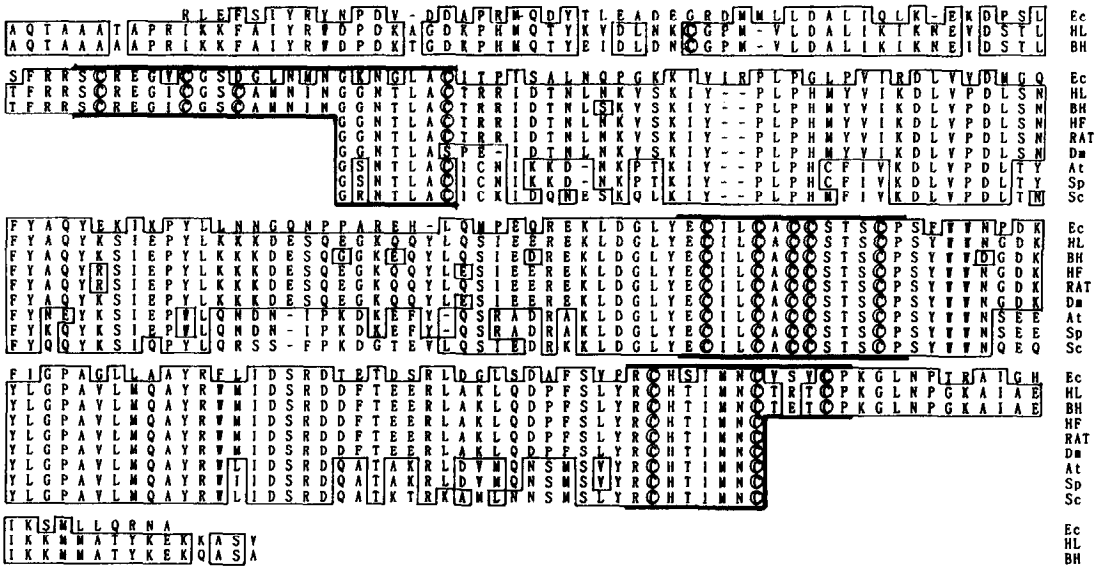

Fig. 2 Comparison of amino acid sequences of Ip subunits from various species. Entire sequence from human liver, HL (this work); bovine heart, BH (12); and *E. coli* (9) are presented. Partial sequences from several species (13) are also aligned. HF, human fibroblast; RAT, rat;, Dm, *Drosophila melanogaster*; At, *Arabidopsis thaliana*; Sp, *Schizosaccharomyces pombe;* Sc, *Saccharomyces cerevisiae.* Identical residues are boxed and the three cysteine-rich clusters are shown, I (Ser64-Cys85), II (Glu157-Pro169) and III (Arg214-Pro226).

of the human Ip subunit was calculated to be 47.2%, in good agreement with the values of mitochondrial Ip peptides obtained from bovine heart (47%) (12) and *Ascaris* muscle (47.4%) (5), and with the characterisitcs of the Ip subunit as a membrane-extrinsic component in complex II.

Iron sulfur center binding sites

Mitochondrial and bacterial complex II, including the fumarate reductase complex, contain three different iron-sulfur clusters (see Refs. 1 and 21 for Reviews). Cysteine residues are clearly essential components for ligation of iron-sulfur centers to the protein. The human Ip subunit contains 13 cysteine residues including a set of cysteine residues which are conserved in bovine (12) and in *E. coli*(9). Distribution of these cysteine residues in three clusters I, II and III (Fig. 2) is consistent with the idea that all three centers are associated with this subunit, although the possibility of the location of S-2 center on Fp or bridging between Fp and Ip cannot be excluded (6). Further, homologies in the regions of the three clusters are higher compared with that of entire peptide in all cases (Table I). Cluster I is similar to that found in [2Fe-2S] clusters of plant and cyanobacterial ferredoxins (24) with a typical alignment of CxxxCxxC-11residues-C, except that the third cysteine is

Table I. Comparison of deduced amino acid sequence of Ip subunit between human liver and other species*

Species	Amino acid sequence homology				
	Whole peptide	S-1	S-2	S-3	Ref.
Bovine heart	94.1	100	100	92.3	12
E. coli sdh B	50.8	68.2	100	69.2	9
B. subtilis sdh B	22.6	45.5	38.5	38.5	11
E. coli frd B	28.2	59.1	46.2	30.8	28
P. vulgaris frd B	29.8	59.1	46.2	30.8	29

* The sequences defined by S-1, S-2 and S-3 are shown in Fig. 2. S-1 (Ser64-Cys85), S-2 (Glu157-Pro169), S-3 (Arg214-Pro226).

replaced by aspartate in *E. coli* (9). These results indicate that cluster I is the binding site of the [2Fe-2S] center S-1. The arrangement of residues in the second cluster resembles that in the bacterial ferredoxins with CxxCxxCxxxCP, which ligates [4Fe-4S] centers (24). Cluster III lacks the second cysteine of the four cysteine residues present in the bacterial ferredoxins, though the arrangement of the other cysteines and the following prolines is similar to that of the ferredoxins. Association of center S-3, containing a [3Fe-4S] cluster, with this region has been suggested from the analogy to the trinuclear iron-sulfur center in *Azotobacter vinelandii* Fd I (1,25). Interestingly, from the hydrophobicity analysis (Fig. 3), these three clusters are found to be located in relatively hydrophobic segments of the Ip polypeptide, as pointed out by Darlison *et al.* in discussing the Ip subunit of *E. coli* (9). This may be relevant to the function of the iron-sulfur centers in mediating electron transport from FAD in the Fp subunit to, a hydrophobic component, cytochrome b and/or ubiquinone.

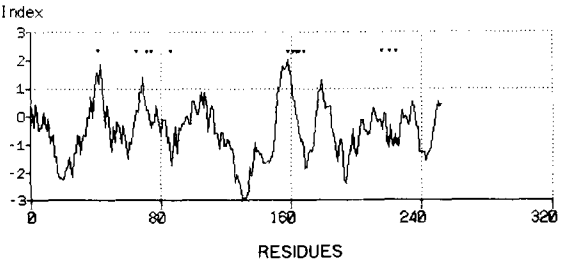

Fig. 3 The hydropathy profile of the human Ip subunit derived according to Kyte and Doolittle (27). The locations of the cysteine residues are indicated by arrows.

CONCLUSION

The result present here is the first report on the cDNA sequence of the Ip subunit in mitochondrial complex II. Striking sequence conservation around the three cysteine-rich clusters indicates their functional importance, and their similarity to ferredoxins emphasizes the idea that the iron-sulfur centers in the Ip subunit of complex II are derived from ferredoxins. The sequence analysis demonstrates extensive homology of the Ip subunits between human mitochondria and *E. coli* (50.8% in amino acid sequence and 52.8% in DNA sequence) (9) compared with *B. subtilis* (22.6% in amino acid sequence) (10). A similar tendency has been found in comparing the sequences of the Fp subunit of *Ascaris* mitochondria and *E. coli* (8) (unpublished observation). These data support the suggestion that mitochondria are more closely related to gram-negative bacteria (26).

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