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Primary structure of a ferredoxin-like iron-sulfur subunit of complex I from *Neurospora crassa*

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Abstract

We have isolated cDNA clones encoding an iron-sulfur polypeptide subunit of the mitochondrial complex I of *Neurospora crassa*. The fungal cDNA library was screened by hybridisation with an heterologous probe from *Paracoccus denitrificans*. The DNA sequence of relevant isolates was determined and revealed an open reading frame encoding a precursor protein of 219 amino acid residues. The gene product is a ferredoxin-like protein that contains two cysteine-rich motives that may each bind a tetranuclear iron-sulfur cluster. The primary structure of the protein is highly homologous to the 23 kDa iron-sulfur subunit of complex I from bovine and *P. denitrificans*. Interestingly, an alanine residue within the second cluster-binding motif, which is conserved in complex I but replaced by tyrosine in similar chloroplast genes, is substituted for serine in *N. crassa*.

Keywords: Mitochondrion; Complex I; Iron-sulfur protein; cDNA; (*Neurospora crassa*)

The mitochondrial H^+-translocating NADH dehydrogenase (Complex I, EC 1.6.5.3) is composed of about 40 polypeptide subunits, 7 of mitochondrial origin and the rest of nuclear origin. This oligomeric enzyme couples rotenone-sensitive transfer of electrons from NADH to ubiquinone to proton translocation across the inner mitochondrial membrane. Protein-bound prosthetic groups are involved in these reactions, a FMN molecule and several iron-sulfur clusters. The exact number of the redox centres is still under discussion, but there are at least one binuclear and three tetranuclear clusters that were characterised by EPR spectroscopy. In addition, there are indications for another cluster of each type, and perhaps even more [1,2]. Enzymes similar to mitochondrial complex I have been identified in prokaryotes and named NDH1. The bacterial operons encoding NDH1 of *Escherichia coli* and *P. denitrificans* were recently cloned and sequenced [3,4]. These enzymes are each composed of 14 proteins, seven of which are homologues of nuclear-coded subunits of bovine complex I, and the rest are homologous to subunits that are encoded in mtDNA. In spite of their presence in both mammalian and bacterial enzymes, two of the nuclear-coded subunits have not yet been identified in complex I of *N. crassa* nor in any of its assembly intermediates. One of these subunits is the 23 kDa iron-sulfur protein (bovine TYKY), and the other is a 20 kDa protein (bovine PSST) that might also bind a prosthetic group [5]. The TYKY subunit may bind two tetranuclear [Fe-S] clusters [6], and thus play a central role in the electron transfer reactions of complex I. On the protein level, there is 72% homology between the 23 kDa subunit of *P. denitrificans* and bovine TYKY [4]. This prompted us to undertake the cloning of the *N. crassa* homologue using the *P. denitrificans* gene as a probe, despite the high GC content of this bacterial DNA.

The *N. crassa* cDNA library [7] was screened by hybridisation, using a digoxygenin-labelled probe that corresponds to the nqo9 gene of *P. denitrificans* encoding the 23 kDa iron-sulfur subunit of NDH1 [4]. The probe was prepared by PCR using the cloned nqo9 gene as template, Vent DNA polymerase (New England Biolabs) and primers from both ends of the coding region. The nucleotide mixture in the reaction was composed of 70% unlabeled nucleotides and 30% DIG-labelling mix (Boehringer) and

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the PCR product was purified with Wizard PCR Preps (Promega). We have screened 5×10^5 library phage and detected 17 positives among them. The cDNA inserts of 6 independent phage were partially sequenced on both ends, and the longest one at the 5′ end was used to rescreen the library. The cDNA inserts of 2 out of 10 positive phage were further analysed, after subcloning in the pGEM4 plasmid vector [7]. The total DNA sequence was obtained by sequencing both strands of different cDNA inserts using Sequenase or Thermo-Sequenase (United States Biochemical) and cDNA-specific oligonucleotides as primers. The Sequenase recommended protocols were applied for sequencing with Thermo-Sequenase with the following modifications: labelling was performed at 42°C and the termination reactions were carried out at 72°C. Computer similarity searches in the EMBL databases were performed with FASTA [8] and other analysis of DNA and protein sequences were performed with the PC/GENE software package (IntelliGenetics).

Fig. 1 depicts the nucleotide sequence of the cDNA and the deduced amino acid sequence of the iron-sulfur subunit of complex I from *N. crassa*. The total DNA sequence is 1116 bp and encodes a precursor protein containing 219 amino acid residues, with a molecular mass of 24902 Da. We assume that the first methionine represents the initial amino acid residue, since no other ATG codon is found in the upstream sequence. The molecular weight of the resulting peptide is close to that of the bovine precursor protein and a mitochondrial targeting signal is predicted for the 34 N-terminal amino acid residues (see below). In addition, if the sequence 5′ to this ATG codon is translated, the resulting protein sequence does not have the characteristics of a mitochondrial presequence. The starting point of the mature polypetide is less clear. A computer analysis predicts that either the first 34 or, less likely, 47 amino acids represent a presequence.

Another possibility is that the mature protein of *N. crassa* results from a two-step cleavage of the precursor polypeptide, because it contains two consensus sequences for such processing [9], RXIXXS (amino acids 20–25) and

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-138                                                    GGCTTGCGACGACAACCA
-120 ACTACCTACCTAGCCACTCCTCTAATATCACACCTGGCGCAACGCATTTGCCTCACCTAC
-60  CCTGCCAGCCAACCTCAAAACCAACAATTAATCAACACCATCCACCGCAACCCGCCCATC

1    ATGCTCACCACCACTGCCTCTTCGGCTGCGGCCGTCGCGCGCCAGCTCACCACCCGCCGA
1     M  L  T  T  T  A  S  S  A  A  A  V  A  R  Q  L  T  T  R  R

61   GTCATCGCCCCCTCCTTCGTCTCTCAGGCCATCCGCACCTACGCAACCCCCGCCGGTCCC
21    V  I  A  P  S  F  V  S  Q  A  I  R  T  Y  A  T  P  A  G  P

121  CCGCCCAAGGGCTTCCGCATTCCGACGCCCAAGACGTGGGACCAAGAGGAGGAACACGTG
41    P  P  K  G  F  R  I  P  T  P  K  T  W  D  Q  E  E  E  H  V

181  CTGGATAAGAACGGACGGTACTTTCTTTTGACGGAGATGTTTAGGGGCATGTATGTGGCT
61    L  D  K  N  G  R  Y  F  L  L  T  E  M  F  R  G  M  Y  V  A

241  ATGGAGCAGTTTTTCAGGCCGCCGTACACAATCTATTACCCCTTCGAAAAGGGTCCCATC
81    M  E  Q  F  F  R  P  P  Y  T  I  Y  Y  P  F  E  K  G  P  I

301  TCCCCCCGCTTCCGCGGCGAGCACGCCCTTCGTCGTTACCCGTCGGGCGAAGAACGCTGC
101   S  P  R  F  R  G  E  H  A  L  R  R  Y  P  S  G  E  E  R  C

361  ATCGCCTGCAAGCTCTGCGAGGCCGTCTGCCCTGCTCAGGCCATCACCATCGAAGCTGAA
121   I  A  C  K  L  C  E  A  V  C  P  A  Q  A  I  T  I  E  A  E

421  GAGCGTGCCGATGGAAGCAGAAGGACGACCCGCTACGATATCGACATGACCAAGTGCATT
141   E  R  A  D  G  S  R  R  T  T  R  Y  D  I  D  M  T  K  C  I

481  TACTGCGGATTCTGCCAGGAGAGCTGTCCCGTGGATGCGATTGTGGAGAGTCCCAATGCG
161   Y  C  G  F  C  Q  E  S  C  P  V  D  A  I  V  E  S  P  N  A

541  GAGTACGCAACGGAGACGAGGGAGGAGTTGTTGTATAACAAGGAAAAGCTACTCTCTAAC
181   E  Y  A  T  E  T  R  E  E  L  L  Y  N  K  E  K  L  L  S  N

601  GGAGACAAGTGGGAGCCTGAGCTTGCGGCTGCTATTCGCGCCGATTCACCTTACAGATAA
201   G  D  K  W  E  P  E  L  A  A  A  I  R  A  D  S  P  Y  R  -

661  AGGGGTTGTGGCTTGAAGACCGGATAACAGGACGGACAATGAAGACAGAGATTCTTTTTT
721  TCGTTGTTGCTATGCTAGAGTTTCGGAACCAGCAGTTTCGGACGGGTTGCGGTATCAACA
781  GGACGCACCATTGTGTTATAGAAGGCAGGCAGGCAGGCGGACAGGCAAAAGAAGGC
841  TGTAGATACATTTGTGAATTGCGTCCCTCATCCAACTTCTTGTCTTTTTGAAGTCATCTC
901  GCCAGGGAAGATCCTATATGCGGCCAAAGGGACGCATCAAGAAACCTAAAGATGCTTGAA
961  AGGCTGAATGTACAATTA
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Fig. 1. Nucleotide sequence of full-length cDNA and deduced primary structure of the iron-sulfur subunit of complex I from *N. crassa*. The consensus sequences for the binding of tetranuclear iron-sulfur clusters are underlined.

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                                                        ..     .
NC  MLTTTASSAAAVARQLTTRRVIAPSFVSQAIRTYATPAGPPPKGFRIPTPKTWDQ -55
BT  ----------------------------------------TYKYVNLREPSM -12

    .      .     *  .  .*  . .        *. ** ****.********** 
NC  EEEHVLDKNGRYFLLTEMFRGMYVAMEQFFRPPYTIYYPFEKGPISPRFRGEHAL -110
BT  DMKSVTDRAAQTLLWTELIRGLGMTLSYLFREPATINYPFEKGPLSPRFRGEHAL -67
PD   MAFDFARATKYFLMWDFIKGFGLGMRYFVSPKPTLNYPHEKGPLSPRFRGEHAL -54

    **** ********************.** * ********************** 
NC  RRYPSGEERCIACKLCEAVCPAQAITIEAEERADGSRRTTRYDIDMTKCIYCGFC -165
BT  RRYPSGEERCIACKLCEAVCPAQAITIEAEPRADGSRRTTRYDIDMTKCIYCGFC -122
PD  RRYPNGEERCIACKLCEAVCPAQAITIDAERREDGSRRTTRYDIDMTKCIYCGFC -109

    **.******** ** *..*** *** * * *** **..** *.*  .  *  **
NC  QESCPVDAIVESPNAEYATETREELLYNKEKLLSNGDKWEPELAAAIRADSPYR -219
BT  QEACPVDAIVEGPNFEFSTETHEELLYNKEKLLNNGDKWEAEIAANIQADYLYR -176
PD  QEACPVDAIVEGPNFEYATETREELFYDKQKLLANGERWEAEIARNLQLDAPYR -164
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Fig. 2. Comparison of the protein sequences of the iron-sulfur subunit of complex I from *N. crassa* (NC), *Bos taurus* (BT) and *P. denitrificans* (PD). Only the mature sequence of bovine TYKY is shown. Identical residues (*) and conservative substitutions (·) are indicated.

RXFXXT (amino acids 66–71). This would yield a mature protein which is shorter than the precursor polypeptide by either 29 or 75 amino acid residues, respectively. It was previously suggested that the bovine homologue is cleaved twice to yield the mature TYKY protein [6].

As with homologues in bovine and bacterial enzymes, and similarly several ferredoxins and chloroplast gene products [5], the *N. crassa* protein includes two sequence motives CXXCXXCXXXCP that may bind tetranuclear [Fe-S] clusters. The 23 kDa subunit of complex I was suggested to bind the [Fe-S] cluster N-2 [6,10], a redox centre that might be directly involved in the proton-translocation activity of the enzyme. Interestingly, a highly conserved (from bacteria to mammals) alanine residue in the second [Fe-S] cluster-binding motif is replaced by serine (residue 168) in the fungal protein (Fig. 2).

In bovine complex I, this protein was suggested to be located at the interface between the 'core' subunits and the hydrophobic ones [10]. This is supported by recent results that locate the protein in the 'connecting fragment' of *E. coli* NDH1 [11]. Since the iron-sulfur centre N-2 was reported to be bound within membrane fragments of Neurospora complex I [12], it will be interesting to determine whether or not this protein is also located in the membrane domain of the enzyme.

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