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Molecular structure of the 8.0 kDa subunit of cytochrome-*c* reductase from potato and its $\Delta\Psi$-dependent import into isolated mitochondria

Hans-Peter Braun, Udo K. Schmitz *

Institut für Genbiologische Forschung GmbH, Ihnestr. 63, 14195 Berlin, Germany

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Abstract

The cytochrome-*c* reductase (EC 1.10.2.2) of the mitochondrial respiratory chain couples electron transport from ubiquinol to cytochrome *c* with proton translocation across the inner mitochondrial membrane. The enzyme from potato was shown to be composed of 10 subunits. Isolation and characterization of cDNA clones for the second smallest subunit reveal an open reading frame of 216 bp encoding a protein of 8.0 kDa. The protein exhibits similarities to a 7.2/7.3 kDa subunit of cytochrome-*c* reductase from bovine and yeast, that is localized on the intermembrane space side of the enzyme complex. It also shows similarity to a previously unidentified 7.8 kDa protein of cytochrome-*c* reductase from *Euglena*. The potato 8.0 kDa protein has a segmental structure, as its sequence can be devided into four parts, each comprising a central Arg-$(Xaa)_5$-Val motif. N-terminal sequencing of the mature 8.0 kDa protein indicates the absence of a cleavable mitochondrial targeting sequence. Import of the in vitro synthesized 8.0 kDa protein into isolated potato mitochondria confirms the lack of a presequence and reveals a dependence of the transport on the membrane potatial $\Delta\Psi$ across the inner mitochondrial membrane. These features are unique among the intermembrane space proteins known so far.

Keywords: Cytochrome-*c* reductase; bc_1 complex; Respiratory chain; Protein transport; Mitochondrion; (*S. tuberosum*)

1. Introduction

The physiological role of the bc_1-complex in bacterial electron transport and energy metabolism relies on three subunits: cytochrome c_1, cytochrome *b* and an iron-sulfur protein (Trumpower, 1990). The equivalent cytochrome-*c* reductase complex of mitochondria also contains these so-called respiratory proteins, but comprises 6–8 additional subunits, the functions of which are less well understood. All of them are rather hydrophilic and are assumed to be peripheral components, that envelope the three respiratory subunits (rewieved in Trumpower, 1990, Bechmann et al., 1992). One of the 'supplementary' proteins is the 'subunit 10' of cytochrome-*c* reductase from bovine (Schägger and von Jagow, 1983). It has a calculated molecular mass of only 7.2 kDa and was reported to be very similar to the 7.3 kDa 'subunit 9' of the respiratory enzyme complex from yeast (Schmitt et al., 1990, Phillips et al., 1990).

The primary structure of the 7.2/7.3 kDa protein is only known for yeast and bovine. In bovine the 7.2 kDa subunit was shown to be part of a cytochrome c_1 subcomplex together with cytochrome c_1 and the so-called 'Hinge' protein upon cleavage of the isolated cytochrome-*c* reductase by treatment with 1.5 M guanidinium (Schägger and von Jagow, 1983, Schägger et al., 1986) or upon a direct chromatographic purification procedure of cytochrome c_1 (Mukai et al., 1985). Consequently the protein is thought to be localized on the intermembrane space side of the inner mitochondrial membrane. As the 7.2 kDa protein can be crosslinked not only with cytochrome c_1, but also with cytochrome *c*, a supportive role of the 7.2 kDa protein in the interaction of the two cytochromes was postulated (Gonzalez-Halphen et al., 1988). In yeast the function of the 7.3 kDa protein was analysed genetically. Overexpression of the gene of the 7.3 kDa protein leads to a suppression of effects of a disruption in the gene encoding the 'Hinge' protein (subunit 6) of the bc_1-complex (Schmitt and Trumpower, 1991). This constitutes evidence of an interaction between these two subunits in yeast and indicates also close neighborhood of the 7.3 kDa protein and cytochrome c_1, as subunit 6 is thought to be associated to

* Corresponding author. e-mail: schmitz@mpimg-berlin-dahlem.mpg.de; Fax: +49 30 83000736.

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cytochrome c_1. Deletion of the gene for the 7.3 kDa protein from yeast causes a petite phenotype. The mutant yeast cells have no bc_1 activity and an altered cytochrome b spectrum (Schmitt et al., 1990, Phillips et al., 1990). The lack of enzymatic activity can be attributed to a loss of electron transfer at the ubiquinol oxidase center (center P), most likely due to an alteration in the insertion of the iron sulfur cluster into the iron sulfur protein (Graham et al., 1992, Phillips et al., 1993, Graham et al., 1993).

Little is known about the small subunits of cytochrome-*c* reductase from higher plants. The enzyme complex is best characterized for potato and was reported to be composed of ten different polypeptides (Berry et al., 1991, Braun and Schmitz, 1992, Braun et al., 1992a). Digestion of the 10 subunits with endoproteinase Lys C and N-terminal sequencing of some of the generated peptides for each component allowed the identification of similarities between the subunits from potato and the sequences of proteins of the equivalent enzyme complex from bovine and yeast (Braun et al., 1994a). Meanwhile, cDNA clones were isolated for all subunits of cytochrome-*c* reductase from potato (Braun et al., 1994b and references therein, Braun and Schmitz, unpublished data). Here we report on the isolation of cDNA clones for the second smallest subunit of cytochrome-*c* reductase encoding an 8.0 kDa protein that resembles the 7.2/7.3 kDa protein from bovine and yeast. We present data on a segmental structure of the potato 8.0 kDa protein and on its $\Delta\Psi$-dependent import pathway to the mitochondrial intermembrane space.

2. Experimental procedures

2.1. Isolation of cDNA clones for the potato 8.0 kDa protein

Copy cDNA clones for the 8.0 kDa protein of cytochrome-*c* reductase from potato were isolated using an amplified λgt11-library of potato tuber (*Solanum tuberosum*, var. 'Desiree') and a mixture of degenerative oligonucleotides which were derived from the N-terminal sequence of a peptide of the second smallest subunit of cytochrome-*c* reductase from potato (Braun et al., 1994a). The mixture contained the full complement of sequences that could potentially encode the octapeptide Lys-Arg-Tyr-Glu-Asp-Ile-Ser-Val (1728 combinations). End-labelling of the oligonucleotides was performed with [γ-^{32}P]dATP using T4 polynucleotide kinase. Screening of the cDNA library was carried out according to standard procedures (Sambrook et al., 1989).

2.2. Cloning, sequencing and computer analysis of DNA

The cDNA inserts were cloned into Bluescript vectors to allow in vitro transcription and translation of the sequences. Overlapping subclones were produced with the exonuclease III deletion strategy using a 'nested deletion kit' (Pharmacia, Uppsala, Sweden). Sequencing of both strands of the DNA inserts was performed following the dideoxynucleotide method (Sanger et al., 1977). The data were analysed with programs of the Staden package on a VAX computer.

2.3. Analysis of proteins

Cytochrome-*c* reductase from potato (*S. tuberosum* L.) was purified by cytochrome *c* affinity chromatography and gel-filtration as described previously (Braun and Schmitz, 1992). Individual subunits of the isolated enzyme complex were separated by SDS/PAGE (Laemmli, 1970) and stained with Coomassie blue. For protein sequencing they were blotted onto Immobilon membranes (Millipore, Bedford, MA, USA) and stained with Ponceau S. The second smallest band was cut out and the N-terminal sequence of the protein was determined by cyclic Edman degradation using an Applied Biosystems pulsed liquid phase sequencer (Kruft et al., 1991).

2.4. In vitro import of the potato 8.0 kDa protein into isolated mitochondria

In vitro transcription of clone pCR8-1, which encodes the complete open reading frame of the potato 8.0 kDa protein, was carried out with a transcription kit (Stratagene, Heidelberg, Germany) according to the supplier's instructions. Translation was performed in the presence of [^{35}S]methionine and [^{3}H]leucine with reticulocyte or wheat-germ lysate (Promega, Madison, USA). Mitochondria for in vitro import reactions were prepared on Percoll step gradients as described previously (Braun et al., 1992b) but washed and resuspended in a buffer containing 0.4 M mannitol, 10 mM KH_2PO_4 (pH 7.2) and 0.1% BSA. For in vitro import reactions 40 μl mitochondria (10 mg protein/ml) were combined with 160 μl import buffer (0.25 M mannitol, 20 mM Hepes (pH 7.5), 80 mM KCl, 1 mM K_2HPO_4, 1 mM ATP, 1 mM malate, 2 mM NADH and 1 mM DTT) and 10 μl radiolabelled precursor protein in reticulocyte lysate. In some import reactions the ionophor valinomycin (final concentration 1 μM) was added to the mitochondria prior to starting the assay. The import mixtures were incubated for 30 min at 20° C and subsequently split into three equal aliquots of 70 μl. One aliquot was kept untreated, while the two others were incubated with proteinase K (final concentration 0.2 mg/ml), one of the latter in the presence of Triton X-100 (final concentration 1%). All aliquots were incubated for 20 min at 20° C, afterwards adjusted to 1 mM PMSF and kept on ice for another 15 min. Mitochondria of the aliquots not treated with detergent were reisolated by centrifugation through a sucrose cushion (0.5 ml 25% sucrose) for 10 min at 13 000 × *g*. The pellets were resuspended directly in loading buffer and the proteins were analysed by the Tricine-

SDS/PAGE system described by Schägger and von Jagow (1987). Gels were treated with 'Amplify' (Amersham, Braunschweig, Germany) and exposed on 'Hyperfilm' (Amersham).

3. Results

3.1. Isolation of cDNA clones encoding the 8.0 kDa subunit of cytochrome-c reductase from potato

Screening of a potato λgt11 cDNA library with an oligonucleotide probe deduced from a partial amino acid sequence of the second smallest subunit of cytochrome-*c* reductase from potato led to the isolation of two positively reacting clones, termed pCR8-1 and pCR8-2. Analysis of the clones revealed inserts of 521 bp with identical sequence (Fig. 1). They contain an open reading frame (orf) of 216 bp encoding a protein of 72 amino acids. The 5′ non-coding region of 103 bp includes an in frame TAA stop codon twelve nucleotides in front of the ATG, indicating that the orf is complete. A poly(A) tail at the end of the 3′ non-coding region of 202 bp is lacking and also a potential polyadenylation signal. Therefore, the 3′ non-coding region of the isolated cDNA clone seems to be incomplete. The amino acid sequence deduced from the open reading frame includes the sequences of the two peptides of the second smallest subunit of cytochrome-*c* reductase from potato, which were published previously (Braun et al., 1994a). It is similar to the sequences of the 7.2/7.3 kDa subunits of cytochrome-*c* reductase from bovine and yeast. Based on the alignment in Fig. 2, 25% of the residues are conserved between potato and bovine and 24% between potato and yeast, while the sequences from bovine and yeast show 35% identity. The subunit of cytochrome-*c* reductase from potato has 11 basic and 8 acidic residues, which are located in the N- and C-terminal parts of the protein (amino acids 1–23 and 39–72). In the middle lies a stretch of uncharged/hydrophobic residues which is not likely to form a membrane spanning helix, as it comprises only 15 amino acids. This tripartite structure is also found in the equivalent proteins from bovine and yeast.

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                                                                  56
CTCAAACCTCAAAGCAAATCATCGCATTTTCTCAAGATTCATCTCTTTTCTCTTCA
                                                   M   E   S      3
AATTAGCAGATCTAAGCAAAACCCAGAAGAGATTTTAAGCTCAGAAA ATG GAA TCA   112

 A   A   R   R   S   G   G   G   V   L   E   G   F   Y   R     18
GCT GCT AGA AGA AGC GGT GGT GGT GTT CTT GAA GGA TTT TAC CGG    157

 L   V   M   R   R   T   P   V   Y   V   T   F   V   I   A     33
CTC GTT ATG CGC CGT ACC CCT GTC TAT GTT ACC TTT GTC ATC GCC    202

 G   A   L   L   G   E   R   A   V   D   Y   G   V   K   T     48
GGC GCT TTG CTC GGC GAA CGG GCG GTG GAT TAT GGG GTT AAA ACA    247

 L   W   E   K   N   N   V   G   K   R   Y   E   D   I   S     63
CTC TGG GAG AAG AAC AAT GTT GGG AAG CGT TAC GAG GAT ATT TCA    292

 V   L   G   Q   R   P   V   D   E   *                        72
GTT CTT GGA CAG CGG CCT GTT GAT GAA TAA AGTTTCAGTAATGAAGTTT    341

ATTTGAGACAAGAGACAGAAGTACTTGTTATCAATATTTCAATAATCGTTTTGCTAATC    400

CTGCATTGCTGGCTTTCTTTTATATATTTGCTGTAGTTTTGAAGGAAATTCATTGAATG    459

AAATATGATGCCAACTTGAAGTTTATGTCATATTGGCTTGTATTTGACACTGTGGGATG    518

ACA                                                            521
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of the insert of clone pCR8-1. The sequence data of the 8.0 kDa subunit of cytochrome-*c* reductase from potato have been submitted to the EMBL sequence data banks and are available under the accession number X79274.

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potato          MESAARRSGGGVLEGFYRLVMRRTPVYVTF
bovine                VAPTLTARLYSLLFRRTSTFALT
yeast                 MSFSSLYKTFFKRNAVFVGT

VIAGALLGERAVDYGVKTLW-EKNNVGKRYEDISVLGQRPV----DE
IVVGALFFERAFDQGADA-IYEHIMEGKLWKHIKHKYENK
IFAGAFVFQTVFDT-AITSWYENHNKGKLWKDVKARIAAGDGDDDDE
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Fig. 2. Comparison of the amino acid sequence of the 8.0 kDa subunit of cytochrome-*c* reductase from potato with the sequences of the 7.2 kDa subunit from bovine (Schägger and von Jagow, 1983) and the 7.3 kDa subunit from yeast (Schmitt et al., 1990, Phillips et al., 1990). Identical residues are boxed and 1–2 gaps per sequence are introduced to optimize the alignment.

3.2. Characterization of the N-terminus of the mature 8.0 kDa protein

Different values varying between 7.5 and 11 kDa were reported for the apparent molecular mass of the second smallest subunit of cytochrome-*c* reductase from potato in SDS-gels using glycine buffers (Berry et al., 1991, Braun and Schmitz, 1992). Reinvestigation of the isolated enzyme complex by the Tricine-SDS/PAGE system of Schägger and von Jagow (1987), which is especially suitable for the separation and size determination of small proteins, revealed an apparent molecular size of about 8

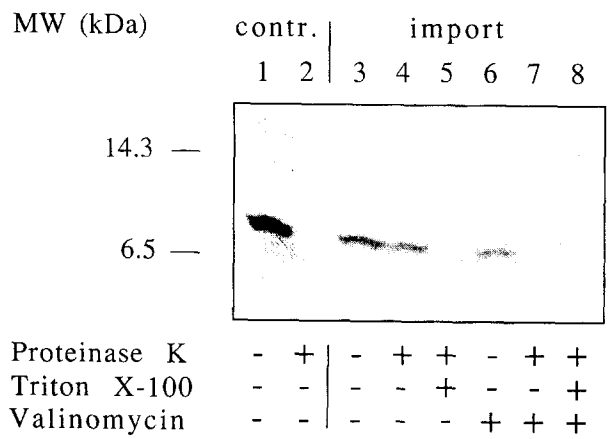

Fig. 3. Import of the 8.0 kDa protein of cytochrome-*c* reductase from potato into isolated mitochondria. The protein was synthesized by transcription of the insert of clone pCR8-1 and by translation of the transcripts in the presence of [^{35}S]methionine and [^{3}H]leucine. The radiolabelled translation product was analysed by SDS/PAGE and fluorography. Synthesis of the 8.0 kDa protein and its sensitivity towards proteinase K is shown in lanes 1 and 2. Lanes 3–8 exhibit import experiments in the presence or absence of proteinase K, Triton X-100 and valinomycin as indicated. The molecular mass of two standard proteins is given on the left (in kDa).

kDa (not shown). As the calculated molecular mass of the deduced amino acid sequence is 8043 Da, a cleavable mitochondrial targeting sequence seems to be lacking. To verify this conclusion, the subunits of the purified cytochrome *c* redcutase complex were separated by SDS/PAGE, blotted onto PVDF membranes and N-terminal sequences of the proteins were determined by cyclic Edman degradation. The obtained sequence for the mature 8.0 kDa protein was Met-Glu-Ser-Ala-Ala-Arg-Arg-Ser-Gly-Gly-Gly-Val-Leu-Glu-Gly-Phe-Tyr, which is identical to the deduced amino acid sequence of the first 17 codons of the corresponding open reading frame.

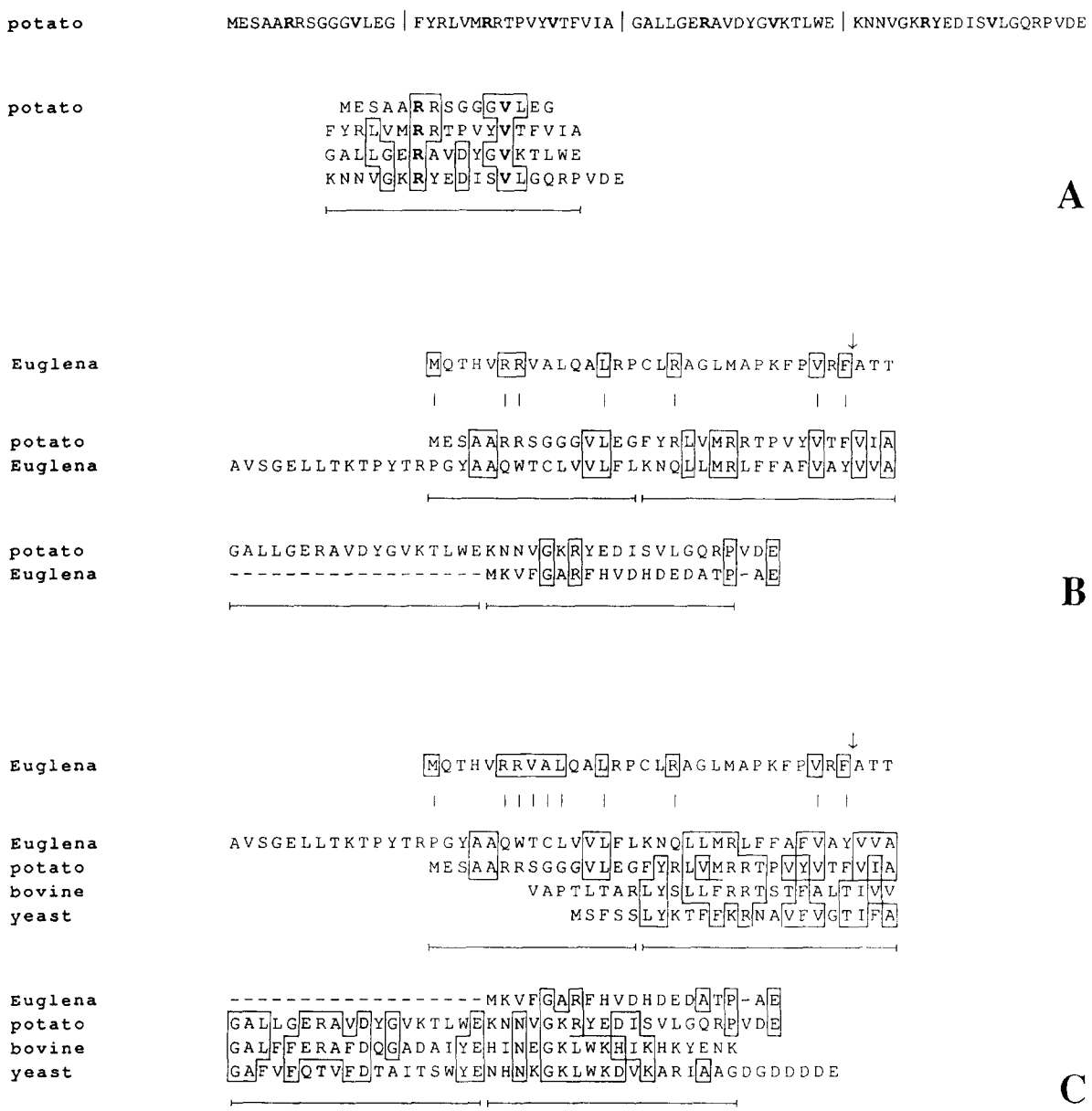

Fig. 4. Segmental structure of the 8.0 kDa subunit of cytochrome-*c* reductase from potato and comparison of the protein with an unidentified 7.8 kDa subunit of the equivalent enzyme complex from *Euglena* (Cui et al., 1994). (A) The amino acid sequence of the potato 8.0 kDa protein can be devided into four parts that exhibit structural similarities. Identical residues between the aligned segments are boxed. The segments comprise 18 amino acids (indicated by the bar), but three amino acids are missing in the first segment and the last segment has three additional residues (B) Sequence comparison of the potato 8.0 kDa protein with the 7.8 kDa subunit of cytochrome-*c* reductase from *Euglena*. Identical residues are boxed, and the segments of the protein from potato are indicated by bars. Two gaps are introduced to the sequence from *Euglena* to optimize the alignment. The *Euglena* protein was reported to have a cleavable presequence (arrow). In addition to the similarity of the mature 8.0 kDa protein from potato and the mature 7.8 kDa protein from *Euglena* the presequence of the Euglena protein resembles the N-terminus of the mature potato protein. Amino acids of the presequence of the 7.8 kDa protein from *Euglena* that are identical to residues of the mature 8.0 kDa protein from potato are also boxed and indicated by vertical marks beneath (top line of part B of the figure). (C) Assembly of the alignments in Fig. 2 and 4B to show similarity between the 7.8 kDa protein from *Euglena* and the 7.2/7.3 kDa proteins from bovine and yeast. Identical residues are boxed and the bars correspond to the segments of the 8.0 kDa protein from potato. Amino acids of the cleavable presequence (arrow) of the 7.8 kDa protein from *Euglena* are boxed and indicated by vertical marks beneath if they are identical to one or more residues of the mature N-termini of the proteins from potato, bovine and yeast (top line of part C of the figure).

3.3. *In vitro import of the 8.0 kDa protein into isolated potato mitochondria*

For in vitro import experiments the insert of clone pCR8-1 was transcribed and the generated mRNA subsequently translated by the use of reticulocyte lysate or wheat germ lysate. As the potato 8.0 kDa protein only contains two methionines, the translation was performed in the presence of [^{35}S]methionine and [^{3}H]leucine. Synthesis and import of the 8.0 kDa protein is shown in Fig. 3. The translation product (lane 1) is sensitive towards proteinase K (lane 2). Upon incubation with isolated mitochondria the precursor cofractionates with the reisolated organelles (lane 3). To examine whether the 8.0 kDa protein is only bound to the mitochondrial surface or actually imported into the organelles, the import reaction was incubated with proteinase K. Most of the radiolabelled protein turned out to be resistant towards the proteinase (lane 4), indicating that it had been internalized by the mitochondria. Upon addition of Triton X-100, the 8.0 kDa protein regained its sensitivity towards proteinase K (lane 5). These data suggest that the in vitro synthesized protein is efficiently imported into isolated mitochondria. The apparent molecular mass of the translation product prior and after import is identical, confirming the absence of a cleavable presequence for mitochondrial targeting. To test whether the translocation of the protein depends on the membrane potential ($\Delta\Psi$) across the inner mitochondrial membrane the import experiments were repeated in the presence of valinomycin. As shown in lane 6 of Fig. 3, the ionophor does not completely prevent cofractionation of some of the radiolabelled protein with reisolated organelles, probably due to binding of the protein to the mitochondrial surface. However, in the presence of valinomycin all the labelled protein is sensitive towards proteinase K (lane 7) and therefore not internalized. Consequently, the import of the 8.0 kDa protein into mitochondria clearly depends on $\Delta\Psi$.

4. Discussion

The 8.0 kDa protein of cytochrome-*c* reductase from potato was sequenced and shown to be similar to the 7.2/7.3 kDa proteins of the respiratory enzyme complex from yeast and bovine. Analysis of sequence motifs within the 8.0 kDa protein also reveals internal similarities, that seem to reflect a segmental structure of the subunit. The sequence of the potato 8.0 kDa protein can be devided into four approximately equal parts, that all have a central Arg-(Xaa)$_5$-Val motif and also show further sequence identities (Fig. 4A). The segmental structure is less visible in the 7.2/7.3 kDa proteins from bovine and yeast (Fig. 2, Fig. 4C). Possibly the original structure of this subunit is most conserved in potato and reflects the distinct events during the molecular evolution of the protein. It is conceivable that the subunit evolved by two or three duplication events of a stretch of about 18 amino acids. On the other hand a random evolution of the repetition of sequence motifs in the 8.0 kDa protein from potato can not be excluded. Sequence analysis of the corresponding subunit of further organisms should allow to distinguish between these possibilities.

Recently isolation and sequencing of a cDNA encoding a 7.8 kDa subunit of cytochrome-*c* reductase from *Euglena* was reported (Cui et al., 1994). As sequence comparison between this protein and subunits of the enzyme complex from yeast and bovine did not reveal significant identities, the 7.8 kDa protein is discussed as a unique component of cytochrome-*c* reductase from *Euglena*. However, the protein does show 20% sequence identity to the 8.0 kDa protein of the respiratory complex from potato (Fig. 4B). Compared to the potato 8.0 kDa subunit the protein from *Euglena* has an extension at the N-terminus and lacks a stretch of 18 amino acids corresponding to the third segment of the potato sequence. The *Euglena* protein comprises a cleavable mitochondrial presequence of 30 amino acids, which shows 23% identity to the N-terminal region of the mature 8.0 kDa protein from potato (Fig. 4B). A reexamination of the identity between the *Euglena* protein and the 7.2/7.3 kDa subunits of cytochrome-*c* reductase from bovine and yeast on the basis of the potato/*Euglena* alignment in Fig. 4B indicates limited similarities, which are at the borderline of significance (Fig. 4C). However, interpretations concerning the 7.8 kDa protein being an unique component of the cytochrome-*c* reductase complex from *Euglena* seem to be questionable.

Like the 7.2/7.3 kDa proteins of cytochrome-*c* reductase from bovine and yeast, which were shown to be physically or functionally linked to cytochrome c_1 or to the iron sulfur protein, the potato 8.0 kDa protein is presumedly localized on the periplasmic side of the inner mitochondrial membrane. Two alternative import pathways are known for nuclear encoded proteins of the mitochondrial intermembrane space (reviewed in Pfanner and Neupert, 1990, Glick et al., 1992a, Hannavy et al., 1993, Neupert, 1994): a direct way accross the outer mitochondrial membrane and an indirect way, that includes partial or complete transport of proteins to the mitochondrial matrix and subsequent reexport accross the inner mitochondrial membrane. Direct transport is known for proteins without cleavable presequences and does not require the membrane potential $\Delta\Psi$ (e.g., cytochrome *c* or cytochrome *c* heme lyase, Stuart and Neupert, 1990, Lill et al., 1992). Indirect transport requires $\Delta\Psi$ and presequences with complex targeting information, that show a typical bipartite polarity profile corresponding to two targeting domains: a hydrophilic N-terminal 'matrix-targeting-domain' with a preponderance of basic and hydroxylated amino acids and an uncharged/hydrophobic 'intramitochondrial-sorting-domain' (e.g., cytochrome c_1 and cytochrome b_2, Glick et al., 1992b, Schwarz et al., 1993). Import of the potato 8.0 kDa protein seems to be indirect,

as its transport into isolated mitochondria is inhibited by the ionophor valinomycin. The N-terminus of the unprocessed 8.0 kDa protein also shows the typical polarity profile of indirectly imported intermembrane space proteins. The stretch from amino acid 1 to 23 is hydrophilic containing 5 basic and two acidic residues, the following region from amino acid 24 to 38 is uncharged and hydrophobic. However, the potential mitochondrial targeting sequence is not removed from the protein upon import, as indicated by direct sequencing of the mature protein. Also the yeast 7.3 kDa protein only lacks the initiator methionine of the deduced sequence from the corresponding open reading frame (Phillips et al., 1990). There are other proteins that are known to be imported to the mitochondrial matrix or to the inner mitochondrial membrane without a cleavable targeting sequence, including some of the small subunits of cytochrome-*c* reductase (reviewed in Trumpower, 1990) and including the adenine nucleotide translocator from beef and fungi (Adrian et al., 1986), but the 8.0 kDa protein from potato seems to be the first example of an intermembrane space protein that is shown to reach its final destination in a $\Delta\Psi$ dependent manner without having a cleavable presequence.

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References

Adrian, G.S., McCammon, M.T., Montgomery, D.L. and Douglas, M.G. (1986) Mol. Cell. Biol. 6, 626–634.

Bechmann, G., Schulte, U. and Weiss, H. (1992) In: Molecular mechanisms in bioenergetics (Ernster, L., ed.), pp. 199–216, Elsevier, Amsterdam.

Berry, E.A., Huang, L. and DeRose, V.J. (1991) J. Biol. Chem. 266, 9064–9077.

Braun, H.P. and Schmitz, U.K. (1992) Eur. J. Biochem. 208, 761–767.

Braun, H.P., Emmermann, M., Kruft, V. and Schmitz, U.K. (1992a) EMBO J. 11, 3219–3227.

Braun, H.P., Emmermann, M., Kruft, V. and Schmitz, U.K. (1992b) Mol. Gen. Genet. 231, 217–225.

Braun, H.P., Kruft, V. and Schmitz, U.K. (1994a) Planta 193, 99–106.

Braun, H.P., Jänsch, L., Kruft, V. and Schmitz, U.K. (1994b) FEBS Lett. 347, 90–94.

Cui, J.Y., Mukai, K., Saeki, K. and Matsubara, H. (1994) J. Biochem. 115, 98–107.

Glick, B.S., Beasley, E.M. and Schatz, G. (1992a) TIBS 17, 453–459.

Glick, B.S., Brandt, A., Cunningham, K., Müller, S., Hallberg, R.L. and Schatz, G. (1992b) Cell 69, 809–822.

Gonzalez-Halphen, D., Lindorfer, M.A., Capaldi, R.A. (1988) Biochem. 27, 7021–7031.

Graham, L.A., Phillips, J.D. and Trumpower, B.L. (1992) FEBS Lett. 313, 251–254.

Graham, L.A., Brandt, U., Sargent, J.S. and Trumpower, B.L. (1993) J. Bioenerg. Biomem. 25, 245–257.

Hannavy, K., Rospert, S. and Schatz, G. (1993) Curr. Opinion Cell Biol. 5, 694–700.

Kruft, V., Kapp, U., Wittmann-Liebold, B. (1991) Biochimie 73, 855–860

Laemmli, U.K. (1970) Nature 227, 680–685.

Lill, R., Stuart, R.A., Drygas, M.E., Nargang, F. and Neupert, W. (1992) EMBO J. 11, 449–456.

Mukai, K., Miyazaki, T., Wakabayashi, S., Kuramitsu, S. and Matsubara, H. (1985) J. Biochem. 98, 1417–1425.

Neupert, W. (1994) Clinical Investigator 72, 251–261.

Pfanner, N. and Neupert, W. (1990) Ann. Rev. Biochem. 59, 331–353.

Phillips, J.D., Schmitt, M.E., Brown, T.A., Beckmann, J.D. and Trumpower, B.L. (1990) J. Biol. Chem. 265, 20813–20821.

Phillips, J.D., Graham, L.A. and Trumpower, B.L. (1993) J. Biol. Chem. 268, 11727–11736.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Habor, Second Edition.

Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Nat. Acad. Sci. USA 74, 5463–5467.

Schägger, H. and von Jagow, G. (1983) Hoppe-Seyler's Z. Physiol. Chem. 364, 307-311.

Schägger, H. and von Jagow, G. (1987) Anal. Biochem. 166, 368–379.

Schägger, H., Link, T.A., Engel, W.D., von Jagow, G. (1986) Methods Enzymol. 126, 181–191.

Schmitt, M.E. and Trumpower, B.L. (1991) J. Biol. Chem. 266, 14958–14963.

Schmitt, M.E., Phillips, J.D. and Trumpower, B.L. (1990) Biochim. Biophys. Acta 1018, 119–123.

Schwarz, E., Seytter, T., Guiard, B. and Neupert, W. (1993) EMBO J. 12, 2295–2302.

Stuart, R.A. and Neupert, W. (1990) Biochimie 72, 115-121.

Trumpower, B.L. (1990) Microbiol. Rev. 54, 101–129.