Molecular Characterisation of the 76 kDa Iron-Sulphur Protein Subunit of Potato Mitochondrial Complex I

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The proton-translocating NADH:ubiquinone oxidoreductase or complex I (EC 1.6.5.3) has been found in mitochondria of all eukaryotes except some fermentative yeasts, whereas genes for a homologous multisubunit complex in chloroplasts have to date only been found on the plastid genome. In potato (Solanum tuberosum L.), the gene coding for the mitochondrial 76 kDa iron-sulphur protein is identified in the nuclear genome. The gene is transcribed into polyadenylated mRNA which is most abundant in flowers, and more frequent in tubers than in leaves. The amino acid sequence is well conserved relative to the mitochondrial and nuclear genomes in different organisms, whereas genes for a homologous multisubunit complex in chloroplasts have to date only been found on the plastid genome. In potato (Solanum tuberosum L.), the gene coding for the mitochondrial 76 kDa iron-sulphur protein is identified in the nuclear genome. The gene is transcribed into polyadenylated mRNA which is most abundant in flowers, and more frequent in tubers than in leaves. The amino acid sequence is well conserved relative to the nuclear-encoded 75 kDa and 78 kDa subunits of Bos taurus and Neurospora crassa, respectively, and to the Paracoccus denitrificans homologue, most prominently in the region presumed to carry the iron-sulphur clusters. Polyclonal antibodies directed against the 78 kDa complex I subunit of N. crassa recognise the 76 kDa polypeptide in potato mitochondrial complex I, and additionally a polypeptide of 75 kDa in solubilised stroma thylakoids from spinach chloroplasts. The 32 amino acid residues long presequence of the potato mitochondrial 76 kDa complex I subunit targets the precursor polypeptide into isolated potato mitochondria but not into isolated chloroplasts. These results suggest that chloroplast stroma thylakoids contain a protein similar in size and antigenicity to, but genetically distinct from, the mitochondrial subunit.

Key words: Chloroplast — NADH:ubiquinone oxidoreductase — NAD(P)H:plastoquinone oxidoreductase — Plant mitochondria — Respiratory chain (Complex I) — Solanum tuberosum.
several mitochondrially encoded complex I subunits (ND 1-6, ND 4L) found in fungi and mammals (Walker 1992), potato mitochondria also code for the NAD7 and NAD9 subunits, the homologues of the bovine nuclear-encoded 49 and 30 kDa subunits (Gäßler et al. 1994, Grohmann et al. 1994). In the protists Acanthamoeba castellanii and Dicysto- stelium discoideum, genes for homologues of the 75 kDa subunit of bovine complex I have been found on the mitochondrial genome (Burger et al. 1995, Cole et al. 1995).

The plastid genomes of several plants contain 11 genes coding for homologues of mitochondrial complex I subunits (Shimada and Sugiiura 1991). The genes are expressed and the protein products localised in the stroma thylakoid membrane, where they may be subunits of a plastoxinone reductase involved in cyclic electron transport (Nixon et al. 1989, Berger et al. 1993, Friedrich et al. 1995, Mi et al. 1995). However, no homologue of the bovine 75 kDa subunit is encoded by the plastid genome. Such a subunit would have to be encoded by the nuclear genome and imported into chloroplasts. A nuclear gene may even encode a protein targeted to, and active in, both mitochondria and chloroplast, as has been shown for glutathione reductase (Creissen et al. 1995). Also for physiological studies of the nuclear-encoded minimal subunits of plant mitochondrial complex I, it is necessary to know if homologous polypeptides exist in chloroplasts.

Here we report the characterisation of the nuclear gene for the 76 kDa subunit of complex I in potato mitochondria, the homologue of the bovine 75 kDa subunit. Furthermore, expression of this gene in different potato plant organs has been investigated, and the intracellular localisation of the protein analysed.

Materials and Methods

Isolation of organelles and sub-organelar fractions—Mitochondria were isolated from potato tubers (Solanum tuberosum L. cv. Bintje) from a local market, and complex I was purified according to Herz et al. (1994). For immunological analysis, spinach chloroplasts were purified (Nakatani and Barber 1977) and fractionated into stroma, thylakoids and envelope (Robinson and Barnett 1988). Stroma and grana thylakoids were separated by differential solubilisation according to Berthold et al. (1981) with the modifications of Völker et al. (1985). Import-competent chloroplasts were isolated from pea leaves according to Schindler et al. (1987).

Protein analysis—The subunit with apparent molecular mass of 75 kDa was purified from isolated potato mitochondrial complex I (Herz et al. 1994) by SDS-PAGE, extracted from blots, digested with endoproteinase Lys-C, fractionated by reverse-phase chromatography, and peptide-sequenced according to Grohmann et al. (1994).

Blotted proteins were immunodetected with the ECL system (Amersham, Little Chalfont, England).

Isolation and sequencing of cDNA clones—Poly(A)+-RNA was isolated from total potato leaf RNA with oligo(dT)-magnetic beads (Dynal, Hamburg, Germany), and first-strand cDNA was synthesised with a preamplification system (Gibco BRL, Life Technologies, Eggenstein, Germany). The first PCR amplifications were done between primers Homol 1 and Lys C 1. The 1.3 kb product was used as template for a second PCR reaction between primers Lys C 2 and Lys C 1. Primer Homol 1, 5'-GAYGTYC- CIATHTGTYGAYCARGG-3', was derived from the peptide sequence of a conserved region in homologues from other species. Primer Lys C 1, 5'-TTTRCTAATTTIACRTCTNCG-3' and Lys C 2, 5'-AARAYGIGITTTCGCNGTNGA-3', were deduced from the peptide sequences of fragments of the purified subunit of potato (Fig. 1). The PCR amplifications were done with Taq-polymerase (Gibco BRL), following the manufacturers instructions, for 30 cycles: 1 min at 94°C, 2 min at 45°C and 2 min at 72°C. The 193 bp product of the second PCR was randomly labelled, and used to screen a potato (cv. Desiree) cDNA library in λZAPII (Koßmann et al. 1992) according to Grohmann et al. (1992).

Sequencing of PCR fragments and cDNA clones was carried out with a T7 sequencing kit (Pharmacia, Uppsala, Sweden) according to the manufacturers instructions. The cDNA clone was sequenced on both strands by a combination of subcloning and oligonucleotide primers. Sequences were analysed with the GCG program package, version 8.1 (University of Wisconsin, Madison, Wi., U.S.A.). The program Pileup was used for amino acid sequence alignments, Gsp for calculating similarities between homologues, and Pepstructure for prediction of α-helices in proteins.

Blotting of nucleic acids—Total RNAs were isolated from potato flowers, leaves, and tubers according to described methods (Chomczynski and Sacchi 1987, Chang et al. 1993). Poly(A)+-RNA was isolated as described above. Northern blotting of total and poly(A)+-RNA, Southern blotting of endonuclease restricted potato DNA, and hybridisation to randomly labelled probes was carried out according to standard procedures (Sambrook et al. 1989).

Organellar import of precursor polypeptides—An [35S]-methionine-labelled protein product of the sequenced cDNA clone in pBluescript II SK was synthesised with a T7/rabbit reticulocyte lysate in vitro transcription/translation system (Amer- sham). For mitochondrial import 10 μl translation product were mixed with 27 μl mitochondria (=0.27 mg protein) and 107 /ul import buffer (Braun and Schmitz 1995) supplemented with 5 mM MgCl2 and 2 mM methionine. Import reactions were incubated at 25°C for 30 min. Half of the reaction was incubated with 20 μg ml-1 proteinase K at 15°C for 15 min, phenylmethylsulphonylfluoride was added to 1 mM, and after 15 min incubation on ice, mitochondria were resolated by centrifugation through 0.5 ml of 25% (w/v) sucrose. Mitochondrial fractions were washed once (0.4 M sucrose, 10 mM potassium phosphate, pH 7.2, 0.1% bovine serum albumin and 1 mM phenylmethylsulphonylfluoride), resuspended in SDS-PAGE loading buffer, and analysed by SDS-PAGE (Laemmli 1970). Where used, valinomycin (1 μM final concentration) was added to mitochondria before starting import.

Import into pea leaf chloroplasts was carried out according to Waegemann and Soll (1991) using 10 μl translation product and chloroplasts corresponding to 30 μg chlorophyll for each double (+thermolysin) import reaction.

Results

The 76 kDa subunit of complex I is nuclear-encoded in potato—The largest complex I subunit, with an apparent...
The 76 kDa subunit of complex I

molecular mass of 75 kDa, was purified by SDS-PAGE from isolated potato mitochondrial complex I. The N-terminal sequence (ADAAAAAAAADA) was obtained by direct peptide sequencing, and internal peptide sequences (DAVFAVDTIAK and FVYLMGADDVNLDK) were obtained after digestion of the protein with Lys-C endoproteinase and separation of the fragments by reverse-phase chromatography.

With derived degenerated primers, an RT-PCR product was obtained and used as probe to screen a potato cDNA library. Eight similar cDNA clones were isolated and partially sequenced. The largest was sequenced in its full length. The amino acid sequence deduced from the cDNA matches the peptide sequences from the isolated subunit, apart from an additional Ala residue, not seen in the protein sequence (Fig. 1). The first ATG triplet is preceded by an in-frame stop codon verifying cloning of the complete reading frame. No alternative start codon is located before the start of the mature polypeptide. Thus, a cleaved peptide of 32 amino acid residues precedes the mature protein which consists of 706 amino acids, with a total molecular mass of 76,475 Da.

Southern analysis with a HindIII fragment of the cDNA clone (Fig. 1) as probe, corresponding to the most highly conserved part of the protein, showed single hybridising fragment sizes in total cellular potato DNA digested with BamHI, EcoRI or HindIII, whereas no signal was seen in potato mitochondrial DNA (results not shown). The analysed cDNA clone has no poly(A) tail but contains a sequence pattern (AATAAG) 25 nucleotides before the 3' terminus with an upstream GT-rich region (Fig. 1) similar to polyadenylation signals in the 3' untranslated regions of other plant mRNAs (Wu et al. 1995). Northern blots of potato total cellular-, and poly(A)+-RNAs from flowers, leaves and tubers probed with the HindIII fragment show expression and in vivo polyadenylation of the mRNA in all three tissues. The steady-state transcript level is highest in flowers, and higher in tubers than in leaves, in both total-, and poly(A)+-RNA (Fig. 2). The results suggest that the 76 kDa subunit of mitochondrial complex I in potato is encoded by a single nuclear gene, and that the polypeptide is translated from polyadenylated mRNA, more abundant in flowers than in tubers and leaves.

Sequence conservation of the 76 kDa subunit—Amino acid sequence alignment of the mature 76 kDa subunit of potato to the corresponding complex I subunits from other species disclosed an N-terminal region of about 270 amino acid residues to be more conserved than the remaining protein. The N-terminal region contains 59 amino acid residues conserved between all these homologous complex I subunits. Of the C-terminal 500 amino acid residues only 8 are completely conserved (not shown). The y-subunit of the NAD + reducing hydrogenase from Alcaligenes eutrophus lacks the C-terminal part entirely (Tran-Betcke et al. 1990).

Fig. 3 depicts the conservation between the N-terminal parts of the 76 kDa subunit homologues. Whereas the mature peptide sequences from most other species align to a common N-terminus, the potato 76 kDa subunit has an

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**Fig. 1** Schematic map of the analysed cDNA for the 76 kDa subunit of potato mitochondrial complex I. The reading frame (boxed) is preceded by an in-frame stop codon (*). The locations of PCR primers and HindIII restriction sites used in the investigation, as well as a potential polyadenylation signal (AATAAG) with an upstream GT-rich region (24 bp, 88% G or T) is denoted on top. The protein has a cleaved pressecence (white box) before the start of the mature polypeptide (grey box). The deduced amino acid sequences corresponding to the peptide sequences determined by N-terminal and internal protein sequencing of the purified 76 kDa subunit are given below. An alanine not detected by peptide sequencing is underlined. The sequences coding for the peptide segments potentially liganding iron-sulphur clusters are denoted in black.

**Fig. 2** Northern analysis of 76 kDa subunit RNAs in different potato organs. In order to compare equal relative amounts, 43, 54 and 30 µg of flower (F.), leaf (L.) and tuber (T.) total RNA were loaded per lane, respectively. For each organ, poly(A)+-RNA isolated from the three-fold amount of total RNA was loaded. The calibration was done by comparing signal intensities when hybridising with a probe against 25S rRNA (not shown).
N-terminal extension of about 30 amino acid residues. The 12 Cys and 2 His residues potentially liganding iron-sulphur clusters are conserved in the potato sequence. The postulated sequence motifs for a tetranuclear (CXXCXX-CXnCP) and a binuclear (CXXCXnCXXC) iron-sulphur cluster (Walker 1992) are positioned at amino acid residues 180–231 and 79–135 in the potato sequence, respectively. The recently suggested motif for a binuclear cluster found in E. coli (Leif et al. 1995) is not conserved in potato.

A chloroplast thylakoid protein is recognised by an antiserum directed against the 76 kDa homologue of N. crassa mitochondrial complex I—To investigate the potential presence of the 76 kDa polypeptide, or a homologue of it, in chloroplasts, various protein fractions of spinach leaf chloroplasts were resolved by SDS-PAGE, blotted, and probed with a polyclonal antiserum against the 78 kDa subunit of mitochondrial complex I from N. crassa (Friedrich et al. 1989). A polypeptide is detected in total and stroma thylakoid fractions, with an apparent molecular mass of 75 kDa, similar to the immunodetected subunit in isolated complex I from potato (Fig. 4A). The 75 kDa immunodetected chloroplast polypeptide is observed neither in the stroma soluble protein fraction nor in the grana thylakoid fraction. Apart from the 75 kDa polypeptide, no protein is detected in the chloroplast fractions, suggesting a high specificity of recognition by the antibodies. In Coomassie stained gels only a very faint band could be seen at 75 kDa, indicating that the antibodies detect minute protein amounts (not shown). The presence of an immunostained polypeptide at 50 kDa in potato mitochondrial complex I (Fig. 4A, lane 5) indicates a partial proteolysis of the 76 kDa subunit during complex I purification. When immunoblotting total protein from isolated potato mitochondria, the 76 kDa subunit is the only protein recognised by
The 76 kDa subunit of complex I

Western blot analysis of the chloroplast protein fractions with an antiserum against the NDH-H gene product of tobacco, the plastid-encoded homologue of the 49 kDa subunit of bovine complex I, shows a similar distribution as the 75 kDa polypeptide (Fig. 4B). A polypeptide of 43 kDa is recognised in stroma thylakoids and total thylakoids, but not in grana thylakoids or soluble stroma proteins, consistent with the observations of Berger et al. (1993).

To control that contaminating mitochondria was not responsible for the immunoreactive 75 kDa polypeptide, the chloroplast fractions were tested with an antibody against the $\alpha$-subunit of ATPase from maize mitochondria. The antibody did not react with any of the chloroplast fractions but gave a very strong positive reaction in potato mitochondrial proteins (Fig. 4C).

The precursor for the mitochondrial 76 kDa subunit is imported into mitochondria but not into chloroplasts—The plastid genome of tobacco contains three reading frames coding for polypeptides of 70–80 kDa (Shimada and Sugiura 1991), but the encoded proteins have no sequence similarity to the 78 kDa subunit of $N.\ crassa$ mitochondrial complex I (not shown). This suggests that the immunoreactive polypeptide should be encoded in the nucleus, either by the gene specifying the 76 kDa subunit of mitochondrial complex I, or by a separate nuclear gene. If the gene for the mitochondrial subunit also codes for a pro-

Fig. 4 Western analysis of chloroplast subfractions. Proteins were separated on 11% polyacrylamide gels, blotted and incubated with antibodies against: (A) the 78 kDa subunit from $N.\ crassa$, (B) the NDH-H polypeptide from tobacco, and (C) the $\alpha$-subunit of mitochondrial F$_1$-ATPase from maize. Lane 1, stroma soluble proteins (80 $\mu$g); lane 2, extracts of stroma thylakoids (20 $\mu$g Chl); lane 3, grana thylakoids (20 $\mu$g Chl); lane 4, total thylakoids (20 $\mu$g Chl); lane 5, isolated mitochondrial complex I from potato (3 $\mu$g protein in (A), 9 $\mu$g in (C)). The positive control (PC) in (C) is potato tuber mitochondria (80 $\mu$g protein).

Fig. 5 In vitro import analysis of the 76 kDa subunit into mitochondria and chloroplasts. In (A), the deduced presequence of the isolated cDNA for the 76 kDa subunit is shown with positively charged amino acids denoted above. In (B), [35S]-labelled translation products of the cDNA for the 76 kDa subunit were incubated with isolated potato mitochondria under different conditions and radiolabelled polypeptides separated on 6–11% SDS-polyacrylamide gels. The different treatments of the translation products are denoted on top, and the positions of the molecular mass standards are given on the left side. In (C), the similar experiment was conducted with isolated pea leaf chloroplasts. On the left, the import analysis for the 76 kDa subunit is depicted, p76; precursor protein for the 76 kDa subunit. On the right, the control for import competence of the chloroplasts by import of in vitro translated small subunit of Rubisco (SSU) and processing from its precursor form (pSSU) to its mature form (mSSU) is shown. The different treatments of the translation products are denoted on top.
tein in chloroplasts, the targeting peptide should be able to
direct the precursor protein into both organelles.

The deduced amino acid sequence of the 76 kDa sub-
unit of mitochondrial complex I has a 32 amino acid resi-
dues long presequence (Fig. 5A). It is rich in Arg, Ser and
Leu residues, and contains only one acidic amino acid resi-
due close to its C-terminus. The N-terminal 12 amino acid resi-
dues of the presequence are predicted to fold into an am-
phiphilic a-helix with the positively charged Arg residues local-
ised on the polar side (not shown). Most of these features
of the presequence are common properties of mitochon-
drial targeting peptides (von Heijne et al. 1989). However,
targeting peptides for mitochondria and chloroplasts are
very heterogeneous, and at the same time share several com-
mon features. We therefore considered it necessary to re-
solve the question by in vitro import experiments with iso-
lated chloroplasts, using mitochondrial import as a positive
control for competence of the translation product to be
recognised by a bona fide molecular interaction.

The precursor of the 76 kDa polypeptide synthesised
in vitro from the analysed cDNA clone (Fig. 1) is imported
into potato mitochondria, as seen from its acquired resist-
ance against degradation by proteinase K (Fig. 5B). In the
presence of the uncoupler valinomycin, the induced prote-
ase-resistance is abolished. This suggests that import into
mitochondria is dependent on the membrane potential, as
generally seen for matrix-targeted proteins.

The location of the N-terminus of the isolated mature
mitochondrial subunit in the cDNA (Fig. 1) suggests that
the presequence is cleaved off post-translationally, most
likely by the matrix peptidase. In the import analysis
(Fig. 5B), precursor and mature forms are not completely
separated by SDS-PAGE due to the large size of the sub-
unit in relation to the presequence. However, the protein re-
main ing after protease treatment was repeatedly seen (3
independent experiments) to migrate slightly faster than
the unimported, protease-sensitive precursor polypeptide
(+ valinomycin), indicating that the targeting sequence is
indeed cleaved off during or after import.

In vitro import analysis of the translation product was
additionally carried out with isolated pea leaf chloroplasts
(Fig. 5C). The precursor of the 76 kDa subunit is bound to
the outer envelope, but is not protected against thermolysin
digestion, and is thus not imported. The import-compe-
tence of the isolated chloroplasts was verified by the effec-
tive import and processing of the precursor protein of the
small subunit of Rubisco. The chloroplast 75 kDa polypep-
tide recognised by the antibody against the N. crassa mito-
chondrial 78 kDa complex I subunit is thus most likely en-
coded by a gene distinct from the gene encoding the 76 kDa
subunit of mitochondrial complex I in plants.

Discussion

The relationship between the 76 kDa subunit of potato
mitochondrial complex I and homologues in other species
—The primary structure of the largest subunit of mitochon-
drial complex I has now been determined in representatives
of all eukaryotic kingdoms, including plants. In potato, the
gene is located in the nucleus, and the protein is translated
as a precursor subsequently imported into mitochondria.
The absence of the gene for the 76 kDa subunit in the
recently analysed mitochondrial genome of Arabidopsis
(Unseld et al. 1997) indicates that its localisation in the
nucleus may be a general feature of plants. The nuclear
genes for the 75-78 kDa subunits of potato, B. taurus and
N. crassa are most likely of mitochondrial origin and were
introduced into the cells by the original endosymbiont, as
indicated by the marked amino acid sequence similarity to
the corresponding P. denitrificans subunit (Table 1). The
mitochondrial localisation of the gene homologues in the
protists A. castellanii and D. discoideum (Burger et al.
1995, Cole et al. 1995), further supports the endosymbiont
origin of this gene. The transfer of the gene from the mito-

Table 1 Amino acid sequence similarities between 76 kDa protein homologues from
different species

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The mature proteins were compared over the maximum region spanned by all sequences. Abbrevia-
tions of species are as in Fig. 3.
chondria to the nucleus, as seen in potato, *B. taurus* and *N. crassa*, has thus most likely occurred after the separation of protists and higher eukaryotes. Unless the transfer of the gene occurred as separate events, the nuclear location in potato indicates a closer relationship between plants and other higher eukaryotes. This would be inconsistent with the proposed affiliation between land plants and *A. castellanii* based on the gene contents of the mitochondrial genomes (Burger et al. 1995).

In representatives of eubacteria and all eukaryotic kingdoms, including the potato mitochondrial 76 kDa subunit, 12 Cys and 2 His residues are found to be completely conserved (Fig. 3). EPR analysis of potato mitochondrial complex I detected 3 tetranuclear iron-sulphur clusters and one binuclear, probably N-1b, a similar cluster conformation as in *N. crassa* complex I, whereas in *B. taurus*, *P. denitrificans* and *E. coli* also a cluster N-1a has been detected (Lin et al. 1995). The extensive sequence conservation of the putative iron-sulphur cluster liganding region of the 76 kDa subunit between potato, *N. crassa* and the other EPR-analysed species is consistent with a localisation of cluster N-1b in this subunit, as previously suggested (Yano et al. 1995).

**Expression of the 76 kDa subunit mRNA**—The steady-state mRNA levels of the gene for the 76 kDa subunit of potato mitochondria are highest in flowers (Fig. 2), as has been observed for other nuclear-encoded mitochondrial proteins (Huang et al. 1994, Landschütze et al. 1995). On the other hand, mRNA levels of the 76 kDa subunit are higher in tubers than in leaves, even though the Northern hybridisation experiments are corrected for the elevated levels of plastid RNA in leaves. Both the matrix processing peptidase subunit of mitochondrial complex III and the TCA-cycle enzyme citrate synthase show considerably higher mRNA levels in leaves than in tubers of potato (Emmermann et al. 1994, Landschütze et al. 1995). This could indicate a difference in relative amounts of respiratory enzymes between tissues where different substrates are available for mitochondrial oxidation. Plant mitochondria also contain a rotenone-insensitive, non-energy-coupled NADH dehydrogenase competing with complex I for matrix NADH (Rasmusson and Möller 1991). Differences in expression of complex I subunits may then also reflect a variation in the relative amounts of the two enzymes.

**Do chloroplasts contain a homologue of the mitochondrial 76 kDa subunit?**—We have observed specific recognition of one polypeptide in spinach leaf chloroplasts by antibodies against the 78 kDa subunit of *N. crassa* mitochondrial complex I. This polypeptide has an apparent molecular mass similar to the potato mitochondrial 76 kDa subunit, and is located in stroma thylakoids as is the NDH-H polypeptide (Fig. 4).

The genes of the "minimal" complex I subunits not present on the plastid genomes correspond to the 24, 51 and 75 kDa subunits of bovine complex I (Shimada and Sugiiura 1991). Based on the structural and evolutionary connection between these subunits and NADH dehydrogenase activity, Friedrich et al. (1995) suggested that the putative complex I in chloroplasts should contain a different setup of subunits to enable the oxidation of ferredoxin or NADPH instead of NADH, which is less abundant in the stroma. Consistent with this hypothesis, the cyanobacterium *Synechocystis* sp. lacks *ndh* genes for homologues to the 24, 51 and 75 kDa subunits (Kaneko et al. 1996). However, NDH proteins encoded by the plastid genome have been reported to co-elute with NADH, but not NADPH, dehydrogenase activity upon partial purification of a complex I-type enzyme from pea chloroplasts (Sazanov et al. 1996). This suggests that the complex I of chloroplasts could have a similar setup of subunits as the mitochondrial enzyme. The chloroplast enzyme may then contain also the immunoreactive 75 kDa polypeptide (Fig. 4). However, an unequivocal answer to this question will be possible only when the active chloroplast enzyme has been purified to homogeneity and all protein constituents have been characterised.

A possible explanation for the presence of the immunoreactive 75 kDa polypeptide in chloroplasts would be if the product of the gene for the mitochondrial subunit is targeted also to chloroplasts. Such dual targeting has been observed for glutathione reductase (Creissen et al. 1995). However, the gene for the mitochondrial subunit is not likely to specify also the immunoreactive 75 kDa polypeptide in chloroplasts. The amino acid presequence deduced from the isolated cDNA has more properties in common with mitochondrial targeting peptides whereas the glutathione reductase presequence shows a bias towards chloroplast targeting peptides (Creissen et al. 1995). Consistently, the mitochondrial 76 kDa subunit of potato is in vitro only imported into mitochondria (Fig. 5). The relatively lower steady-state mRNA levels in leaves additionally imply that the protein product of the gene for the 76 kDa subunit of mitochondrial complex I plays no role in chloroplasts.

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References


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