Plant mitochondrial pyruvate dehydrogenase complex: purification and identification of catalytic components in potato

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The pyruvate dehydrogenase complex (mPDC) from potato (*Solanum tuberosum* cv. Romano) tuber mitochondria was purified 40-fold to a specific activity of 5.60 $\mu\text{mol}/\text{min}$ per mg of protein. The activity of the complex depended on pyruvate, divalent cations, NAD^+ and CoA and was competitively inhibited by both NADH and acetyl-CoA. SDS/PAGE revealed the complex consisted of seven polypeptide bands with apparent molecular masses of 78, 60, 58, 55, 43, 41 and 37 kDa. N-terminal sequencing revealed that the 78 kDa protein was dihydrolipoamide transacetylase (E2), the 58 kDa protein was

dihydrolipoamide dehydrogenase (E3), the 43 and 41 kDa proteins were α subunits of pyruvate dehydrogenase, and the 37 kDa protein was the β subunit of pyruvate dehydrogenase. N-terminal sequencing of the 55 kDa protein band yielded two protein sequences: one was another E3; the other was similar to the sequence of E2 from plant and yeast sources but was distinctly different from the sequence of the 78 kDa protein. Incubation of the mPDC with $[2\text{-}^{14}\text{C}]\text{pyruvate}$ resulted in the acetylation of both the 78 and 55 kDa proteins.

INTRODUCTION

The mitochondrial pyruvate dehydrogenase complex (mPDC) occupies a central point in cellular metabolism, linking cytosolic glycolytic metabolism with the tricarboxylic acid cycle. The reaction catalysed by mPDC is essentially irreversible, the enzyme activity is regulated by covalent modification and metabolite concentrations [1–3] and its activity, in plants at least, is reported to be low relative to other enzymes of the tricarboxylic acid cycle [1,4]. Hence this complex represents both the committed step of carbon entry to the tricarboxylic acid cycle and potentially the key to understanding the control of respiratory metabolism.

The mPDC has been well characterized in mammals, fungi and bacteria as a large multi-enzyme assembly that catalyses the oxidative decarboxylation of pyruvate to form acetyl-CoA and NADH by the sequential operation of three separate enzymes: pyruvate dehydrogenase (E1; EC 1.2.4.1), dihydrolipoamide transacetylase (E2; EC 2.3.1.12) and dihydrolipoamide dehydrogenase (E3; EC 1.8.1.4). The E1 enzyme contains two polypeptide chains, α and β , whereas both the E2 and E3 enzymes contain a single subunit. E1 kinase (EC 2.7.1.99) and phospho-E1 phosphatase (EC 3.1.3.43) are associated regulatory proteins, which in mammals both consist of two subunits. Mammalian and yeast mPDCs also contain small amounts of a 50 kDa protein, known as component X, which contain a single lipoyl domain. Component X shares some structural, immunological and functional similarities with E2, and has a structural role in E3 binding and the assembly of the E2 core [5,6].

In contrast, little information is available on the structure and subunit composition of plant mPDC. Purification of mPDC to near homogeneity has been achieved only from floral buds of one dicotyledonous plant species, *Brassica oleracea* var. *italica* (broccoli) [7] and var. *botrytis* (cauliflower) [8]. However, neither the sizes of individual polypeptides nor definitive subunit assignments were presented for either of these preparations. Antibodies raised

against the broccoli mPDC holoenzyme of Rubin and Randall [7] have been shown to react with five proteins in pea mitochondrial extracts separated by SDS/PAGE [9]. However, two later reports with anti-(broccoli mPDC) and anti-(bovine mPDC) antibodies in different laboratories have detected a different set of immunoreactive polypeptides with various apparent molecular masses in pea mitochondrial extracts [10,11]. Taylor et al. [11] have also suggested that one of the proteins identified by Camp and Randall [9] might be a spurious reaction with the E1 subunit from the 2-oxoglutarate dehydrogenase complex.

Further attempts to identify the components of plant mPDC have used antibodies raised against mammalian, yeast and several plant mPDC polypeptides. The E1 α component has been identified as a 41–43 kDa protein on the basis of reaction with antibodies raised against E1 α from mammals, yeast and *Zea mays* [11–13] and on the basis of phosphorylation studies [14,15]. Using anti-(pig mPDC E3) antibodies the E3 component in pea mitochondria had been identified as a 58 kDa protein [1] or a 67 kDa [11] protein. More recent results suggest that plants contain a single nuclear-encoded E3 gene and that its 56 kDa product is targeted to the mitochondrion and is a component of glycine decarboxylase, 2-oxoglutarate dehydrogenase and mPDC [16–18]. In contrast with these two subunits, the other components of mPDC from plants are the source of considerable disagreement in the literature: various claims have been made that the plant and mammalian complexes contain catalytic subunits of very different sizes. E1 β -specific antibodies raised against protein from non-plant sources have not been found to cross-react with any pea mitochondrial proteins [11]. For E2, Taylor et al. [11] reported an anti-(mammalian E2) antibody reacting with a 50 kDa protein from pea mitochondria. In contrast, a monoclonal antibody, which serendipitously led to the isolation of a cDNA encoding E2 from *Arabidopsis*, cross-reacts with an 80 kDa protein in pea mitochondria [10]. Immunological studies with antibodies raised against the mammalian X

Abbreviations used: E1, pyruvate dehydrogenase; E2, dihydrolipoamide transacetylase; E3, dihydrolipoamide dehydrogenase; mPDC, mitochondrial pyruvate dehydrogenase complex; MTS, mitochondrial targeting sequence; PEG-6000, 6 kDa poly(ethylene glycol); TPP, thiamine pyrophosphate.

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The N-terminal sequences reported in this paper appear in the Swiss-Prot Database at EMBL under the accession numbers P52903 (E1 α), P81419 (E1 β), P81420 (E2, 55 kDa component), P81421 (E2, 78 kDa component), P80503 (E3).

component have also been used as evidence that a 67 kDa [11] or, in contrast, a 50 kDa [10] immunoreactive protein might be the structural X component of mPDC in pea seedlings.

Very recently, Thelen et al. [19] reported a partial purification of mPDC from the monocotyledonous *C₄* species *Zea mays*. The E1 β (40 kDa), E1 α (43 kDa) and E3 (62–63 kDa) subunits were identified on the basis of immunoreactivity with subunit-specific antibodies raised against mPDC subunits. These authors also identified 52–53 kDa proteins in the partial purification as E2 subunits on the basis of immunoreactivity and N-terminal sequencing. Thelen et al. [19] suggest that the 52–53 kDa proteins probably represent single-lipoyl-domain E2 subunits in contrast with two-lipoyl-domain E2 subunits found in other plant and mammalian mPDCs.

Our aim in this study was to identify definitively the subunits of mPDC from a higher plant source. We present the purification of mPDC to near-homogeneity from potato tubers, present the sizes of the major polypeptide components and identify them by N-terminal sequencing and acetylation studies, rather than simply on the basis of immunoreactivity. The remarkable similarity between the size of component polypeptides of the plant and mammalian mPDC is discussed in the light of earlier conflicting reports to the contrary.

MATERIALS AND METHODS

Isolation of potato mitochondria

Potato tubers (*Solanum tuberosum* cv. Romano) were purchased locally; 5–10 kg was used for mitochondrial preparation. Peeled, sliced tuber tissue was homogenized with a commercial juice extractor and the extract was mixed 4:1 (v/v) with a grinding medium [0.3 M mannitol/80 mM tetrasodium pyrophosphate/KOH (pH 7.5)/10 mM K₂PO₄/1% (w/v) PVP-40/1% (w/v) BSA/2 mM EGTA/25 mM cysteine/HCl]. The homogenate was centrifuged at 2000 *g* for 5 min and the supernatant was centrifuged at 15000 *g* for 20 min. The resultant pellet was resuspended in wash medium [0.3 M mannitol/10 mM Tes/KOH (pH 7.5)/0.1% (w/v) BSA] and centrifuged at 40000 *g* for 45 min layered over a step gradient consisting of 20%, 28% and 50% (v/v) Percoll [20]. Purified mitochondria were aspirated from the 28%/50% (v/v) Percoll interface and washed twice by centrifugation at 15000 *g* for 15 min.

Purification of mPDC

The methods of Randall et al. [8] and Rubin and Randall [7] were modified for the purification of potato mPDC. Isolated mitochondria were diluted to 3–5 mg/ml protein in a solution consisting of 25 mM K₂PO₄/KOH, pH 6.7, 1 mM NAD⁺, 0.1 mM thiamine pyrophosphate (TPP) and 5 mM dithiothreitol, freeze-thawed once in liquid N₂, stirred on ice for 15 min, and then centrifuged at 40000 *g* for 30 min. The supernatant was then centrifuged at 200000 *g* for 180 min. The amber pellet was resuspended in PDC buffer [25 mM Tes/KOH (pH 7.0)/1 mM NAD⁺/0.1 mM TPP/5 mM dithiothreitol] to a final concentration of 4–5 mg/ml protein. After being warmed to 25 °C, 10 mM MgCl₂ was added and the precipitated material was then removed by centrifugation at 20000 *g* for 15 min. The supernatant was again warmed to 25 °C and 0.1 vol. of 50% (w/v) 6 kDa poly(ethylene glycol) (PEG-6000) was added, after which it was centrifuged at 20000 *g* for 15 min. The pellet, which contained the mPDC activity, was resolubilized in PDC buffer supplemented with 2 mM EDTA. Insoluble material was removed by further centrifugation at 20000 *g* for 15 min. This PEG-purified mPDC was then layered on a 1.8 ml 10–50% (v/v)

glycerol gradient [containing nine 0.2 ml steps each increasing by 5% (v/v) glycerol] in PDC buffer and centrifuged in a swing-out rotor at 100000 *g* for 14 h. The addition of as little as 0.003% protamine sulphate completely inhibited potato mPDC activity and thus this step was omitted from purification procedures in contrast with those previously reported for *B. oleracea* purifications (see [7]). Unless otherwise stated, all manipulations were performed at approx. 4 °C.

Activity of mPDC

The activity of the potato mPDC during purification was determined by monitoring the formation of NADH at 340 nm and 25 °C. The reaction medium contained 75 mM Tes/NaOH, pH 7.5, 0.5 mM MgCl₂, 2 mM NAD⁺, 0.2 mM lithium CoA, 0.2 mM TPP, 2.5 mM cysteine/HCl and 1 mM sodium pyruvate [1]. The reaction was initiated by the addition of enzyme or pyruvate. Protein concentrations were determined by the method of Bradford [21] with BSA as standard. Kinetic values are presented as means $\pm$ S.D. (*n* = 3).

Protein gel electrophoresis, N-terminal sequencing and FPLC

The protein subunit composition of the purified potato mPDC was further characterized by electrophoresis under denaturing and reducing conditions on 0.1% SDS/12% (w/v) polyacrylamide gels by the method of Laemmli [22]. The apparent molecular masses of the component protein subunits are based on the average of three independent experiments with the following protein standards: phosphorylase β (97.4 kDa), BSA (66 kDa), catalase (57.5 kDa), ovalbumin (45 kDa), aldolase (40 kDa) and carbonic anhydrase (29 kDa). For N-terminal sequence analysis, proteins were separated by SDS/PAGE, electroblotted on PVDF membranes and sequenced by automated Edman degradation on an Applied Biosystems protein sequencer (Model 494A). Superose 6 gel-filtration and Mono Q anion-exchange columns were used on a Pharmacia LKB (Uppsala, Sweden) FPLC system.

Acetylation of mPDC

Acetylation was conducted by a modification of the procedure reported by Jilka et al. [23]. Purified potato mPDC (30 μ g) was incubated at 30 °C for 2 min in the presence of 0.5 mM [2-¹⁴C]pyruvate (35000 d.p.m./nmol) in a 30 μ l solution also containing 1 mM MgCl₂, 1 mM NAD⁺, 0.2 mM TPP and 60 mM Tes/NaOH, pH 7.5. Reactions were terminated by the addition of *N*-ethylmaleimide to a final concentration of 10 mM. After 30 min at 25 °C, samples were denatured in sample buffer at 50 °C for 2 h and then subunits separated by SDS/PAGE. Gels were stained with Coomassie Blue, destained and then incubated in Amplify (Amersham, Little Chalfont, Bucks., U.K.), in accordance with the manufacturer's instructions. After vacuum-drying on Whatman No. 3MM paper, gels were directly exposed to Kodak Biomax X-ray film, stored at –80 °C and developed after 1–3 days in accordance with standard protocols.

RESULTS

Purification of potato mPDC

A typical scheme for the purification of mPDC from potato tubers is summarized in Table 1. Freeze-thawing purified mitochondria released the matrix protein including mPDC and the membranous material was removed by centrifugation at 40000 *g*. Large protein complexes, including mPDC, were then

Table 1 Purification of mPDC from potato tubers

Fraction	Volume (ml)	Activity ($\mu\text{mol}/\text{min}$)	Protein (mg)	Specific activity ($\mu\text{mol}/\text{min}$ per mg of protein)	Recovery (%)
Freeze-thawed mitochondria	36	24.5	180	0.135	—
Matrix fraction	35	22.0	110	0.210	90
Ultracentrifugation	5.8	14.0	26.0	0.530	57
Mg ²⁺ /PEG fractionation	0.58	10.5	2.90	3.65	43
Glycerol gradient	0.40	5.10	0.910	5.60	21

Figure 1 Profile of potato mPDC activity on glycerol step-gradient

Gradient was fractionated into 0.1 ml samples from top [fraction 1, 10% (v/v) glycerol] to bottom [fraction 18, 50% (v/v) glycerol].

separated from other matrix components by centrifugation at 200000 *g*. These steps led to a 3–4-fold purification of the mPDC with a 50–70% recovery depending on the preparation. Sequential fractionation of the resolubilized protein pellet with 10 mM MgCl₂/5% (w/v) PEG-6000 resulted in a 40–50% recovery of mPDC with a specific activity 25–30-fold that found in whole potato mitochondria. A final centrifugation on a 10–50% (v/v) glycerol step-gradient yielded a fraction of mPDC activity in 35–40% glycerol with a specific activity of 5.6 $\mu\text{mol}/\text{min}$ per mg of protein (Figure 1). Fractions with similar specific activities were pooled, precipitated with the MgCl₂/PEG fractionation procedure and resolubilized to a final concentration of 1.5–2 mg/ml protein. Aliquots of mPDC could be stored for several weeks at -80°C with only 5–15% losses in activity. No ATP-dependent inhibition of the activity of the glycerol-purified complex was observed (results not shown), suggesting that regulatory kinase subunits were either dissociated or inactivated during the purification protocol. The purified complex passed through a Superose gel-filtration column immediately after the void volume, suggesting a size in excess of the 5–5000 kDa separation range of this column (results not shown). The mPDC complex could also be bound on a Mono Q anion-exchange column equilibrated with PDC buffer and was eluted as an active complex in 300–350 mM NaCl (results not shown).

Kinetics of purified potato mPDC

The activity of the glycerol gradient purified mPDC was completely dependent on pyruvate, CoA and NAD⁺. Approx. 20% of control mPDC activity was maintained when TPP was not

Figure 2 SDS/PAGE separation, N-terminal sequencing and subunit assignment of potato mPDC polypeptides

added to the reaction medium (results not shown). This was probably due to the presence of TPP throughout the purification, its inclusion in the final resuspension buffer of the glycerol purified complex, and the tight binding of this cofactor to the complex. Divalent cations did not need to be added to the preparation to obtain maximal activity; however, titration with EDTA abolished activity at 50 μM (results not shown), suggesting that cations were tightly bound to the complex during the purification. No reduction of NAD⁺ by the purified complex was observed with 2-oxoglutarate as substrate. The activity of the complex was linear with time for several minutes at low mPDC concentrations (less than 1.5 $\mu\text{g}/\text{ml}$ protein) but was substantially inhibited in the first minute of reaction as the mPDC concentration in the assay medium was increased to more than 3 $\mu\text{g}/\text{ml}$ protein. The K_m values of the mPDC for the substrates pyruvate, NAD⁺ and CoA were 62 ± 6.0 , 270 ± 30 and 29 ± 4.2 μM respectively. The competitively inhibitory products, NADH and acetyl-CoA, had K_i values of 24 ± 4.8 and 79 ± 11 μM respectively. NADH is clearly a potent inhibitor of the mPDC of potato, with a K_i that is one-tenth of the K_m for NAD⁺. In contrast, the K_i for acetyl-CoA is similar to the K_m for CoA. Similar observations have been reported in a variety of plant tissues (reviewed in [1]).

Protein gel electrophoresis and N-terminal sequencing of potato mPDC

SDS/PAGE of the purified mPDC from potato revealed that the complex consisted of seven major polypeptide bands with apparent molecular masses of 78, 60, 58, 55, 43, 41 and 37 kDa (Figure 2). The protein bands were electroblotted on PVDF membranes and excised; the N-terminal sequence of each band was then determined.

Figure 3 Coomassie-stained SDS/PAGE separation of potato mPDC (A) and autoradiograph showing acetylated polypeptides (B)

mPDC (40 µg of protein) was acetylated with [2-¹⁴C]pyruvate. Molecular masses (in kDa) are indicated.

The sequence of the 78 kDa protein did not resemble any known sequence in the first 10 or 11 residues, but the latter sequence, MPALSPTM (one-letter codes), matches exactly a predicted sequence near the N-terminus of the full-length cDNA sequences of mPDC E2 from *Arabidopsis*, *Saccharomyces cerevisiae* and *Neurospora crassa* (accession numbers EMBL Q39082, SP P12695 and SP P20285 respectively). This sequence, with a single amino acid change, is also found in a 94-residue fragment of an *Oryza sativa* (rice) clone identified by similarity to be E2 (EMBL accession number Q42979). On the basis of similarity to the human E2 protein [24] this latter region in the sequence of the 78 kDa protein is the beginning of a lipoyl-binding domain.

The sequence of the 60 kDa protein was identified as potato mitochondrial HSP60 by virtue of 100% identity with potato and pumpkin sequences (accession numbers SP P80502 and SP Q05045 respectively). This protein might be co-purifying with mPDC as it can be found as a large complex in potato mitochondria [25], or it might be attached to the mPDC complex. During MonoQ ion-exchange chromatography the 60 kDa protein co-eluted with the other mPDC subunits in an active complex, suggesting the latter (results not shown).

The sequence from the 58 kDa protein was identical with the first 20 residues of a 40-residue N-terminal sequence from a potato mitochondrial matrix protein (accession number SP P80503) reported by Jansch et al. [25], which was identified as E3 of mPDC on the basis of greater than 90% identity with predicted amino acid sequences derived from soybean and pea full-length cDNA sequences (accession numbers EMBL Q41219 and SP P31023 respectively).

The main sequence of the Edman degradation of the 55 kDa protein band was identical with the sequence of the 58 kDa protein identified as mPDC E3. A secondary sequence from the 55 kDa protein band showed significant similarity to residues in the N-terminal sequences for maize E2 subunits [19], and also residues in the deduced amino acid sequences from cDNA species encoding yeast, *Arabidopsis* and human E2 (see alignments in [19]).

The N-terminal amino acid sequences of the 43 and 41 kDa proteins were identical and matched a sequence beginning 27 residues from the start codon of a predicted amino acid sequence derived from a full-length cDNA clone (accession number GB

Z26949) encoding E1α of mPDC from potato [12]. It is not clear whether the 43 and 41 kDa proteins in the purified potato mPDC represent isoenzymes of E1α, the products of C-terminal proteolysis of a single polypeptide or differential post-transcriptional processing products. The inclusion of the protease inhibitor 4-(2-aminoethyl)benzenesulphonyl fluoride-HCl (Pefabloc) at 1 mM during purification did not change the apparent abundance of the 41 kDa protein in potato mPDC (results not shown).

The N-terminal 16-residue sequence of the 37 kDa protein shared a region of 12 residues with over 90% identity with translated cDNA sequences from *Pisum sativum* (accession number SP P52904), *Arabidopsis* (GenBank accession number U09137), and *Schizosaccharomyces pombe* (accession number SP Q09171) encoding the E1β subunit of mPDC.

Acetylation of mPDC

To determine which of the E2-like proteins identified by N-terminal sequencing were functional dihydrolipoamide trans-acetylases of mPDC, the purified complex was incubated with [2-¹⁴C]pyruvate and TPP in the absence of CoA to acetylate the lipoyl chains of proteins accepting acetyl groups from E1. Autoradiographic detection of ¹⁴C incorporation revealed near-equal labelling of the 78 and the 55 kDa proteins (Figure 3).

DISCUSSION

The 40-fold purification of potato PDC has yielded a complex with a specific activity 5–15-fold those described in other reports of partial purifications of plant mPDC [4,26,27] and very similar to the reported activities of the *B. oleracea* mPDC preparations [7,8]. Kinetic analysis reveals typical competitive inhibition of mPDC activity by its products, NADH and acetyl-CoA. Separation of the component polypeptides by SDS/PAGE shows that this near-homogeneous complex contains seven protein bands, and six major proteins have been identified as the catalytic components of the complex by N-terminal amino acid sequence analysis (Figure 2).

The E2 component of *Escherichia coli* PDC is reported to have retarded electrophoretic mobility owing to its three large lipoyl acid-containing regions [28]. This probably explains the aberrantly higher apparent molecular masses of multi-lipoyl chained E2 proteins from mammalian, plant and bacterial mPDC calculated from SDS/PAGE compared with predictions from cDNA sequence analysis [10,11,28]. By using only apparent molecular masses from SDS/PAGE separation, the size and identity of the potato mPDC components can be directly compared with those in the complex from other organisms. Table 2 shows that the component polypeptides of the potato

Table 2 mPDC protein subunit sizes based on SDS/PAGE separation

Abbreviations: —, not present; n.d., not determined. References are referred to by superscript numbers as follows: ¹this study, ²[32], ³[33], ⁴[19], ⁵[11], ⁶[1], ⁷[10], ⁸[34], ⁹[28].

Subunit	Subunit size (kDa)					
	Potato ¹	Bovine ^{2,3}	Maize ⁴	Pea ^{5,6,7}	Yeast ⁸	<i>E. coli</i> ⁹
E2	78/55	74	52/53	50 ⁵ /80 ⁷	58	83
X	n.d.	50	n.d.	50 ⁷ /67 ⁵	50	—
E3	58/55	53	62/63	67 ⁵ /58 ⁶	56	56
E1	—	—	—	—	—	96
E1α	43/41	42	43	41 ^{5,6}	45	—
E1β	37	37	40	n.d.	35	—

mPDC show a striking similarity in size to those of the bovine mPDC but have less similarity in size with yeast and *E. coli* PDCs. The combined data from Camp and Randall [9], Taylor et al. [11] and Guan et al. [10] provide contradictory information regarding the identity of the component subunits of PDC from pea leaf mitochondria. The recently characterized maize mPDC shows slight differences in E3 size from the potato preparation, and only a 50–55 kDa E2 class has been identified in this plant species [19].

The N-terminal sequencing data in Figure 2 suggests the presence of 55 and 58 kDa E3 proteins in potato mPDC. On the basis of evidence from pea, it has been widely proposed that only one mPDC E3 gene exists in plants and that the single encoded 56 kDa polypeptide participates in the catalytic function of PDC, oxoglutarate decarboxylase and glycine decarboxylase in mitochondria [16–18]. Two recent reports have, however, suggested the presence of multiple E3 proteins in plant mitochondria. Thelen et al. [19] presented evidence of 62 and 63 kDa E3 polypeptides in maize mPDC. In a preliminary report, Conner et al. [29] has also observed two E3 populations in potato mitochondria with apparent molecular masses of 56 and 58 kDa. Further work is required to determine whether these E3 protein bands represent multiple E3 isoenzymes or are due to post-translational modifications or proteolytic cleavages of a single polypeptide.

Taylor et al. [11] suggested that a small E2 component in plants might be analogous to the E2 in yeast mPDC or the E2 in mammalian 2-oxoglutarate dehydrogenase, which only have a single lipoyl domain, whereas the larger mammalian mPDC E2 component has two lipoyl domains. Since that suggestion, results have been presented for the presence in plant mPDC of E2 proteins with single and double lipoyl domains. The predicted amino acid sequence as derived from the full-length *Arabidopsis* E2 cDNA sequence includes two highly conserved repeats with a high degree of similarity to the lipoyl domains in the mammalian sequences [10]. Antibodies used to isolate this *Arabidopsis* clone react with an 80 kDa protein in pea leaf mitochondria [10], similar in size to the 76 kDa mammalian E2 and much larger than the 50 kDa single lipoyl chain E2 found in yeast (Table 2). The identification here of a 78 kDa E2 protein in purified potato mPDC is also indicative of a two-lipoyl-domain E2 component analogous to that found in the mammalian complex. The original immunological evidence of Taylor et al. [11] for a 50 kDa E2 in plant mPDC has been recently strengthened by the identification of 52–53 kDa E2 proteins in maize mPDC purifications [19]. Here we have shown that both 78 and 55 kDa E2-like proteins exist in potato mPDC (Figure 2) and that they can be acylated to a similar extent (Figure 3). Further work will be required to determine whether plant mPDC contains two classes of E2 proteins, whether one class fulfils a more structural role analogous to the X component of mammalian and yeast mPDC, or whether the 55 kDa protein is a proteolytic cleavage product of the 78 kDa protein. The last possibility cannot be discounted until genes encoding the two species have been identified or the two proteins have been shown to be distinct, immunologically or on the basis of internal peptide sequences.

Before this report the only directly determined N-terminal sequences available for plant mPDC were for the E3 [16–18,25] and the E2 [19] subunits, although full-length cDNA clones for all the catalytic subunits of mPDC from at least one plant source have been published [10,12,17,30,31]. Given the high degree of identity between the predicted amino acid sequences derived from these cDNA species and the set of directly determined N-terminal sequences of the subunits in the potato mPDC, the latter can be used as additional tools for the prediction of the

cleavage sites of mitochondrial targeting sequences (MTSs) from translated precursor proteins. The potato E1 α N-terminal sequence shows that cleavage occurs between residues 26 and 27 in the precursor protein predicted from the potato E1 α cDNA [12] at a –2 arginine position. The resultant MTS can be folded as an amphiphilic α -helix. Alignment of the predicted sequence of the *Arabidopsis* E1 α precursor with the potato E1 α N-terminal sequence suggests cleavage seven or eight residues before the Thr-33 predicted by Luethy et al. [30]. Alignment of the potato E1 β N-terminal sequence (Figure 2) with the predicted amino acid sequence derived from the E1 β *Arabidopsis* and *P. sativum* cDNA species also predict cleavages at the –2 arginine residue, very close to the site in the potato E1 α precursor. The predicted amino acid sequence from the E2 cDNA from *Arabidopsis* does not have a clear MTS with a large number of acidic residues in the region after the methionine residue. However, alignment with the potato N-terminal sequence for the 78 kDa E2 predict the same Thr-52 cleavage site proposed for this E2 precursor protein by Guan et al. [10].

In conclusion, our purification of the potato mPDC has provided direct identification and size determination of the mPDC catalytic components in plants, and also the first complete set of N-terminal sequences of the mature subunits from plants. We hope to exploit further this potato mPDC preparation in attempts to identify the elusive kinase and phosphatase subunits of plant mPDC that are lost during protein purification.

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