





Short sequence-paper

Cloning and molecular analyses of the *Arabidopsis thaliana* plastid pyruvate dehydrogenase subunits ¹

Mark L. Johnston ^a, Michael H. Luethy ^{a,2}, Jan A. Miernyk ^a, Douglas D. Randall ^{b,*}

^a The Department of Biochemistry and the Interdisciplinary Plant Group, University of Missouri, Columbia, MO 65211, USA

^b 117 Schweitzer Hall, Columbia, MO 65211, USA

Received 4 March 1997; revised 11 June 1997; accepted 27 June 1997

Abstract

Herein we report the first molecular description of the pyruvate dehydrogenase component of the higher plant plastid pyruvate dehydrogenase complex. The full-length cDNAs for the $E1\alpha$ (1530 bp) and $E1\beta$ (1441 bp) subunits of the Arabidopsis thaliana plastid pyruvate dehydrogenase contain open reading frames that encode polypeptides of 428 and 406 amino acids, respectively, with calculated molecular weight values of 47120 and 44208. The deduced amino acid sequences for Arabidopsis plastid $E1\alpha$ and $E1\beta$ have 61% and 68% identity to the odpA and odpB genes of the red alga Porphyra purpurea, respectively, but only 31% and 32% identity to the plant mitochondrial counterparts. Results of Southern analyses suggest that each subunit is encoded by a single gene. Northern blot analyses indicate expression of mRNAs of the appropriate size in Arabidopsis leaves. © 1997 Elsevier Science B.V.

Keywords: Pyruvate dehydrogenase; Plastid; Lipid metabolism; Multi-enzyme complex; (Arabidopsis thaliana)

The pyruvate dehydrogenase complex (PDC) is a large multi-enzyme structure composed of three primary component enzymes, pyruvate dehydrogenase (PDH) (E1, EC 1.2.41); dihydrolipoamide acetyltransferase (E2, EC 2.3.1.12); and dihydrolipoamide dehydrogenase (E3, EC 1.8.1.4)[1]. In the well-characterized mammal complex, 60 subunits of E2 comprise the central core and the E1 and E3 components decorate the outer surface of this core [2]. E1 is a heterotetramer composed of two α and two β sub-

units. The E3 component, a homodimer, associates with the complex via an E3 binding protein. [3].

The PDC catalyzes the irreversible oxidative decarboxylation of pyruvate according to the equation:

$$\label{eq:pyruvate} \begin{split} \text{Pyruvate} + \text{CoA} + \text{NAD}^+ &\rightarrow \text{Acetyl-CoA} + \text{CO}_2 \\ &\quad + \text{NADH} + \text{H}^+ \end{split}$$

In mitochondria, this reaction represents the irreversible commitment of carbon to the citric acid cycle, and therefore is a logical point for regulation. Previous experiments have shown that plant mitochondrial PDC activity is, in fact, regulated by product inhibition, metabolites, and reversible phosphorylation [4–7] as is the mammalian complex [2].

In prokaryotes, PDC is localized in the cytoplasm, while in eukaryotes it is within the mitochondrial

^{*} Corresponding author. Fax: +1 573 8825635. E-mail: bchemdr@showme.missouri.edu

¹ The nucleotide sequence data in this article have been entered into the GenBank database under the accession numbers U80185 and U80186.

² Present address: DEKALB Genetics Corp., 62 Maritime Drive, Mystic, CT 06355-1958, USA.

matrix. Plants, however, are unique in that a second form of the complex exists in the plastids [8–10]. Based upon enzymology [11–13] and immunochemical analyses [14,15], it is clear that plastid PDC is distinct from its mitochondrial counterpart. In plants de novo fatty acid biosynthesis occurs exclusively in the plastids [16–20]. The plastid form of PDC can provide the fatty acid precursor, acetyl-CoA [16,17,21].

The cDNAs that encode the E1 α and E1 β subunits of plant mitochondrial PDH have been cloned [22–24]. Recently, Reith and Munholland [25] reported the sequence of the entire plastid genome of the red alga *P. purpurea*. Encoded in this genome are open reading frames homologous to PDH α and β subunits. We obtained expressed sequence tag (EST) clones [25] from the Arabidopsis Biological Resource Center (ABRC), at Ohio State University, that have significant homology to the algal plastid sequences. These partial cDNAs were used to isolate full-length cDNAs for both the plastid α and β subunits from an *A. thaliana* cDNA library.

Selection of *A. thaliana* EST clones was accomplished searching the *Arabidopsis* EST database using the BLASTP PROGRAM of the National Center for Biotechnology Information. We reasoned that EST clones encoding the plastid PDH subunits would have relatively high homology to the *P. purpurea odpA* and *odpB* genes [25], and at the same time relatively low homology to mitochondrial PDH E1 α and E1 β [22–24] sequences. Two clones (GenBank accessions T75600 and N65566) were thus identified as potentially encoding the plastid E1 α and E1 β subunits.

Oligonucleotides were designed based on sequences common to P. $purpurea\ odpA$ and odpB and the two Arabidopsis EST sequences and synthesized: E1 α 5' primer, (CGGTAC \blacktriangledown CAAGTCTGACTCTGTCGTT), 3' primer, (CCTTCGA \spadesuit AGGTTCCATCTCCGAAAAA); E1 β 5' primer, (CGGTAC \blacktriangledown CTTCGAGGCTCTTCAGGAA), 3' primer, (CCTTCGA \spadesuit ACGGGCCTTAGACCAGT). The symbols denote restriction sites (\blacktriangledown KpnI and \spadesuit HindIII) added for subcloning. Thermal cycling was used to amplify cDNA fragments from A. thaliana using first strand cDNA. Thermal cycling reactions (50 μ l total volume) contained 10 mM Tris-HCl, pH 7.9, 1.25 mM MgCl₂, 25 μ M dNTPs, 5 units Taq polymerase (Promega, Madison, WI), 2 μ g A.

thaliana first strand cDNA, and 10 ng of each primer. Thermal cycling was performed with a Perkin-Elmer model 480, with rapid ramp times set at 1°C/s. Cycling conditions were 94°C for 20 s, 50°C for 30 s, 72°C for 2 min with 6 s extensions each cycle and 30 rounds of cycling. Under these conditions, products of the expected size, 288 base pairs (E1 α) and 215 base pairs $(E1\beta)$ were obtained. The products were subcloned into pGEMT (Promega, Madison, WI) and sequenced to confirm their identity. Thermal cycling was also used to generate probes radiolabelled with $(\alpha^{32}P)$ -dCTP, using reaction mixtures identical to those previously described except for a 1000-fold reduction in the concentration of non-radioactive dCTP. Before use, the probes were desalted using Sephadex G-50 columns to remove unincorporated nucleotides. An Arabidopsis cDNA library (λ-PRL2, obtained from the ABRC) was plated at a density of 2.25×10^4 plaques per plate for a total of 2.25×10^5 plaques. BioTrace NT nylon filters (Gelman, Ann Arbor, MI) were used for plaque-lifts and were processed according to the manufacturer's specification. Hybridizations were done according to Current Protocols in Molecular Biology [26]. After three rounds of screening, 7 potential $E1\alpha$ and 12 potential $E1\beta$ cDNA clones were isolated, ranging in size from 1100 to 1550 base pairs. Plaque-purified λ phage were treated according to the manufacturer's instructions (Gibco BRL, Gaithersburg, MD) in order to excise the pZL-1 recombinant clones.

DNA sequencing was performed using an ABI prism Model 377 sequencer, and analyzed using IntelliGenetics GeneWorks DNA analysis program version 2.5 on a Macintosh computer. Dye-deoxy terminating cycle sequencing reactions were carried out on both strands of full-length cDNA inserts and deletion fragments derived therefrom.

DNA isolation, Northern and Southern blotting, and Southern hybridizations were carried out according to Current Protocols in Molecular Biology (2.9.1, 4.3.1 and 4.9.1) [26]. RNA isolation was accomplished with the RNAgents, total RNA isolation kit (Promega, Madison, WI). Northern blot prehybridization (3 h), hybridization (12 h), and 4 washes were done with 2.5 × SSPE (1 × = 0.15 mM NaCl, 0.02 mM Na₂PO₄, 2 μ M EDTA, pH 7.4), 1% SDS, 1% non-fat dry milk, and 250 μ g/ml salmon sperm DNA at 68°C. Blots were exposed on Kodak X-

OMAT/AR film (Rochester, New York) at -70° C with an intensifying screen.

Two EST clones (accessions T75600 and N65566) which encode proteins more highly related to the P. purpurea odpA and odpB sequences than to the Arabidopsis mitochondrial sequences were used to isolate two cDNAs as potential E1 α and E1 β clones. $E1\alpha$ cDNA (1530 bp) has a 106 bp 5' untranslated region, a 1284 bp open reading frame encoding a polypeptide of 428 amino acids (Fig. 1A), and a 140 bp 3' untranslated region. The E1 β cDNA (1441 bp) has a 6 bp 5' untranslated region, a 1218 bp open reading frame encoding a polypeptide of 406 amino acids (Fig. 1B), and a 217 bp 3' untranslated region. The calculated molecular weight and isoelectric point values for the $E1\alpha$ and $E1\beta$ polypeptides encoded by the open reading frames are 47 120 with a pI of 7.25, and 44 208 with a pI of 5.89, respectively. The deduced amino acid sequence for $E1\alpha$ has 61% and $E1\beta$ 68% identity with P. purpurea odpA and odpB, respectively.

The first 68 residues of E1 α and 73 residues of E1 β exhibit characteristics of chloroplast transit pep-

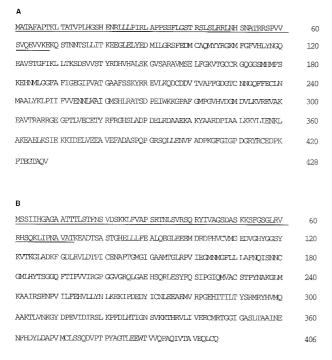


Fig. 1. A and B. The deduced amino acid sequences of the A. thaliana plastid E1 α and E1 β cDNA clones receptively. Presumptive targeting sequences are underlined.

tides but not those of mitochondrial targeting sequences [27,28]. To determine structural motifs of the transit peptides, GeneWorks (IntelliGenetics, Mountain View, CA) protein algorithm was used to identify possible α -helix and β -strands. Both plastid E1 α and E1 β have the potential to form amphiphilic β -strands consistent with plastid targeting sequences, but did not fit the amphiphilic α -helix which is characteristic of mitochondrial targeting sequences (data not shown).

Overall, there is 28% sequence identity between *Arabidopsis* plastid PDH $E1\alpha$ and its mammalian counterparts. However, in specific regions the degree of sequence conservation is much higher. The PDH component of PDC requires thiamin pyrophosphate (TPP) as a cofactor for decarboxylation of pyruvate [2]. It has been reported that TPP binds to the $E1\alpha$ subunit of mammalian PDH at a site containing a structural motif common to pyrophosphate-binding enzymes [1]. A similar motif (50% identity with the bovine $E1\alpha$ TPP-binding domain) is found in the *A. thaliana* plastid $E1\alpha$ sequence at residues 160-213 (Fig. 2A).

A highly conserved Cys residue (Cys 62 of mature human $E1\alpha$, Fig. 2A) has been identified in eukaryotic PDH $E1\alpha$ sequences, and it has been proposed that this Cys is an essential component of the enzyme's active site [29]. The *A. thaliana* plastid $E1\alpha$ sequence contains a similar motif, i.e. the same immediate flanking residues at 112-116, but the otherwise conserved Cys is replaced with a Val (Fig. 2A). Additional experiments will be necessary to determine if a distal Cys fulfills the role of the residue absent in the plastid sequence.

Mitochondrial PDCs are regulated in part by reversible phosphorylation of three conserved Ser residues in the $E1\,\alpha$ sequence by a specific, complex-associated PDH-kinase [1]. The Ser residues phosphorylated in mammalian mitochondrial PDH are also conserved in the plant mitochondrial [23], yeast [30], and nematode [31] amino acid sequences. However, while the plant mitochondria PDC is reversibly phosphorylated [5,6], all evidence to date indicates that plastid PDC activity is not regulated by phosphorylation [15]. Despite this difference, the regulatory Ser residues and their flanking sequences are present in plastid $E1\,\alpha$ sequence (Fig. 2A). Korotchkina and Patel [32] have reported the results from

В

Α

								_						
Plast	tid A.t. alpha	MATAFAPTKL TÄ	ATVPLHGSH 1	ENRLLLPIRL	APPSSFLGST	RSLSLRRLNH	50	Plastid A.t. beta						50
P.p.	odp alpha alpha						25	P.p. odp beta A.t. beta						
H.s.	alpha II			MRKMLAA	VSRVLSGASQ	KPASRVLVAS	27	H.s. beta						2
	alpha alpha I	MLAASFK RO		MIF	VFANIFKVPT	VSPSVMAISV	47 23	S.c. beta A.s. beta		:				. 3
M.c.	alpha					MTYL	4	M.c. beta						
	alpha					-	12 50	B.s. beta Consensus						50
Conse	ensus						30	COMPANIAN						50
	tid A.t. alpha	SNATRRSPVV SV	QEVVKEKQ S	STNNTSLLIT	KEEGLELYED	MILGRSFEDM	100 40	Plastid A.t. beta P.p. odp beta	KKSFGSGLRV	RHSQKLIPNA	VATKEADTSA	STGHELLLFE MSKVEMED	ALQEGLEEEM	100 18
	odp alpha alpha	STDTTPITIE TS	SLPFTAHLC I	DPPSRSVESS	SQELLDFFRT	MALMRRMEIA	75	A.t. beta	MLGILRQRAI	DGASTLRRTR	FALVSARSYA	AGAKEMTVRD	ALNSAIDEEM	50
H.s.	alpha II	RNFANDATFE IK	KKCDLHRLE I	EGPPVTTVLT	REDGLKYYRM	MQTVRRMELK	77	H.s. beta		VRRPLREVSG ARRAPTSFVR				47
	alpha alpha I	EGSDTVQIEL PE RLASTEATFQ TK					97 73	S.c. beta A.s. beta		LLRNGLTSAC				. 53 48
M.c.	alpha	GKFDPLKNEK VC	ZVLDKDGKV :	INPKLMPKIS	DQEILEAYKI	MNLSRRQDIY	54	M.c. beta				MATTNNTK	AVTIDATIDOAM	18
B.s.	alpha	QLEKVAEQFP TF					62	B.s. beta						. 18
Conse	ensus	Motif 1	0	P	LY	MRR.E	100	Consensus		•••••	Region		ALA.DEE.	100
Plast	tid A.t. alpha	CAQMYYRGKM FO					149	Plastid A.t. beta	DRDPHVCVMG	EDVGHYGGSY	KVTKGLADKF	GDLRVLDTPI	CENAFTGMGI	150
P.p.	odp alpha	CAQMYYKGKM FG ADSLYKANVI RG	FVHLYNGQ I	EAVSTGVIKL	L-DSKDYVCS	TYRDHVHALS	89 124	P.p. odp beta A.t. beta		EDVGHYGGSY EEVGQYQGAY				68 100
H.s.	alpha alpha II	ADQLYKQKII RG					126	H.s. beta	ERDEKVFLLG	EEVAQYDGAY	KVSRGLWKKY	GDKRIIDTPI	SEMGFAGIAV	97
s.c.	alpha	CDALYKAKKI RG	SFCHLSVGQ 1	EAIAVGIENA	I-TKLDSIIT	SYRCHGFTFM	146	S.c. beta	DRDDDVFLIG	EEVAQYNGAY	KVSKGLLDRF	GERRVVDTPI	TEYGFTGLAV	103
	alpha I alpha	AGNLYKEKKV RO QNTMQRQGRL LS	SFCHLYSGQ I	EACAVGTKAA EACEVAVINA	M-DAGDAAVT	AYRCHGWTYL CYRNNAAWIA	122 103	A.s. beta M.c. beta		EEVAQYDGAY EDVGTEGGVF				98 68
B.s.	alpha	RSISLNRQGR LG					111	B.s. beta		EDVGVNGGVF				68
Conse	ensus	Y	GF.HLGQ 1			.YR.H	150	Consensus	.RDVG	E,VG.Y.G,Y	K.TKGLK.	GRV.DTPI	.EF.G	150
Dlace	tid A.t. alpha	KGVSARAVMS EI	FCKVTYCC 1		nding ste	ARTGEGTPVA	199	Plastid A.t. beta	GAAMTGLEPV	TECHNIMGELL	LAFNOTSNING	CMT.HYTTSGGO	FTTPVVTRCP	200
	odp alpha	KGVPSQNVMA EL	LFGKETGCS 1	RGRGGSMHIF	SAPHNFLGGF	AFIAEGIPVA	139	P.p. odp beta	GAAITGLRPI	VEGMNMSFLL	LAFNQISNNA	GMLRYTSGGN	FTLPLVIRGP	118
A.t.	alpha	RGGSLHEVFS EL RGLSVREILA EL	MGROAGCS I	KGKGGSMHFY	KKESSFYGGH	GIVGAOVPLG	174 174	A.t. beta H.s. beta	GAAYAGLKPV	VEFMTFNFSM CEFMTFNFSM	QAIDHIINSA	AKSNYMSAGQ	INVPIVFRGP	150 147
	alpha II alpha	RGASVKAVLA EL	LMGRRAGVS !	YGKGGSMHLY	APG - FYGGN	GIVGAQVPLG	194	S.c. beta		VEFMSFNFSM				153
A.s.	alpha I	SGSSVAKVLC EL	LTGRITGNV :	YGKGGSMHMY	GEN FYGGN	GIVGAQQPLG	170	A.s. beta	GAAMNGLRPI	CEFMSMNFSM	QGIDHIINSA	AKAHYMSAGR	FHVPIVFRGA	148
	alpha alpha	MGQLVRNIML YW HGLPLYQAFL FS	WIGNEAG-G I	KAPEG-VNCL OT PEG-VNVI	PPO	IVIGSQYSQA	144 152	M.c. beta B.s. beta		LEMQFEGLGL PEIQFFGFVY				118 118
	ensus	.G.SV EL					200	Consensus		.E.MF				200
COLLE	ensus .				PDH B B	inding ste						Region	2	
	tid A.t. alpha	TGAAFSSKYR RE	VLKQDCD- I	DVTVAFFGDG	TCNNGQFFEC	LNMAALYKLP	248 189	Plastid A.t. beta P.p. odp beta		HSQRLESYFQ HSQRLEAYFQ				250 168
	odp alpha alpha	TGAAFQSIYR QQ CGIAFAQKYN KE	EEA	-VTFALYGDG	AANOGOLFEA	LNISALWDLP	217	A.t. beta	NGAAAGVGAQ	HSQCYAAWYA	SVPGLKVLAP	YSAEDARGLL	KAAIRDPDPV	200
H.s.	alpha II	AGIALACKYN GK	KDE	-VCLTLYGDG	AANQGQIFEA	YNMAALWKLP	217	H.s. beta	NGASAGVAAQ	HSQCFAAWYG	HCPGLKVVSP	WNSEDAKGLI	KSAIRDNNPV	197
S.c.	alpha alpha I	AGLAFAHQYK NE TGIAFAMKYR KE	EDA	-CSFTLYGDG	ASNOGOVES ATNOGOVES	FNMAKLWNLP MNMAKLWDLP	237 213	S.c. beta A.s. beta		HSQDFSPWYG HSQDFTAWFM				203 198
	alpha	TGIAFADKYR KI	rGG	-VVVTTTGDG	GSSEGETYEA	MNFAKLHEVP	187	M.c. beta	MGGGIRALEH	HSEALEAVYA	HIPGVQIVCP	STPYDTKGLI	LAAIDSPDPV	168
	alpha	AGVALGLKMR GK					195	B.s. beta		HSDSLEGLVA				168
Conse	ensus	.G.AFA.KYR	*3	-VTGDG	NQGQ.FE.	.NMA.LW.LP	250	Consensus	.GA.	HSQA	PGLKVV.P	DAKGLL	KAAIRD.NPV	250
	tid A.t. alpha	IIFVVENNLW AI					298 239	Plastid A.t. beta						296
P.D.	odp alpha alpha	IIFVVENNQW AI AILVCENNHY GM	GTAEWRAA F	KSPSYYKRGD	-Y-VPGLKVD	GMDAFAVKOA	265	P.p. odp beta		LQEEIP				214
H.s.	alpha II	CIFICENNRY GM	igtsveraa <i>i</i>	ASTDYYKRGD	-F-IPGLRVD	GMDILCVREA	265	A.t. beta H.s. beta		ESFPISEEAL VPFEFLPEAQ				250 247
	alpha alpha I	VVFCCENNKY GM VLYVCENNGY GM	IGTAASRSS A	AMTEYFKRGQ	-Y-IPGLKVN -Y-VPGTWVD	GMDILAVYQA	285 261	S.c. beta	VFLENELLYG	ESFEISEEAL	SPEFTLPY-K	AKIEREGTDI	SIVTYTRNVQ	252
	alpha	CIFVIENNKW AI	STARSEQT F	KSINFAVKGI .	ATGIPSITVD	GNDYLACIGV	237	A.s. beta M.c. beta	ICLENEILYG	MKFPVSPEAQ AFKQEVP	SPDFVLPFGQ	AKIQRPGKDI	TIVSLSIGVD	248 215
B.s.	alpha	AIFVVQNNRF AI					245	B.s. beta		SFRQEVP				215
Conse	ensus	.IFV.ENN	GTAR			CMD.LAVA	300	Consensus	LELLY.	E			TIVTYSV.	300.
Plast	id A.t. alpha	AKEAVTRARR GE	GPTLVECE I	*1 PYRFRGHSLA 1	•2 DPDELRDAAE	KAKYAAR D	- 346					gion 3		244
P.p.	odp alpha	AEKAVERARQ GQ	GPTLIEAL T	PYRFRGHSLA 1	DPDELRSRQE	KEAWVAR D	287	Plastid A.t. beta P.p. odp beta	HVTEALPLLI.	NKGYDPEV NDGYDPEV	TDTISPKELD	IDSISVSVKK	THRVLIVEEC	344 262
	alpha alpha II	CKFAKQHALE -K TRFAAAYCRS GK					314 315	A.t. beta	FALKAAEKLA	EEGISAEV	INLRSIRPLD	RATINASVRK	TSRLVTVEEG	298
	alpha	SKFAKDWCLS GK	GPLVLEYE 1	TYRYGGHSMS I	DPGTTYRTRD	EIQHMRSKND .	335	H.s. beta S.c. beta	HCLEAAAVLS	KE GVECEV KKY - GVSAEV	INMRTIRPMD	METIEASVMK	TNHLVTVEGG	295 301
	alpha I	VRWAKEWCNA GK FKEVVEYVRK GN	GPLMIEMA I	TYRYSGHSMS I	DPGTSYRTRE	EVQEVRKTRD	311 286	A.s. beta	VSLHAADELA	KSGIDCEV	INLRCVRPLD	FQTVKDSVIK	TKHLVTVESG	296
B.s.	alpha alpha	VKAARERAIN GE					295	M.c. beta		ETHPNATIDL				265
Conse	ensus	.K.A G.	GP.L.E I	TYRY.GHSMS	DPYR	ED	350	B.s. beta Consensus		KEGISAEV	_		-	263 350
Plast	tid A.t. alpha	PIAALKKYLI EN	KLAKEAEL F	KSIEKKIDEL '	VEEAVEFADA	SPOPGRSOLL	396			Regio	n 4			
P.p.	odp alpha	PIKKLKKHIL DN	QIASSDEL N	NDIQSSVKID :	LEQSVEFAMS	SPEPNISELK	337	Plastid A.t. beta P.p. odp beta		TAAINE-NFH IAQINE-HLF				393 311
	alpha	PIERIKKLVL SH	DLATEKEL F	KDMEKEIRKE '	VDDAIAKAKD	CPMPEPSELF	364 365	A.t. beta		CASVVE-ESF				347
	alpha II alpha	PIAGLKMHLI DL	GIATEAEV F	KAYDKSARKY	VDEQVELADA	APPPEAKLSI	385	H.s. beta		CARIMEGPAF				345
A.s.	alpha I	PITGFKDKIV TA					361	S.c. beta A.s. beta	WPNCGVGAEI	VAQVMESEAF SARVTESDAF	GYLDGPILRV	TGVDVPMPYA	QPLETAALPO	351 346
	alpha alpha	PLIRLKQYLI DK PLVRFRKFLE AK	COLWSEEEE N	NVIEGAKEE	IKEAIKKADE	TEKOKALDTI NVMXDPIDIL	336 345	M.c. beta	VKSFSVSAEI	IATVNE-ECF	EYIKAPLSRC	TGYDVITPFD	RG-EGYFOVN	313
Conse		PILK					400	B.s. beta Consensus		VAEINE-RAI				311 400
D1 - c-	tid A.t. alpha	ENVFADPKGF GI	מפוומפאסק ב	אישהאמערע א	OV		428				· IUDAF · · K	.G.DVF.FIA		
P.p.	odp alpha	RYLFADN					344	Plastid A.t. beta P.p. odp beta	PAQIVTAVEQ	LCQ IVNSSKTITT				406 331
A.t.	alpha	TNVYVKGFGT ES YHIYSSDPPF EV	FGPDRKEV F	KASLP			389 390	A.t. beta	IEDIVRASKR	ACYRSK				363
S.C.	alpha II alpha	LEEDVYVKGT ET	RGRTP F	SDUMDERKOG :	FASRD		420	H.s. beta	VKDIIFAIKK	TLNI				359
A.s.	alpha I	TOTYVNTPAO VV	RCTTDEVI C	OKYLTSEEAV '	KATAK		396	S.c. beta A.s. beta	TPTIVKAVKE PADVVKMVKK					366 361
	alpha alpha	KYQYDKMDIF LE SIMFEELPFN LK	EOVEIAKE P	FEKYPESKÉ (KESK	GGHH		370 369	M.c. beta	PKKVLVKMQE	LLDFKF				329
	arpha ensus						450	B.s. beta	FKDVIETAKK					325
COLLEGE								Consensus	IA.K.					420

Fig. 2. A and B. Alignment of the deduced amino acid sequences of PDH E1 α and E1 β . Abbreviations are the same as in Fig. 5. *, conserved; •, non-conserved phosphorylation sites; \bigcirc , the conserved Cys 62 of the mature H.s. E1 α sequence.

mutagenesis of these phosphorylation sites, and concluded that site one is closer to the active site or lies on the pathway to the main catalytic conformational change. This might explain why this region is so highly conserved. The amino acid-motif corresponding to phosphorylation site one in mitochondrial PDH sequences is present in the plastid polypeptide (Tyr 320-Pro 330 or Tyr 287-Pro 297 in the H.s. sequence, Fig. 2A). Two of the four substitutions are by residues with conserved properties. The sequence of the plastid E1 α corresponding to phosphorylation site two lacks a Ser and the region is dominated by five acidic and two basic residues (Asp 329-Asp 339). The Arabidopsis plastid $E1\alpha$ sequence contains a Ser at site 3 (Ala 259-Ala 267), but the flanking residues are dissimilar to the mammalian site 3 (Fig. 2A). While two of the three Ser are in the appropriate positions, it is most likely then that plastid PDC is not regulated by phosphorylation due to the lack of plastid PDH-kinase [15].

Wexler et al. [33] compared alignments of three PDH and three branched-chain α -keto acid dehydrogenase sequences. Among $E1\beta$ sequences, four regions of sequence conservation were observed. Region one, the proposed E2 interaction site, is present in the Arabidopsis plastid PDH E1 β sequence (Fig. 2B). Conserved regions two and three share high homology with other decarboxylating enzymes, suggesting a role in decarboxylation of pyruvate [33]. A functional role has not yet been attributed to region four (Fig. 2B). Eswaran et al. [34] have described Arg 239 as being an essential residue near or at the active site of the bovine $E1\beta$. This residue is conserved throughout the eukaryotic PDH sequences (e.g. Arg 269 of H.s. sequence in Fig. 2B) and is present in the A. thaliana plastid $E1\beta$ sequence at position 318.

The genomic organization of *Arabidopsis* $E1\alpha$ and $E1\beta$ was determined by Southern blot analysis. An $E1\alpha$ cDNA probe hybridized to a single restriction fragment in each lane, suggesting one gene (Fig. 3A). An $E1\beta$ cDNA probe, on the other hand, hybridized to multiple fragments in a pattern consistent with the restriction digest of $E1\beta$ cDNA (data not shown). The Xba I lane contained multiple hybridizing bands which could be due to a second gene or an intron containing an Xba I restriction site (Fig. 3B).

In order to evaluate expression of the A. thaliana

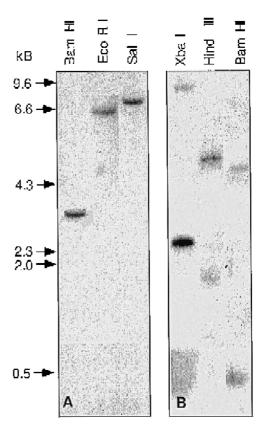


Fig. 3. Southern analyses of genomic DNA isolated from mature *A. thaliana* leaves. Each lane was loaded with 10 μ g of DNA digested with *Bam*HI, *Hind*III, *Sal*I, *Eco*RI or *Xba*I as indicated. A and B. Genomic Southern blots hybridized with random primed probes generated from gel-excised E1 α and E1 β cDNAs, respectively. (α^{32} P)-dCTP was incorporated using an oligolabelling kit (Pharmacia, Uppsala, Sweden). The positions of λ DNA markers digested with *Hind*III are indicated to the left of the figure.

plastid PDH genes, 10 μ g total RNA obtained from young leaves were resolved by formaldehyde gel electrophoresis. Northern blot analyses confirmed the expression of a single mRNA species of 1.65 kb for E1 α and 1.5 kb for E1 β (Fig. 4A and B).

The two cDNAs reported here have been tentatively identified as encoding plastid rather than mitochondrial proteins based on their high homology with the P. purpurea chloroplast genes, the presence of N-terminal sequences characteristic of plastid transit peptides, and their relatively low homology with plant mitochondrial E1 subunits [22–24]. Predictions of the mature N-terminal sequences were based on homology with the mature odp and mitochondrial E1 sequences. The mature A. thaliana plastid E1 α and

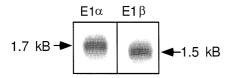


Fig. 4. Northern blot analyses of *A. thaliana* RNA. Total RNA was isolated from young leaves of *A. thaliana* plants. 10 μ g of total RNA was run on formaldehyde gels then transferred to nylon membranes. Probes were prepared as described in the legend for Fig. 3. RNA markers were used to determine the sizes of the hybridizing bands.

E1 β amino acid sequence (Fig. 2A and B), have the highest homology (68%) with the *P. purpurea* chloroplast *odpA* and *odpB* sequences, respectively, but only 31 and 32% identity with the respective *A. thaliana* mitochondrial E1 sequences. The homology with other eukaryotic mitochondrial E1 sequences is lower yet. Additionally, a monoclonal antibody prepared against mitochondrial E1 α does not recognize chloroplastic E1 α [35] nor does the monoclonal antibody recognize the recombinant plastid E1 α on immunoblots (data not shown).

Dendrogram analyses show that *A. thaliana* plastid E1, *P. purpurea* chloroplast *odp* and *Synechocystis sp.* (a cyanobacterium) *pdh* sequences segregate as a family distinct from mitochondrial and bacterial sequences (Fig. 5A and B). A similar separation has also been shown for plastid and mitochondrial ribosomal RNA sequences [36]. The *A. thaliana* plastid cDNAs and *P. purpurea odp* genes are the only sequences reported thus far for plastid forms of PDH.

As additional cDNAs and genes for plastid and mitochondrial specific isozymes are determined, insight as to the lineage of plastid genes will be gained. Mitochondrial rRNA genes show convincing similarity to purple-photosynthetic bacterial rRNA sequences. In contrast, plastid rRNA has similarity with cyanobacterial rRNA. This relationship between plastids and cyanobacteria also has been noted for genes encoding the transcriptional and translational apparatus [36]. The new sequences reported here should contribute to understanding if the emergence of mitochondria and plastids was the result of single or multiple primary (i.e. eubacteria/eukaryotic) endosymbioses, or if secondary (i.e. eukaryotic/ eukaryotic) endosymbioses led to the establishment of these organelles [36].

In conclusion, we have cloned and sequenced A. thaliana cDNAs whose properties indicate that they are the plastid forms of PDH E1 α and E1 β subunits. Having these cDNAs will allow us to perform trans-

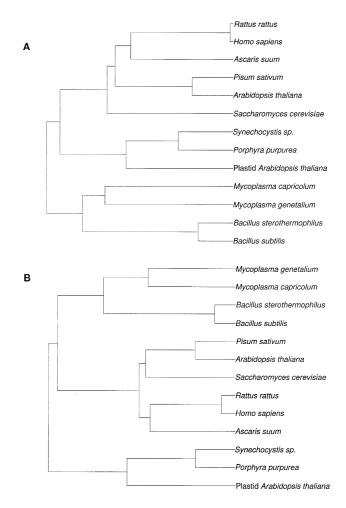


Fig. 5. A and B. Dendrogram analysis of the deduced amino acid sequence of PDH E1 α and E1 β subunits, respectively. Abbreviations and accession numbers to the sequences are: P. p., Porphyra purpurea odp (U38804); S. sp., Synechocystis sp. (D90915); A. t., Arabidopsis thaliana (U21214, U09137); P. s., Pisum sativum (U51918, U56697); H. s., Homo sapiens (L13318, D90086); R. r., Rattus rattus (Z12158, P49432); S. c., Saccharomyces cerevisiae (P16387, M98476); A. s., Ascaris suum (M76554, M38017); M. gen., Mycoplasma genetalium (U39706); M. c., Mycoplasma capricolum (U62057); B. su., Bacillus subtilis (M57435); and B. s., Bacillus stearothermophilus (X53560). Dendrogram analyses was accomplished with GeneWorks CLUSTAL V method (IntelliGenetics, Mountain View, CA). CLUSTAL V parameters were as follows: cost to open gap = 5, cost to lengthen gap = 25, gap penalty = 3, number of top diagonals = 5, window size = 5, PAM matrix = PAM250, K-tuple = 1, and consensus cutoff = 50%.

genic plant and developmental expression experiments to determine the role of plastid PDC in fatty acid biosynthesis.

The authors thank J. Stone, R. Larkin, Q. Lin for their assistance with Northern and Southern analyses. This work was supported by the National Science Foundation Grant IBN-941948 and the University of Missouri Food for the 21st Century Program. This is journal report 12598 for the Missouri Agriculture Experiment Station.

References

- [1] L.J. Reed, Acc. Chem. Res. 7 (1974) 40-46.
- [2] M.S. Patel, T.E. Roche, FASEB J. 4 (1990) 3224-3233.
- [3] S. Gopalakrishnan, M. Rahmatullah, G.A. Radke, S. Powers-Greenwood, T.E. Roche, Biochem. Biophys. Res. Commun. 160 (1989) 715–721.
- [4] D.D. Randall, P.M. Rubin, M. Fenko, Arch. Biochem. Biophys. 178 (1977) 342–349.
- [5] D.D. Randall, J.A. Miernyk, T.K. Fang, R.J.A. Budde, K.A. Schuller, Ann. N.Y. Acad. Sci. 573 (1989) 192–205.
- [6] D.D. Randall, J.A. Miernyk, N.R. David, J. Gemel, M.H. Luethy, Proc. Phytochem. Soc. Europe 39 (1996) 87–107.
- [7] R.J.A. Budde, T.K. Fang, D.D. Randall, J.A. Miernyk, Plant Physiol. 95 (1991) 131–136.
- [8] E.E. Reid, C.R. Lyttle, D.T. Canvin, D.T. Dennis, Biochem. Biophys. Res. Commun. 62 (1975) 42–47.
- [9] E.E. Reid, P. Thompson, C.R. Lyttle, D.D. Dennis, Plant Physiol. 59 (1977) 842–848.
- [10] P. Thompson, E.E. Reid, C.R. Lyttle, D.T. Dennis, Plant Physiol. 59 (1977) 854–858.
- [11] P. Thompson, E.E. Reid, C.R. Lyttle, D.T. Dennis, Plant Physiol. 59 (1977) 849–853.
- [12] M. Williams, D.D. Randall, Plant Physiol. 64 (1979) 1099– 1103.
- [13] P.J. Camp, J.A. Miernyk, D.D. Randall, Biochim. Biophys. Acta 933 (1988) 269–275.
- [14] A.E. Taylor, R.J. Cogdell, J.G. Lindsay, Planta 188 (1992) 231–255.

- [15] P.J. Camp, D.D. Randall, Plant Physiol. 77 (1985) 571–577.
- [16] J.A. Miernyk, D.T. Dennis, J. Exp. Bot. 34 (1983) 712-718.
- [17] F. Kang, S. Rawsthorne, Plant J. 6 (1994) 795-805.
- [18] B. Zilkey, D.T. Canvin, Biochem. Biophys. Res. Commun. 34 (1969) 646–653.
- [19] C.H. Drennan, D.T. Canvin, Biochim. Biophys. Acta 187 (1969) 193–200.
- [20] J.B. Ohlrogge, D.N. Kuhn, P.K. Stumpf, Proc. Natl. Acad. Sci. USA 76 (1979) 1194–1198.
- [21] R.G. Smith, D.A. Gauthier, D.T. Dennis, D.H. Turpin, Plant Physiol. 98 (1992) 1233–1238.
- [22] C.P.L. Grof, B.M. Winning, T.P. Scoysbrook, S.A. Hill, C.J. Leaver, Plant Physiol. 108 (1995) 1623–1629.
- [23] M.H. Luethy, J.A. Miernyk, D.D. Randall, Gene 164 (1995) 251–254.
- [24] M.H. Luethy, J.A. Miernyk, D.D. Randall, Biochim. Biophys. Acta 1187 (1994) 95–98.
- [25] M. Reith, J. Munholland, Plant Molec. Biol. Rep. 13 (1995)
- [26] F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, K. Struhl, Current Protocols in Molecular Biology, Vol. 1, 8 ed., John Wiley and Sons, Boston, MA, 1994.
- [27] Y. Gavel, G. von Heijne, FEBS 261 (1990) 455–458.
- [28] G. von Heijne, J. Steppuhn, R.G. Herrmann, Eur. J. Biochem. 180 (1989) 535–545.
- [29] M.S. Ali, T.E. Roche, M.S. Patel, J. Biol. Chem. 268 (1993) 22353–22356.
- [30] R.H. Behal, K.S. Browning, L.J. Reed, Biochem. Biophys. Res. Commun. 164 (1989) 941–946.
- [31] K.R. Johnson, R. Komuniecki, Y. Sun, M.J. Wheelock, Molec. Biochem. Parasitol. 51 (1992) 37–48.
- [32] L.G. Korotchkina, M.S. Patel, J. Biol. Chem. 270 (1995) 14304–14397.
- [33] I.D. Wexler, S.G. Hemelatha, M.S. Patel, FEBS Lett. 282 (1991) 209–213.
- [34] D. Eswaran, M.S. Ali, B.C. Shenoy, L.G. Korotchkina, T.E. Roche, M.S. Patel, Biochim. Biophys. Acta 1252 (1995) 203–208.
- [35] M.H. Luethy, N.R. David, T.E. Elthon, J.A. Miernyk, D.D. Randall, J. Plant Physiol. 145 (1995) 443–449.
- [36] J.D. Palmer, in R.G. Herrmann (Ed.), Plant Gene Research: Cell Organelles Vol. 7, Springer-Verlag, Vienna, 1992, pp. 99–133.