Biochimica et Biophysica Acta 810 (1985) 310–318
Elsevier

BBA 41889

Coupling factor activity of the purified pea mitochondrial F_1-ATPase

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(Received May 23rd, 1985)
(Revised manuscript received September 16th, 1985)

Key words: Coupling factor; Mitochondrial F_1-ATPase; Chloroplast CF_1-ATPase; (Pea)

The pea cotyledon mitochondrial F_1-ATPase was released from the submitochondrial particles by a washing procedure using 300 mM sucrose/2 mM Tricine (pH 7.4). The enzyme was purified by DEAE-cellulose chromatography and subsequent sucrose density gradient centrifugation. Using polyacrylamide gel electrophoresis under non-denaturing conditions, the purified protein exhibited a single sharp band with slightly lower mobility than the purified pea chloroplast CF_1-ATPase. The molecular weights of pea mitochondrial F_1-ATPase and pea chloroplast CF_1-ATPase were found to be 409 000 and 378 000, respectively. The purified pea mitochondrial F_1-ATPase dissociated into six types of subunits on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Most of these subunits had mobilities different from the subunits of the pea chloroplast CF_1-ATPase. The purified mitochondrial F_1-ATPase exhibited coupling factor activity. In spite of the observed differences between CF_1 and F_1, the mitochondrial enzyme stimulated ATP formation in CF_1-depleted pea chloroplast membranes. Thus, the mitochondrial F_1 was able to substitute functionally for the chloroplast CF_1 in reconstituting photophosphorylation.

Introduction

Although the coupling factors isolated from a variety of organisms have similar structure and function, they are not identical molecules. Mitochondrial coupling factors from bovine heart and yeast show structural similarities, yet immunological and functional specificities [1]. Antiserum prepared against purified yeast mitochondrial coupling factor inhibited the yeast ATPase activity, but had no effect on the bovine heart mitochondrial enzyme. Yeast coupling factor, when added to bovine heart submitochondrial particles, substituted for the bovine heart enzyme only with respect to its structural function, but was not active as a catalyst [1].

In many respects, the coupling factor from mitochondria (F_1) is remarkably similar to the coupling factor from chloroplasts (CF_1). Yet these proteins also exhibit specificity with regard to immunological and functional properties. Spinach chloroplast CF_1 reacted only with an antibody raised against CF_1 and not with an antibody against bovine heart F_1 [2]. Stimulation of photophosphorylation by CF_1 could not be duplicated by addition of F_1, nor did the chloroplast enzyme stimulate oxidative phosphorylation [3]. On the other hand, in the nonsulfur purple photosynthetic bacterium *Rhodopseudomonas capsulata*, where both photosynthetic and respiratory ATP formation occur on the plasma membrane bound coupling factor, the coupling factor for photophosphorylation and the coupling factor for oxidative

Abbreviations: CF_1, chloroplast coupling factor; F_1, mitochondrial coupling factor; DCCD, *N,N'*-dicyclohexylcarbodiimide; Chl, chlorophyll; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; Tes, 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-amino}ethanesulphonic acid.

phosphorylation are identical proteins [4]. They are immunologically identical and functionally interchangeable.

In higher plants, photophosphorylation and oxidative phosphorylation occur in two different cell compartments (chloroplasts and mitochondria). Are these two processes catalyzed by an identical type of a coupling factor molecule or does a plant form two types of coupling factors, one specific for photophosphorylation and the other specific for oxidative phosphorylation? If two specific types of coupling factors exist, to what degree do they differ?

The chloroplast coupling factor has been studied in great detail, while relatively little information is available on the plant mitochondrial enzyme. Partial purification of the F_1-ATPase from pea cotyledon mitochondria has been reported by Malhotra and Spencer [5]. Grubmeyer [6] obtained further purification of this enzyme, using DEAE-cellulose chromatography. Recently, purification and the subunit composition of the F_1-ATPase from sweet potato [7], maize [8,9,] and Fava bean [10] mitochondria has been described. None of the above-mentioned investigations, however, measured the physiological coupling factor activity of the enzyme preparation. Structurally, the plant mitochondrial F_1-ATPase seems to resemble closely its chloroplast counterpart, though, at present, the subunit composition of the mitochondrial enzyme is not firmly established [7–10]. Iwasaki et al. [11] prepared an antibody against purified sweet potato mitochondrial F_1-ATPase. The antibody reacted with β, but not α subunit of sweet potato chloroplast CF_1, suggesting differences between α subunits of the mitochondrial and chloroplast enzyme in the same plant species.

In the present paper we report the purification to homogeneity of the pea mitochondrial F_1-ATPase and demonstrate its coupling factor activity. We compare the mitochondrial enzyme to the pea chloroplast CF_1 and show that, although the two proteins can be separated by disc gel electrophoresis and exhibit different molecular weights, they must be closely related as the mitochondrial enzyme can substitute for the chloroplast coupling factor in the reconstitution of photophosphorylation in CF_1-depleted chloroplast membranes.

Materials and Methods

Preparation of chloroplasts and purification of chloroplast coupling factor (CF_1)

Chloroplasts were isolated from leaves of two week old pea plants (*Pisum sativum* L. cv. Homesteader) and CF_1 was extracted from the chloroplasts at 20°C with 300 mM sucrose/2 mM Tricine buffer (pH 7.4) as described by Strotmann et al. [12]. After centrifugation at 48 000 × *g* for 30 min at 20°C, the supernatant was concentrated by ultrafiltration and CF_1 purified further using a modification of the sucrose density gradient centrifugation of Lien and Racker [13]. The concentrated supernatant (1 ml) was loaded on a linear sucrose gradient (35 ml of 12.5% to 35% w/v sucrose) containing 2 mM EDTA/1 mM ATP/20 mM Tricine buffer (pH 7.1) and centrifuged at 44 000 × *g* for 41 h at 20°C. The fractions with highest ATPase activity were pooled, salted out with $(NH_4)_2SO_4$ (100% saturation) and stored frozen at −20°C until further use.

Preparation of mitochondria, submitochondrial particles and purification of mitochondrial coupling factor (F_1)

Pea seeds (*Pisum sativum* L. cv. Homesteader) were soaked in water for 6 h, then germinated in vermiculite for four days at 27°C in the dark. The cotyledons were harvested and mitochondria isolated by differential centrifugation as described by Solomos et al. [14]. The submitochondrial particles were prepared by sonication of the mitochondria using the conditions of Grubmeyer and Spencer [15]. The mitochondrial F_1 was released from the particles by washing them with a low-ionic strength sucrose solution (300 mM sucrose/2 mM Tricine, pH 7.4) at 20°C as in the case of chloroplast CF_1. After centrifugation at 100 000 × *g* for 60 min at 20°C, the F_1 was purified from the supernatant by chromatography on DEAE-cellulose as described by Grubmeyer [6]. The supernatant was loaded on a 1.5 × 17 cm DEAE-cellulose (Whatman DE-52) column equilibrated with 'buffer A' containing 300 mM sucrose/2 mM EDTA/2 mM ATP/20 mM Tris-H_2SO_4 (pH 7.4). After washing with 50 ml of this buffer, the proteins were eluted from the column with 150 ml of a linear 0–0.2 M K_2SO_4 gradient in buffer A. The fractions with the highest

ATPase activity were pooled and salted out with $(NH_4)_2SO_4$ (100% saturation). The $(NH_4)_2SO_4$ precipitate was dialyzed extensively against buffer A and the F_1 further purified by sucrose density gradient centrifugation, using the conditions described above for CF_1 purification. The fractions with ATPase activity were pooled, salted out with $(NH_4)_2SO_4$ (100% saturation) and stored at $-20°C$ until further use.

Preparation of CF_1-depleted chloroplast membranes

Leaves from two week old pea plants were homogenized at 5°C with a Polytron homogenizer in a medium containing 400 mM sucrose, 10 mM NaCl, 40 mM Tricine-KOH buffer (pH 8.0), 0.2% fatty acid poor bovine serum albumin and freshly added 20 mM sodium ascorbate. The homogenate was filtered through Miracloth (Calbiochem) and centrifuged for 5 min at $3500 \times g$ at 5°C. The chloroplast sediment was resuspended in a solution of 3 mM NaCl, 0.2% fatty acid poor bovine serum albumin (pH 8.0) and after chlorophyll determination, the suspension was adjusted to 2 mg chlorophyll/ml. In order to release CF_1, 2 ml of the chloroplast suspension were mixed with 30 ml 3 mM NaCl/1 mM EDTA/0.2% fatty acid poor bovine serum albumin (pH 8.0) and incubated for 10 min at 20°C. After centrifugation at 35000 $\times g$ for 20 min (20°C) the resulting CF_1-depleted chloroplast membranes were suspended in a buffer containing 20 mM Tricine-KOH (pH 8.0)/10 mM NaCl/0.2% fatty acid poor bovine serum albumin (Tricine-NaCl-bovine serum albumin buffer) for use in reconstitution studies.

Reconstitution of photophosphorylation in CF_1-depleted chloroplast membranes

CF_1-depleted chloroplast membranes (50 µl suspension containing 50 µg chlorophyll) were preincubated for 10 min at 20°C with 150 µl 100 mM $MgCl_2$ and 0.6 ml Tricine-NaCl-bovine serum albumin buffer containing various amounts of purified, desalted CF_1 or F_1. Subsequently, 0.7 ml of the incubation mixture, comprising 150 µmol Tricine-KOH (pH 8.0)/3 µmol ADP/4.5 µmol sodium phosphate containing 1 µCi $^{32}P_i$/1.5 mg fatty acid poor bovine serum albumin/25 µmol glucose/50 units hexokinase/45 nmol phenazine methosulfate, was added and incubation carried out for 5 min at 20°C at 100000 lx light intensity. After deproteination with 0.1 ml 60% trichloroacetic acid the amount of ATP formed was measured by $^{32}P_i$ incorporation into ATP according to Nielsen and Lehninger [16].

ATPase activity of CF_1 and F_1

ATPase activity of the isolated CF_1 was measured after activation with dithiothreitol according to McCarty and Racker [17], using conditions described previously [18]. The ATPase activity of the mitochondrial F_1 was determined as described by Grubmeyer [6]. The mixture consisting of 300 µmol sucrose/25 µmol Tes-KOH (pH 8.0)/6 µmol $MgCl_2$/2 µmol phosphoenolpyruvate/10 units of pyruvate kinase/5 µmol ATP/F_1-ATPase in a final volume of 1 ml was incubated for 15 min at 37°C. The reaction was stopped by adding 2 ml 0.72 N H_2SO_4, containing 0.7% ammonium molybdate and the released P_i determined according to Serrano et al. [19]. One unit of ATPase activity is defined as the amount of enzyme which released 1 µmole P_i/min under the assay conditions.

Chlorophyll and protein determinations

Chlorophyll was measured in 80% acetone extracts as described by MacKinney [20]. Protein was determined according to Bradford [21].

Molecular-weight determination of CF_1 and F_1 by polyacrylamide gel electrophoresis

The procedure for molecular-weight estimation using non-denaturing polyacrylamide gel electrophoresis has been used by Hedrick and Smith [22] and later modified by Bryan [23]. We have carried out the electrophoresis in polyacrylamide slab gels using conditions described in Sigma Technical Bulletin No. MKR-137. The molecular-weight standards included ovalbumin (45000), bovine serum albumin monomer (66000) and dimer (132000), urease dimer (240000) and tetramer (480000), ribulose bisphosphate carboxylase (565000). The molecular-weight standards and the two coupling factors (CF_1 and F_1) were electrophoresed in 4, 5, 6, 7, 8 and 9% polyacrylamide gels. After electrophoresis, the gels were stained for protein with Coomassie brilliant blue R-250 and the R_F of each protein in each gel was

determined. A Ferguson plot [24] for each protein was constructed by plotting 100 $\log(R_F \times 100)$ vs. percentage of acrylamide concentration. The slopes of these plots, referred to as retardation coefficients, were determined using linear regression analysis. The molecular weights of CF_1 and F_1 were determined from the plots of the logarithm of retardation coefficients vs. logarithm of molecular weight of the above standards [23].

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis was carried out in slab gels containing a linear 7.5–15% acrylamide concentration gradient under conditions described by Chua [25]. The gels were stained for protein with Coomassie brilliant blue R-250 and the apparent molecular weights of CF_1 and F_1 subunits were determined (using linear regression analysis) from the plot of the logarithm of molecular weight of standard proteins vs. the electrophoretic mobilities of these standards. The molecular weight standards (Sigma) included bovine serum albumin (66 000), ovalbumin (45 000), glyceraldehyde 3-phosphate dehydrogenase (36 000), carbonic anhydrase (29 000), trypsinogen (24 000), soybean trypsin inhibitor (20 100), α-lactalbumin (14 200) and aprotinin (6500).

Results and Discussion

Purification of CF_1-ATPase from chloroplasts of pea leaves

CF_1-ATPase was purified as described under Materials and Methods to a final specific activity of 20 units/mg protein (after dithiothreitol activation). When electrophoresed in 7% polyacrylamide gels under the conditions of Davis [26], the preparation gave a single protein band containing ATPase activity.

Purification of F_1-ATPase from pea cotyledon mitochondria

The purification procedure from the pea submitochondrial particles is summarized in Table I. Special care was taken during the solubilization of the F_1-ATPase from the submitochondrial particles. In order to preserve the integrity of the enzyme, the more drastic solubilization procedures, such as sonication or chloroform extraction [27] were avoided. The ATPase was released from the membranes by a low ionic strength solution of buffered sucrose, a procedure that resulted in the release of approx. 25% of the enzyme. Two step purification of the released enzyme, employing DEAE-cellulose chromatography followed by sucrose density gradient centrifugation, resulted in

TABLE I

PURIFICATION OF F_1-ATPase FROM PEA COTYLEDON MITOCHONDRIA

Material	Total activity (units)	Total protein (mg)	Specific activity (units/mg protein)	Yield (%)	Purification
Submitochondrial particles	299	243	1.2	100	1
Sucrose-Tricine wash of submitochondrial particles	78	52	1.5	26	1.3
DEAE-cellulose eluate	24.1	3.6	6.7	8	5.6
Gradient purified enzyme	20.7	0.9	23.0	7	19.2

a 20-fold purification of the F_1-ATPase with the final specific activity of 23 units/mg protein. Significant enhancement of the specific activity occurred after both purification steps. When the proteins were eluted from the DEAE-cellulose column using a 0–0.2 M K_2SO_4 gradient, the single peak of the ATPase activity eluted at approx. 0.1 M K_2SO_4 preceding the major protein peak (not shown). Sucrose density gradient centrifugation resulted in the appearance of a sharp protein peak (with a maximum at 24% sucrose) containing all of the ATPase activity (not shown). Practically no loss of activity occurred in this step, while purification increased 3.5-fold. The activity of the final preparation was comparable to that of the dithiothreitol activated chloroplast ATPase (see above).

The specific activity value of 23 units/mg protein is somewhat lower than the one reported for purified sweet potato mitochondrial F_1-ATPase (39 units/mg protein) [7], while being considerably higher than the activity of purified maize mitochondrial F_1-ATPase obtained by Hack and Leaver [8] or Spitsberg et al. [9]. Whether the observed differences are attributable to the differences in the species used or in the isolation and assay methods employed remains to be seen. It should be noted that, in agreement with the results of Grubmeyer [6], the activity of the purified pea cotyledon ATPase could be further increased by including 100 mM NaCl or 50 mM $NaHCO_3$ in the incubation medium. A preparation of our purified F_1-ATPase which had a specific activity of 28 units/mg protein when assayed as described in Materials and Methods, had a specific activity of 46 units/mg protein when 100 mM NaCl was included in the assay medium and a specific activity of 60 units/mg protein when 50 mM $NaHCO_3$ was included during the assay. The activities reported for F_1-ATPases from animal mitochondria are in the order of 100 units/mg protein for either bovine heart [28] or rat liver [29] enzyme, thus, so far, being higher than those exhibited by plant mitochondrial enzymes.

The purified pea cotyledon F_1-ATPase was analyzed by polyacrylamide gel electrophoresis using the conditions of Davis [26]. The preparation exhibited a single sharp protein band containing the ATPase activity. As in the case of chloroplast CF_1, the enzyme could be stored for at least two months as an $(NH_4)_2SO_4$ precipitate at −20°C without any loss of the activity.

Coupling factor activity of the purified pea mitochondria F_1-ATPase

The preparation of pea mitochondrial ATPase described above represents the first preparation of a plant mitochondrial enzyme with coupling factor activity. It could substitute for the chloroplast coupling factor in stimulating ATP formation in pea chloroplast membranes, which are depleted in CF_1 (Fig. 1). We have performed the reconstitution experiments with the chloroplast membranes as they reconstituted very well with chloroplast CF_1 and at the same time the system afforded a comparison between CF_1 and F_1. It is known that EDTA, which was used in this study to remove the CF_1 from the thylakoids, does not completely remove the coupling factor from the membranes [30]. However, the complete removal of CF_1 usually results in irreversible damage to the membranes [31,32], while if less than 50% of the total CF_1 pool is removed, complete activity can be recovered upon reconstitution with purified protein [31,33].

It is known that the coupling factor molecules that reconstitute thylakoid membranes can fulfill a

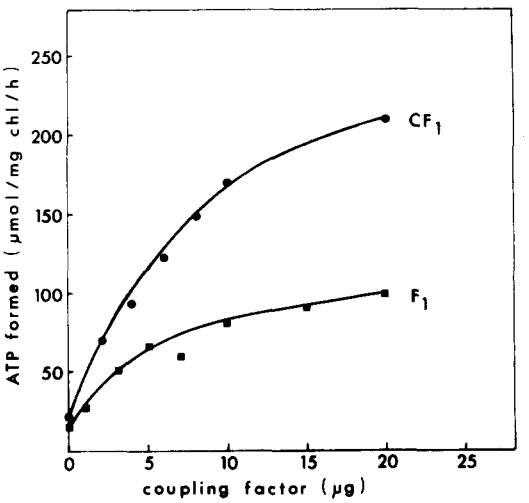

Fig. 1. Reconstitution of photophosphorylation in CF_1-depleted chloroplast membranes. Reconstitution and measurements of ATP formation were performed as described in Materials and Methods using 0–20 μg of either purified CF_1 or purified F_1.

dual role: a structural one in sealing the proton channels created by previous removal of CF_1 and a functional one in catalyzing the synthesis of ATP [34]. Since the F_1 was less efficient than CF_1 in reconstituting photophosphorylation (Fig. 1), the question immediately came to mind whether the F_1 was fulfilling only the structural role and thus enabling the residual CF_1 on thylakoid membranes to form more ATP by utilizing the increased proton gradient, or whether it was also catalytically active itself. The proton leak resulting upon removal of CF_1 from the chloroplast membrane can be blocked by the energy transfer inhibitor *N,N'*-dicyclohexylcarbodiimide (DCCD) [35], which binds to the proton-conducting part (subunit III) of the chloroplast proteolipid [36]. In order to elucidate the role of F_1 in the reconstitution experiments, we have used DCCD in an experiment analogous to that of McCarty and Racker [35]. In agreement with their results, DCCD at low concentrations stimulated photophosphorylation in CF_1-deficient chloroplast membranes (Table II). This can be explained by DCCD sealing the proton channels exposed by CF_1 removal. In the absence of added coupling factor, the highest ATP formation occurred with 3 nmol DCCD per incubation tube. However, considerably higher rates of photophosphorylation were observed when the membranes were reconstituted with 40 μg CF_1 (saturating amount) indicating that the CF_1 was catalytically active. Similarly, when the purified mitochondrial F_1-ATPase was added, the ATP formation was stimulated to a higher degree than by DCCD alone.

Further evidence that the CF_1 and F_1 added in the reconstitution experiments were catalytically active in ATP formation, rather than just sealing the thylakoid membranes was obtained when DCCD-modified CF_1 and DCCD-modified F_1 were used to reconstitute photophosphorylation in CF_1-depleted chloroplast membranes. Shoshan and Selman [37] have shown that incubation of soluble spinach CF_1 with DCCD results in inactivation of CF_1 and that this DCCD-modified CF_1 could restore proton uptake in EDTA-extracted chloroplasts, but was not catalytically active in ATP synthesis. In our experiments, DCCD-modified CF_1 and DCCD-modified F_1 reconstituted pho-

TABLE II

STIMULATION OF PHOTOPHOSPHORYLATION IN CF_1-DEPLETED CHLOROPLAST MEMBRANES BY DCCD, CF_1 AND F_1

CF_1-depleted chloroplast membranes were prepared as described in Materials and Methods, except that the NaCl concentration in the EDTA-containing extraction medium was decreased to 2 mM NaCl in order to remove more CF_1 and to obtain thylakoids with low residual ATP-forming capacity. Reconstitution was done as described in Materials and Methods, except that the indicated amount of DCCD (dissolved in 10 μl ethanol) was added to each test tube immediately after the addition of the Tricine-NaCl-bovine serum albumin buffer containing neither CF_1 nor F_1, or containing 40 μg of either CF_1 or F_1.

DCCD (nmol)	ATP formed (μmol/mg chlorophyll per h)			
	Experiment I		Experiment II	
	no CF_1	40 μg CF_1	no F_1	40 μg F_1
0	1	92	5	79
1	5	87	8	54
2	6	81	13	36
3	9	47	23	19
5	8	14	13	8
10	3	1	1	2

TABLE III

RECONSTITUTION OF ATP FORMATION IN CF_1-DEPLETED THYLAKOIDS BY NATIVE AND DCCD-MODIFIED CF_1 AND F_1

80 μg of purified CF_1 and F_1 were incubated at 37°C for 60 min in 0.34 ml of an incubation mixture, containing 50 mM Tes-KOH (pH 7.0), 1 mM ATP, 2 mM EDTA and no DCCD or 0.3 mM DCCD. Free DCCD was removed from the incubated samples by the Sephadex chromatography-centrifugation procedure of Penefsky [38] using Sephadex G-50 centrifuge columns equilibrated with a buffer containing 50 mM Tricine-KOH (pH 8.0)/10 mM NaCl. DCCD inhibited the ATPase activity of CF_1 and F_1 by 91% and 90%, respectively. The native and DCCD-modified coupling factors eluted from the centrifuge columns were used to reconstitute ATP synthesis in CF_1-depleted thylakoids (cont. 50 μg Chl) as described in Materials and Methods.

Additions to CF_1-depleted thylakoids	ATP formed (μmol/mg Chl per h)
No additions	9
30 μg Native CF_1	57
30 μg DCCD-modified CF_1	24
30 μg Native F_1	44
30 μg DCCD-modified F_1	19

tophosphorylation in CF_1-depleted thylakoids, but the resulting rates of ATP formation were only about 40% of the rates obtained with native CF_1 and F_1 (Table III).

Thus, it can be concluded that the native CF_1 and native F_1 were both catalytically active in ATP synthesis in addition to fulfilling the structural role in the thylakoid membranes.

Electrophoresis of F_1 and CF_1 in nondenaturing polyacrylamide gels

The ability of the mitochondrial F_1 to substitute for the chloroplast CF_1 suggested that the two proteins must be closely related. Yet, they could be separated by polyacrylamide gel electrophoresis using the conditions of Davis [26], with CF_1 exhibiting a slightly higher mobility than F_1 (Fig. 2). Thus, the two proteins are not identical and differ to some degree in their charge and/or size. We tried therefore to determine their molecular weights using polyacrylamide gel electrophoresis under nondenaturing conditions as described in Materials and Methods. The retardation coefficients for the standard proteins were determined to be 5.3, 7.0, 10.5, 12.9, 20.9, and 21.5 for ovalbumin, bovine serum albumin monomer, bovine serum albumin dimer, urease dimer, urease tetramer, and ribulose bisphosphate carboxylase, respectively. Plotting the logarithm of the retardation coefficients against the logarithm of molecular weight [23] of each standard and analyzing the data by linear regression produced a straight line. The retardation coefficients for the chloroplast CF_1 and mitochondrial F_1 were found to be 17.7 and 18.5. Using these values, the molecular weights of CF_1 and F_1 were determined from the standard curve to be 378 000 and 409 000, respectively. The value of 378 000 for the chloroplast enzyme is close to the molecular weight of spinach CF_1 estimated by Moroney et al. [40], using sedimentation equilibrium centrifugation and light scattering, and supports the enzyme subunits stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$ suggested by the authors. Molecular weight for the sweet potato mitochondrial F_1-ATPase has been estimated at 370 000 from polyacrylamide gel electrophoresis by Iwasaki and Asahi [7]. Spitsberg et al. [9] recently estimated the molecular weight of corn mitochondrial F_1-ATPase using apparent subunit molecular weight estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and a subunit stoichiometry suggested by densitometric analysis of the gels. The suggested stoichiometry of $\alpha_3\beta_3\gamma\delta_2\varepsilon$ gave a molecular weight of 430 000, which is close to the value obtained by us for the pea mitochondrial enzyme.

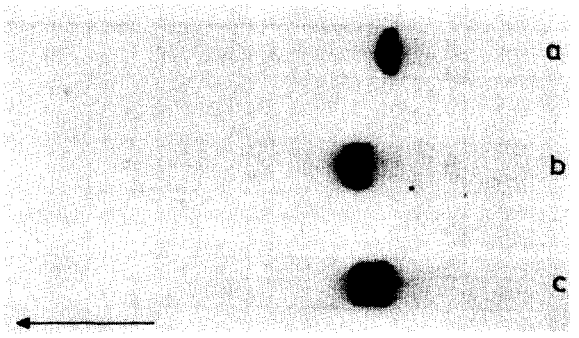

Fig. 2. Polyacrylamide gel electrophoresis of purified CF_1 and F_1. Electrophoresis was carried out in 10% polyacrylamide cylindrical gels according to Davis [26]. Gels were stained for ATPase activity as described previously [39]. Lane (a), 10 μg mitochondrial F_1; lane (b), 10 μg chloroplast CF_1; lane (c), 10 μg F_1 + 10 μg CF_1. The arrow indicates the direction of the run.

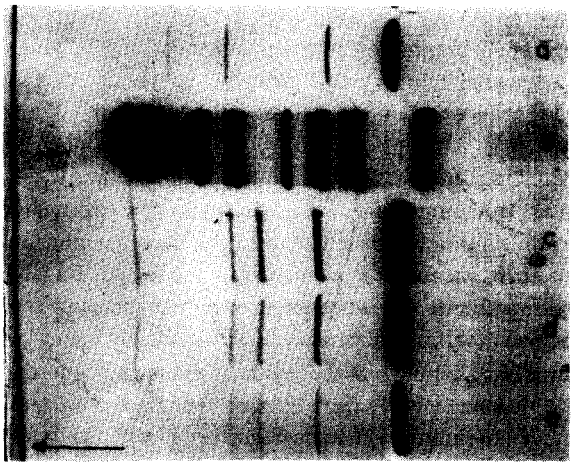

Fig. 3. SDS-polyacrylamide gel electrophoresis of purified CF_1 and F_1. Electrophoresis was carried out as described in Materials and Methods. Lane (a), 3 μg CF_1; lane (b), 3.5 μg of each molecular-weight standard (bovine serum albumin, ovalbumin, glyceraldehyde 3-phosphate dehydrogenase, carbonic anhydrase, trypsinogen, soybean trypsin inhibitor, α-lactalbumin, aprotinin); lane (c), 12 μg F_1; lane (d), 7 μg F_1; lane (e), 2.5 μg F_1. The arrow indicates the direction of the run.

SDS-polyacrylamide gel electrophoresis of purified F_1 and CF_1

The subunit composition and apparent molecu-

lar weights of the subunits of purified pea mitochondrial F_1 were compared to those of pea chloroplast CF_1 using slab gel electrophoresis in the presence of SDS as described by Chua [25]. The results are shown in Fig. 3. It can be seen that most of the subunits of mitochondrial F_1 had a different mobility than the subunits of chloroplast CF_1. The purified mitochondrial F_1 preparation contained six types of subunit, while the chloroplast CF_1 contained only five polypeptides. Although the two largest subunits of CF_1 migrated together as one protein band on these gels (Fig. 3, lane a), these two subunits separated on SDS-polyacrylamide gel electrophoresis under conditions described by Weber and Osborn [41] in gels containing 10% acrylamide (not shown). This was in contrast to the two largest subunits of mitochondrial F_1 which did not separate on electrophoresis performed according to Weber and Osborn [41] (not shown), but did separate on electrophoresis performed according to Chua [25] (Fig. 3, lane e). The apparent molecular weights of the subunits were determined from the data shown in Fig. 3. Subunits of CF_1 were found to have apparent molecular weights of 54000 (α and β), 39000 (γ), 22500 (δ) and 13000 (ε). The subunits of pea mitochondrial F_1 had apparent molecular weights of 57000 (α), 55000 (β), 36500 (γ), 26500 (δ), 22500 (δ') and 8000 (ε).

Although the hydrophilic, peripheral component of the coupling factor complex of most examined organisms contains usually five different polypeptides, it should be noted that our pea cotyledon mitochondrial F_1 preparation resembles in its subunit composition and subunit size the F_1-ATPase purified from sweet potato root tissue by Iwasaki and Asahi [7]. Their F_1 preparation also contained six kinds of subunits with molecular weights of 52500, 51500, 35500, 26000, 23000 and 12000. While the preparation of Fava bean mitochondrial F_1-ATPase was reported to contain only five types of subunits [10], the molecular weight of the Fava bean ε subunit (22900) was closer to that of the δ' subunit (22500) in our preparation, than to the usual molecular weight of ε subunits present in coupling factors from a variety of organisms (6000–16000). However, the maize mitochondrial F_1-ATPase was found to have the usual complement of five types of polypeptide with molecular weights ranging from 58000 to 8000–12000 [8,9]. Since most of the F_1 proteins contain only five types of subunit, it is possible, that the sixth polypeptide seen in our preparation is due to contamination with a protein not related to the coupling factor complex. Alternatively, this polypeptide may be a component of the coupling factor complex which is involved in linking the F_1 to the membrane sector of this complex. Such a component could be released together with F_1 during the F_1 solubilization from submitochondrial particles. Lastly, the sixth polypeptide could be a proteolytic product formed during the F_1 isolation from one of the larger subunits. Contamination with an unrelated protein is not very likely, since when the purified F_1 was electrophoresed in nondenaturing gels, stained for ATPase activity (as in Fig. 2), the ATPase zones excised and the protein from these zones electrophoresed in SDS-containing gels, it still contained all six polypeptides. The other possibilities mentioned above will be further investigated.

We conclude that the pea chloroplast CF_1 and pea mitochondrial F_1 are close, but not identical in size, with the mitochondrial enzyme exhibiting a higher molecular weight and somewhat different subunit composition than its chloroplast counterpart. Nevertheless, in spite of the differences between pea CF_1 and F_1, the mitochondrial enzyme must be structurally very similar to the chloroplast enzyme, as it can functionally substitute for the chloroplast coupling factor in reconstituting photophosphorylation in CF_1-depleted chloroplast membranes.

Acknowledgments

This research was supported in part by a grant from the Agriculture Canada Research Fund to the Dean. We would like to thank Dr. H. Horak for her help in preparing the manuscript, Mr. H. Bestman for linear regression analysis of the data, Mr. B. Zytaruk for preparing the figures and Mr. I. Duncan for technical assistance.

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