Plant mitochondrial F_1 -ATPase

The presence of oligomycin-sensitivity-conferring protein (OSCP)

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Purified pea (*Pisum sativum*) cotyledon F_1 -ATPase contains six subunits rather than the five usually reported for F_1 -ATPases. The additional 26.5 kDa (δ) subunit is shown by immunoblotting and *N*-terminal amino acid sequencing to be similar to bovine oligomycin-sensitivity-conferring protein (OSCP). It is concluded that the δ subunit of plant mitochondrial F_1 -ATPase is the plant OSCP. This OSCP subunit occurs in all mono- and di-cotyledonous species of plants tested (maize, oats, peas, potatoes, sweet potatoes and turnips).

INTRODUCTION

F_1 -ATPase has been purified from mitochondria isolated from a number of different species of plants. Although several of these preparations [maize (*Zea mays* Hack & Leaver, 1983; Partridge *et al.*, 1985), fava bean (*Vicia faba*; Boutry *et al.*, 1983), oat (*Avena sativa*) root (Randall *et al.*, 1985) and cuckoo-pint (*Arum maculatum*; Dunn *et al.*, 1985)], like those obtained from mammalian sources, contain five subunits, other preparations contain six subunits [sweet-potato (*Ipomoea batatas*) root (Iwasaki & Asahi, 1983, 1985) pea (*Pisum sativum*) cotyledon (Horak & Packer, 1985; Horak *et al.*, 1987) and turnip (*Brassica napus*; O'Rourke, 1988)]. Horak *et al.* (1987) and Kimura *et al.* (1989) have suggested that the additional (sixth) subunit (26–27 kDa) may be equivalent to the oligomycin-sensitivity-conferring protein (OSCP) from bovine heart F_1/F_0 -ATPase. Norling *et al.* (1988) demonstrated a requirement for an OSCP-like protein to confer oligomycin sensitivity to potato (*Solanum tuberosum*) F_1 -ATPase in cross-reconstitution experiments with bovine heart mitochondrial membranes. Horak *et al.* (1989) have shown that the 26.5 kDa protein present in the six-subunit pea F_1 -ATPase increases oligomycin sensitivity of the membrane-bound enzyme and therefore functions as the plant OSCP. The present paper demonstrates that the δ -subunit of pea F_1 -ATPase is closely related to bovine OSCP and that this plant OSCP occurs in mitochondria from all species of plants tested.

MATERIALS AND METHODS

Mitochondria were isolated and purified from 4-day-old etiolated pea (c.v. Homesteader) cotyledons and the F_1 -ATPase purified from submitochondrial particles (SMP) as described by Horak & Packer (1985). SMP were also obtained by the same methods from 4-day-old etiolated oat (cv. Victory) shoots and roots, maize (cv. Sunnyvee) shoots as well as from sweet potatoes, turnips and potatoes.

Bovine OSCP was purified from submitochondrial

particles prepared from bovine heart muscle mitochondria as described by Senior (1979).

Rabbit antiserum against bovine OSCP was generously donated by Dr. A. Dupuis, Centre d'Etudes Nucleaires, Grenoble, France. The antibody, which with bovine F_1 -ATPase is specific for OSCP (Dupuis *et al.*, 1985), was checked by blotting against extracts of bovine SMP before use. SDS/polyacrylamide-gel electrophoresis was carried out as described by Laemmli (1970), and immunoblotting was performed essentially as described by Dunn (1986). After electrophoresis (180V for 60 min) in a Bio-Rad Mini Gel system [Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ontario, Canada], using 0.5 mm-thick 13 %-(w/v)-acrylamide gels, the proteins were transferred to Schleicher and Schuell nitrocellulose membranes (0.2 μ m pore size) using a Bio-Rad Mini Trans-Blot apparatus operated at 75V for 2 h (250–300 mA) at 0 °C. Molecular-mass standards were stained after transfer with 0.5 % (w/v) Ponceau S Red stain in 1 % (v/v) acetic acid for 5–10 min before destaining in water. The transfer was carried out in a buffer consisting of 10 mM- NaHCO_3 , 3 mM- Na_2CO_3 and 20 % (v/v) methanol, pH 9.8. The Blot Rinse Buffer (BRB) consisted of 10 mM-Tris/HCl, pH 7.4, 150 mM-NaCl, 1 mM-EDTA, 0.1 % (v/v) Tween 20 and 0.01 % sodium ethylmercurithiosalicylate (Thimerosal). The blots were blocked overnight at 4 °C in BRB + 3 % (w/v) bovine serum albumin (BSA). After treating the blots with anti-OSCP serum [diluted 1:1000 with BRB + 0.3 % (w/v) BSA] for 150 min at 20 °C and washing three times with BRB, the blots were incubated with Bio-Rad goat anti-rabbit IgG (H + L) horseradish-peroxidase-conjugated antibody, diluted 1:3000 in BRB + 0.3 % (w/v) BSA. Excess second antibody was removed by washing twice for 10 min in BRB and twice for 10 min in 10 mM-Tris/HCl (pH 7.4)/150 mM-NaCl before the colour was developed with freshly prepared 4-chloro-1-naphthol (6 mg in 2 ml of methanol) + 6 μ l of 30 % (v/v) H_2O_2 in 10 ml of 10 mM-Tris/HCl (pH 7.4)/150 mM-NaCl. Colour development was stopped by rinsing with water.

For protein sequencing, samples of purified pea cotyledon F_1 -ATPase were first dialysed against a large

Abbreviations used: OSCP, oligomycin-sensitivity-conferring protein; BSA, bovine serum albumin; SMP, submitochondrial particles; PTH, phenylthiohydantoin.

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volume of distilled water before freeze-drying and redissolving in gel-electrophoresis sample-preparation buffer containing 1% (w/v) SDS. F_1 -ATPase subunits were resolved by SDS/polyacrylamide-gel electrophoresis, according to the conditions described in Applied Biosystems User Bulletin no. 25, on 13%-(w/v)-polyacrylamide gels using a Mighty Small II apparatus (Hofer Scientific Instruments Ltd., Newcastle, Staffs., U.K.). The gel was then blotted on to Immobilon-P membrane [Millipore (U.K.) Ltd., Watford, Herts., U.K.] as described by Matsudaira (1987) and the subunit bands were carefully excised with a scalpel.

N-Terminal protein sequencing was carried out on the excised bands by automatic Edman degradation using the standard 03R PTH (phenylthiohydantoin) program in an Applied Biosystems model 470A gas-phase sequencer fitted with a 120A on-line PTH analyser.

RESULTS AND DISCUSSION

Fig. 1 shows an immunoblot obtained with purified pea F_1 -ATPase. The enzyme contains six subunits, α , β , γ , δ , δ' , ϵ (lane 1); the α - and β -subunits migrate close to each other and are resolved only when small amounts of protein (1 μ g) are used for electrophoresis. The δ -subunit (26.5 kDa) has a mobility similar to that of bovine OSCP (lane 4) and reacts with the anti-(bovine OSCP) antibody (lanes 2 and 3). Whereas the antibody against bovine OSCP reacted readily with the pea mitochondrial δ -subunit, it did not react with any of the subunits of pea chloroplast CF_1 -ATPase (results not shown). To ascertain whether this OSCP-like protein was also present in other plant mitochondria, SMP were prepared from several dicotyledonous (turnip, potato and sweet potato) and monocotyledonous (oats and maize) plants. The proteins from these SMP were separated by SDS/

Fig. 1. Immunoblot of purified pea F_1 -ATPase and of bovine OSCP with anti-(bovine OSCP) antiserum

Immunoblotting was carried out, as described in the text, with 1:1000-diluted anti-(bovine OSCP) antiserum. Lane 1, polyacrylamide-gel electrophoresis of 10 μ g of purified pea F_1 -ATPase, silver-stained; lanes 2–4, immunoblots with anti-(bovine OSCP) antiserum: lane 2, 5 μ g of purified pea F_1 -ATPase; lane 3, 1 μ g of purified pea F_1 -ATPase; lane 4, 1 μ g of crude bovine OSCP.

Fig. 2. Immunoblot of proteins from SMP from several plant species

A 10 μ g portion of SMP was solubilized in sample-preparation buffer, electrophoresed and immunoblotted with 1:1000-diluted anti-(bovine OSCP) antiserum as described in the text. Lane 1, 4-day etiolated oat shoot SMP; lane 2, 4-day oat root SMP; lane 3, potato SMP; lane 4, turnip SMP; lane 5, sweet-potato SMP; lane 6, maize SMP; lane 7, pea cotyledon SMP.

polyacrylamide-gel electrophoresis and immunoblotted. Fig. 2 shows that, in all the species tested, the antibody against bovine OSCP recognized a protein which had a mobility similar to that of bovine OSCP. The results indicate that mitochondria from both mono- and dicotyledonous plants contain an OSCP-like protein. Fig. 2 shows that the strength of the reaction of the plant OSCP with the anti-(bovine OSCP) antibody varied from one plant species to another. This variation could be due to differences in the amounts or the structure of the OSCP present in these SMP. In addition to the OSCP-like protein, other polypeptides from potato SMP (lane 3) and sweet potato SMP (lane 5) also reacted with the antibody. The additional two (smaller) OSCP-like polypeptides from potato SMP may be proteolytic fragments produced during the preparation of the SMP. The faint band of an apparently larger OSCP-like polypeptide observed in the sweet-potato SMP may represent non-specific binding of the antibody.

A 38-residue *N*-terminal amino acid sequence was obtained for the pea δ -subunit (Fig. 3), which apart from a six-residue *N*-terminal extension, is very similar to that of the bovine OSCP (Ovchinnikov *et al.*, 1984), with 12 identical residues within the 32-residue overlapping sequence. The sequence similarity is even greater when the sequence obtained is compared with the *N*-terminal sequence of sweet-potato δ -subunit (Kimura *et al.* 1989), which also has an *N*-terminal extension of six residues when compared with the bovine OSCP. The similarity to the *Escherichia coli* (Walker *et al.*, 1984) and spinach (*Spinacia oleracea*) chloroplast CF_1 (Hermans *et al.*, 1988) δ -subunits is less than that to bovine OSCP.

The evidence presented here, together with that published elsewhere (Horak *et al.*, 1987, 1989; Kimura *et al.*, 1989), clearly shows that the subunit identified on the basis of its electrophoretic mobility as the δ (26.5/27 kDa)-subunit in six-subunit preparations of plant F_1 -ATPase is equivalent to OSCP in mammalian mito-

Fig. 3. Similarity between the N-terminal amino acid sequence of the δ -subunit of pea mitochondrial F_1F_0 -ATPase and those of F_1F_0 -ATPase subunits from other sources

The N-terminal amino acid sequence for the pea δ -subunit (M_r 26500) was obtained as described in the text. Residue yields were 56 pmol (residue 1) declining progressively to 1 pmol (residue 38), after which yields were insufficient to permit further identification. Amino acid sequences of δ -subunits from *E. coli* (Walker *et al.*, 1984), sweet-potato F_1 (Kimura *et al.*, 1989), spinach chloroplast CF_1 (Hermans *et al.*, 1988) and bovine heart OSCP (Ovchinnikov *et al.*, 1984) are taken from the references cited. Residues identical with those in the pea δ (OSCP)-subunit sequence are boxed.

chondria. The experiments with submitochondrial particles (Fig. 2) demonstrate that this plant OSCP is present in all the mono- and di-cotyledonous plant species tested. The absence of the OSCP subunit from some purified preparations of plant F_1 -ATPase probably results from minor differences in the extraction and purification procedures or from a different degree of partitioning of OSCP between the F_0 and F_1 sectors of the plant ATPase rather than an inherent absence of OSCP from some types of plant mitochondria. Consequently, in six-subunit preparations of plant F_1 -ATPase, the 20–23 kDa subunit (usually designated as δ') should be regarded as the true δ -subunit. OSCP is often considered to be part of the F_0 sector of the ATPase, since it is not usually isolated together with the mammalian and yeast F_1 -ATPases. Since, in plants, OSCP can be isolated associated with F_1 -ATPase, it is likely that this component is more closely associated with F_1 than F_0 and that slight changes in the structure of components of the plant ATPase complex increase the relative binding to F_1 rather than to F_0 . Despite the observed differences in N-terminal amino acid sequences (Fig. 3), plant OSCP is sufficiently similar to mammalian OSCP to be recognized by antibodies to the mammalian protein. The results presented here reinforce the similarities between the F_1/F_0 -ATPases of plant, yeast and mammalian mitochondria. The results show that the plant F_1/F_0 -ATPase is more closely related to the corresponding enzyme complex in mammalian mitochondria than to the chloroplast CF_0/CF_1 complex from the same plant.

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