The plant mitochondrial F_1-ATPase

The identity of the δ' (20 kDa) subunit

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Received 5 September 1990

The N-terminal amino acid sequence of the 20 kDa (δ') subunit of the turnip (*Brassica napus* L.) mitochondrial F_1-ATPase has been determined. Comparison of the sequence obtained with those of the ε subunits of chloroplast CF_1, *E. coli* F_1 and the δ subunit of bovine F_1 shows that the turnip δ' subunit is another member of this family of homologous proteins. The δ' subunit of sweet potato F_1-ATPase [(1989) J. Biol. Chem. 264, 3183–3186] is very similar to the turnip sequence and thus can also be considered to belong to this family.

Plant mitochondria; Mitochondrial F_1-ATPase; F_1-ATPase; Subunit; Plant F_1-ATPase

1. INTRODUCTION

The mitochondrial F_1-ATPase has been purified from a number of different species of plants. While the enzymes obtained from maize (*Zea mays*) [1,2], faba bean (*Vicia fava*) [3], oat root (*Avena sativa*) [4] and cuckoo pint (*Arum maculatum*) [5], like that from mammalian sources, contain five subunits, those from sweet potato root (*Ipomoea batatas*) [6,7], pea cotyledon (*Pisum sativum*) [8,9] and turnip (*Brassica napus*) [10] have six subunits. ATPase subunits in the five subunit preparations are designated on the basis of their mobility in SDS polyacrylamide gel electrophoresis as α, β, γ, δ, and ϵ with approximate molecular masses of 55000, 52000, 30000, 20000 and 8000 with variations depending on the tissue source. Confusion exists in the identification of the subunits in the six subunit preparations of plant F_1 because these preparations contain two polypeptides in the δ region, the δ (M_r 25000–27000) and the δ' (M_r 20000–23000). The δ subunit of the six-subunit pea ATPase has been identified as the plant oligomycin-sensitivity-conferring protein [11], indicating that the δ' (20 kDa) subunit may be the plant equivalent of the δ subunit of the mammalian F_1-ATPase. However, the δ' (23 kDa) subunit in the six-subunit sweet potato F_1-ATPase may have no analogue in other ATPases since the N-terminal amino acid sequence could not be matched with any sequence in the EMBL data base [12]. This paper provides the N-terminal amino acid sequence of the turnip δ' subunit and shows it has a significant homology with other published F_1-ATPase subunit sequences as well as with that of the sweet potato δ' subunit.

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Abbreviations: OSCP, oligomycin-sensitivity-conferring protein; BSA, bovine serum albumin; SDS, sodium dodecyl sulphate; Tes, *N*-tris[hydroxymethyl]methyl-2-aminoethanesulphonic acid; PMSF, phenylmethylsulphonyl fluoride

2. MATERIALS AND METHODS

Mitochondria were isolated and purified from turnips purchased locally as described by Gauvrit and Wilson [13] except that BSA was omitted from the media. Mitochondria were only used if they had good respiratory control and ADP:O ratios characteristic of intact plant mitochondria. Osmotic shock particles were prepared by shocking the mitochondria in 20 vols of 2.5 mM Tes-KOH, pH 7.5, 1 mM EDTA at 4°C. All subsequent steps were carried out at room temperature. The ATPase was released from the membranes by solvent treatment [14] in the presence of 1 mM freshly prepared PMSF, except that dichloromethane was used as a solvent. The ATPase was purified from the dichloromethane-solubilised material after centrifugation at $100000 \times g$ for 40 min in a Beckman 40 rotor by the method of O'Rourke [10]. The soluble ATPase was precipitated between 30% and 50% saturated ammonium sulphate, collected by centrifugation and redissolved in 250 mM sucrose, 20 mM Tes-KOH, pH 7.5, 1 mM EDTA containing 4 mM ATP and fractionated on a 20–35% glycerol gradient at 39000 rpm for 18 h in a Beckman SW 41 rotor. The resulting ATPase band from the gradient was subjected to ion-exchange chromatography on a DEAE-Sephacel (Pharmacia Ltd, Milton Keynes, UK) column (0.9×13 cm) using a linear gradient of 0 to 0.5 M KCl in 30% (w/v) glycerol, 20 mM Tris-HCl, pH 8.0, 1 mM EDTA and 4 mM ATP. The purified ATPase migrated as a single protein band on non-denaturing polyacrylamide gel electrophoresis which coincided with the ATPase activity band located by activity staining [15]. Turnip ATPase prepared by this method normally contains six subunits [10] (approximately 52, 48, 30, 24, 20, and 9 kDa (O'Rourke and Wilson, unpublished data), although some preparations lack the δ (24 kDa) subunit.

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00145793/90/$3.50

Amino Acid Sequences of δ, δ′ and ε Subunits of F_1 - ATPases

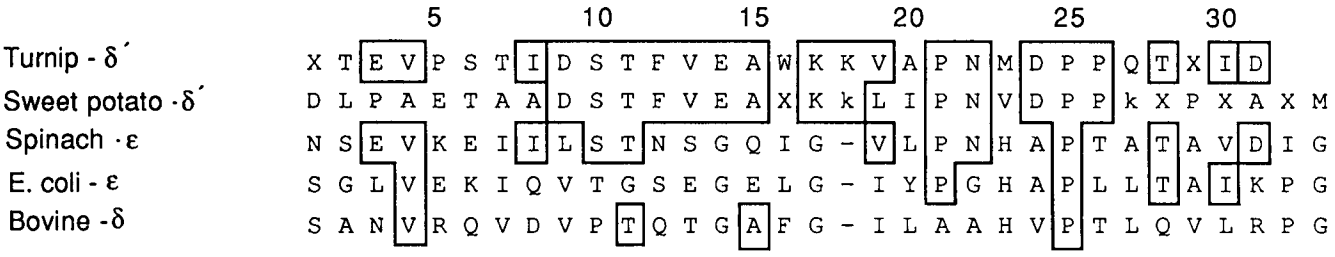

Fig. 1. The N-terminal amino acid sequence of the δ′ subunit of the turnip mitochondrial F_1-ATPase and comparable sequences of other F_1-ATPase subunits. The sequence of the turnip δ′ subunit (20 kDa) was obtained as described in the text. Residue yields were approximately 90 pmol for the first residues, gradually declining to 1 pmol for residue 31 after which yields were insufficient to enable further identification. Residue 4 of the turnip sequence gave 46 and 39 pmol of valine and leucine, respectively. Comparison is made with positions 16–47 of the spinach chloroplast ε subunit [19], 18–49 of the *E. coli* ε subunit [20,21], residues 30–61 of the bovine δ subunit [18] and residues 3–35 of the sweet potato δ′ subunit [12]. Alignments with the sweet potato δ′ were carried out manually, those of the chloroplast, *E. coli* and bovine sequences are taken from Walker et al. [18]. A break of 1 residue was incorporated into the alignments of the chloroplast, *E. coli* and bovine sequences to maximise homology. Sequences identical to those of the turnip are boxed.

For protein sequencing, samples of purified turnip F_1-ATPase were first dialysed against a large volume of distilled water before treating with gel electrophoresis sample preparation buffer containing 1% w/v SDS [16]. 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 (Hoefer Scientific Instruments Ltd, Newcastle, Staffs, UK). The gel was then blotted onto Immobilon-P membrane (Millipore UK Ltd, Watford Herts., UK) [17] and the δ′ (20 kDa) subunit band carefully excised with a scalpel.

N-Terminal protein sequencing of the excised δ′ subunit band was performed using automatic Edman degradation with the standard 03R PTH (phenylthiohydantoin) program in an Applied Biosystems model 470A gas-phase sequencer fitted with a 120A on-line PTH analyser.

3. RESULTS AND DISCUSSION

Fig. 1 shows the 31 residue N-terminal amino acid sequence of turnip F_1-ATPase δ′ subunit, manually aligned with sweet potato δ′ [12], and with spinach chloroplast CF_1 ε, bovine δ and *E. coli* ε subunits (aligned by Walker et al. [18]). Residue 4 showed evidence of heterogeneity with approximately equal yields of valine and leucine. There are 11 identical residues when the turnip sequence is compared with that of the spinach chloroplast CF_1-ATPase ε subunit, showing a close relationship between these two proteins. Similarities, albeit weaker, also exist between the turnip δ subunit and comparable regions of the bovine F_1 δ and *E. coli* F_1 ε subunit N-terminal sequences. Since the chloroplast CF_1 ε, the *E. coli* ε and the bovine δ subunits have been shown to be homologous proteins [18], the turnip F_1 δ′, which shows significant homology with the chloroplast CF_1 ε subunit, must also be considered homologous to the bovine δ and *E. coli* ε subunits despite the comparatively small number of identical residues in the segment of sequence compared in Fig. 1. There is a marked homology between the sweet potato and turnip sequences, demonstrating that these are also related subunits. However, when the sweet potato δ′ sequence is compared directly with the comparable regions of the spinach CF_1 ε subunit and other related sequences there are only five identical residues in the overlapping region of 35 residues. This low number of identical residues probably explains why the sweet potato δ′ sequence could not be matched with sequences in the literature [12]. It is notable that both plant δ′ subunits are at least 13 residues shorter at the N-terminus than the comparable chloroplast, bovine and *E. coli* subunits. Of the three residues which are common in all the relevant parts of the ε subunit alignments of Walker et al. [18], only proline-53 (in the bovine sequence) is conserved through the two plant mitochondrial δ′ sequences. The similarities between the plant mitochondrial δ′ and other ATPase subunit sequences show that this subunit is not unique to plant F_1-ATPase and confirm an earlier suggestion [11] that the plant δ′ subunit is the plant equivalent of the bovine δ and the chloroplast and bacterial ε subunits.

Acknowledgements: We thank Aileen Flett for her excellent technical assistance, the Science and Engineering Research Council (UK) and the Natural Sciences and Engineering Research Council of Canada (operating grant to A.H.) for their financial support.

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