Putative amino-terminal presequence for β-subunit of plant mitochondrial F_1ATPase deduced from the amino-terminal sequence of the mature subunit

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The α- and β-subunits of sweet potato mitochondrial F_1ATPase were purified from the F_1 complex by gel filtration and ion-exchange high-performance liquid chromatography. Isoelectric focussing and N-terminal amino acid sequencing indicated that the purified β-subunit contains at least two polypeptides similar to each other. The N-terminal 18 amino acid sequence of the β-subunit showed homology to the amino acid sequence of the tobacco mitochondrial F_1ATPase β-subunit precursor deduced from the nucleotide sequence [(1985) EMBO J. 4, 2159–2165] between residues 56 and 73, suggesting that the N-terminal 55 amino acids of the tobacco precursor constitute the presequence required for mitochondrial targetting.

(Plant) Mitochondria Organelle biogenesis F_1ATPase β-Subunit Nuclear-encoded subunit Presequence

1. INTRODUCTION

In higher plants, both mitochondria and chloroplasts have F_1ATPases with structure and catalytic functions similar not only to each other, but also to F_1ATPases from other organisms. However, by producing ATP via oxidative phosphorylation in one case or photosynthetic phosphorylation in the other, the role of these two enzymes in the supply of cellular ATP greatly varies depending on the tissues, and the growth or developmental stage of the tissue. Since the subunits of mitochondrial (F_1) and chloroplast (CF_1) F_1ATPases are encoded by nuclear and each organelle's genome, the function of each ATPase must be dependent on the co-operated gene expression among nuclear and each organelle's genes, and proper assembly of subunits synthesized in the different locations within the cell.

All of the five subunits (α-, β-, γ-, δ- and ε-subunits) from fungal and mammalian F_1 are encoded by nuclear genes [1]. In the case of CF_1, which also consists of five subunits, only the γ- and δ-subunits are encoded by nuclear genes, whereas the other subunits are encoded by chloroplast genes [2]. Higher plant mitochondrial F_1 has been reported to contain five [3–6] or six [7,8] subunits depending on the plant species. Among these subunits, the α-subunit is encoded by the mitochondrial genome and its gene structures have recently been determined in maize [9,10] and pea (Morikami, A. and Nakamura, K., in preparation). The other subunits are encoded by nuclear genes [4,11].

The nuclear-encoded proteins of mitochondria and chloroplast are generally synthesized on cytosolic ribosomes as larger precursors having N-terminal presequence, and post-translationally

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Abbreviation: DEAE, diethylaminoethyl

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targetted to proper organelle [1,2]. These presequences are cleaved from the mature protein during or after transport into organelle. It is supposed that the specific targetting depends on the nature of the presequence and its interaction with each organelle. Indeed, we have previously shown by in vitro translation of poly(A)$^+$ RNA from sweet potato tuberous roots that at least three subunits (β-, δ- and δ'-subunits) of mitochondrial F_1 are synthesized as larger precursors [11].

Although the presequence, or 'transit peptide' [12], of several chloroplast proteins has been determined and characterized in detail [13], none of the presequence of nuclear-encoded plant mitochondrial protein has been determined so far. Recently, Boutry and Chua [14] determined the nucleotide sequence of a nuclear gene coding for the β-subunit of tobacco mitochondrial F_1. From a comparison of the deduced amino acid sequence of the precursor with the yeast or *E. coli* F_1 β-subunit sequence, they suggested that the N-terminal 60–86 residues of the precursor may comprise the presequence. In this study, we purified the α- and β-subunits of sweet potato mitochondrial F_1. A comparison of the N-terminal 18 amino acid sequence of the β-subunit with the sequence of the tobacco precursor suggests that the N-terminal 55 residues of the tobacco precursor constitute the presequence.

2. MATERIALS AND METHODS

2.1. *Purification of sweet potato mitochondrial F_1ATPase (F_1)*

Mitochondrial F_1 was purified from the parenchymatous tissue of sweet potato (*Ipomoea batatas* Lam. var Kokei no.14) tuberous roots as described in [7].

2.2. *Purification of F_1 α- and β-subunits*

Lyophilized F_1 was dissolved to give a final concentration of 5 mg/ml in 6 M guanidine hydrochloride (Sigma), 10 mM sodium phosphate (pH 6.5), 1 mM EDTA, and 4 mM dithiothreitol. Dissociated subunits (300 μg) were separated by gel filtration high-performance liquid chromatography using a GlasPac TSK G3000SW column (8 × 300 mm, LKB) equilibrated with the same buffer without dithiothreitol at a flow rate of 0.15 ml/min. The peak fraction containing both α- and β-subunits was dialyzed against 8 M urea, 20 mM Tris-HCl (pH 7.0), and injected onto an Ultropac TSK 545 DEAE column (7.5 × 150 mm, LKB) equilibrated with the same buffer. The α- and β-subunits were separated by elution with a linear 30 min gradient of NaCl from 0 to 0.45 M at a flow rate of 0.7 ml/min.

2.3. *Isoelectric focussing*

Isoelectric focussing was performed on 4% (w/v) polyacrylamide, 0.2% (w/v) *N,N'*-methylenebisacrylamide disc gels containing 8.5 M urea, 2% (w/v) Nonidet P-40, 1.6% (w/v) Ampholine (pH 5–7), and 0.4% (w/v) Ampholine (pH 3.5–10) at 300 V for 10 h.

2.4. *Amino-terminal amino acid sequence analysis*

Lyophilized β-subunit (~5 nmol) dissolved in 0.1% SDS was applied to an automated amino acid sequence analyser (JEOL JAS-47K) using 0.1 M Quadrol buffer (pH 9.0) as a coupling buffer and polybrene as a carrier. The phenylthiohydantoin (PTH) amino acids were identified by high-performance liquid chromatography using TSK ODS-120T column (4.6 × 250 mm, Toyo Soda, Tokyo) by an isocratic elution system at 30°C [15].

2.5. *Other methods*

Protein concentration was determined by a Bio-Rad protein assay system with bovine serum albumin as the standard. SDS-polyacrylamide gel electrophoresis on a 10% (w/v) polyacrylamide slab gel containing 2 mM EDTA was performed according to Laemmli [16].

3. RESULTS AND DISCUSSION

When purified sweet potato mitochondrial F_1 complex was dissociated with 6 M guanidine hydrochloride and subjected to gel filtration high-performance liquid chromatography, the α- and β-subunits were separated from other small subunits and minor contaminants which copurified with F_1ATPase in this experiment (fig.1). After the dissociating agent was replaced with 8 M urea, the α- and β-subunits were separated from each other by DEAE ion-exchange high-performance liquid chromatography (fig.2). The α- and β-subunits were eluted at about 0.12 and 0.17 M NaCl,

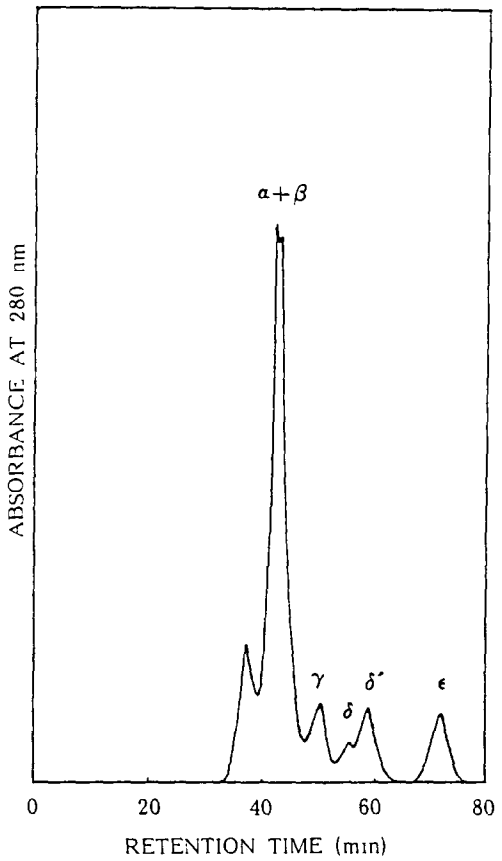

Fig.1. Separation of sweet potato mitochondrial F_1 subunits by high-performance liquid chromatography using a GlasPac TSK G3000SW column with 6 M guanidine hydrochloride. Polypeptide composition of each peak fraction was determined by SDS-polyacrylamide gel electrophoresis (not shown).

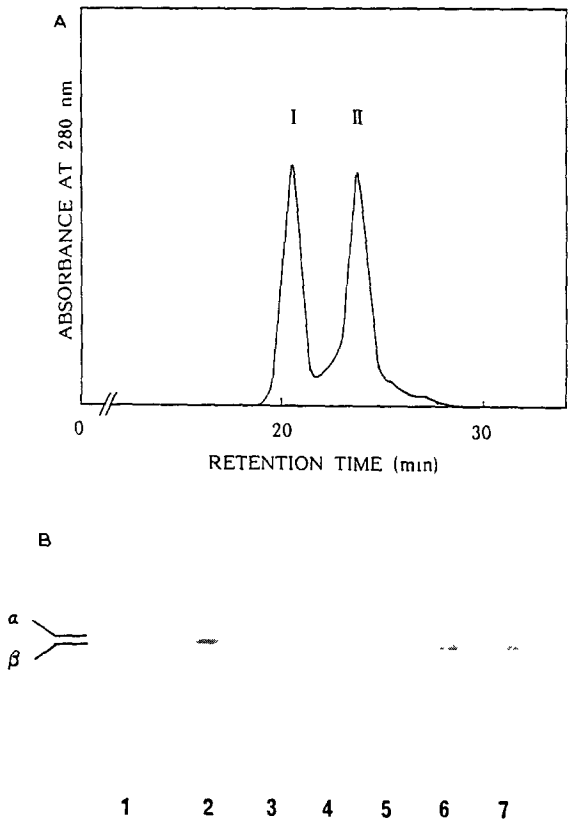

Fig.2. (A) Purification of the α- and β-subunits by high-performance liquid chromatography using an Ultropak TSK-545 DEAE column with 8 M urea. (B) SDS-polyacrylamide gel electrophoresis of purified subunits. Lanes: 1, α- plus β-subunit fraction from a GlasPac TSK-G3000 SW column; 2,3 and 4, purified α-subunit (peak I); 5,6 and 7, purified β-subunit (peak II).

respectively. The purified α- and β-subunits migrated as a single band with an apparent M_r of 52500 and 51500, respectively, on SDS-polyacrylamide gel electrophoresis (fig.2).

As shown in fig.3, isoelectric focussing of the purified α- and β-subunits demonstrated the existence of charge heterogeneity in each subunit. The α-subunit showed one major band with a p*I* value of about 6.4 and several minor bands. The β-subunit was dissolved into two major bands with p*I* values of 5.4 and 5.5, and one minor band. These p*I* values of the α- and β-subunits are similar to those reported for mitochondrial F_1 of maize [4]. The charge heterogeneity of the F_1 α- and β-subunits has also been reported in other organisms [3,4,17]. These charge variant components may result from the microheterogeneity in primary sequences, or the proteolytic cleavage or the chemical modification of the identical polypeptide [17].

The N-terminal amino acid sequence of the α-subunit could not be determined possibly because of the *N*-formylation of the Met residue [18]. The N-terminal 18 amino acid sequence of the β-subunit was determined (fig.3). Although amino acid residues at positions 1, 2 and 16 could not be determined, the amino acids at these positions could be Ser because of the lability of phenylthiohydantoin-Ser. Equimolar amounts of Glu and Asp were detected at the eighth position of

Fig.3. Isoelectric focussing of purified sweet potato mitochondrial F_1ATPase α- and β-subunits. Lanes: 1, α-subunit (10 μg); 2, β-subunit (10 μg).

the sequence. Taken together with the fact that the purified β-subunit shows two major bands with different p*I* values (fig.2), these results suggest that the β-subunit of sweet potato mitochondrial F_1 contains at least two polypeptides similar to each other. Two larger precursors for the β-subunit previously detected by in vitro translation of poly(A)$^+$ RNA from sweet potato tuberous roots [11] may correspond to precursors for these two polypeptides. Boutry and Chua [14] have recently reported that tobacco F_1 β-subunit is encoded by two nuclear genes, both of which are expressed. Likewise, the β-subunit of sweet potato F_1 may also be coded for by at least two nuclear genes.

The amino acid sequence of the β-subunit from various organisms is highly conserved, except for the N-terminal and the C-terminal part of the sequence [17]. The N-terminal sequence of sweet potato F_1 β-subunit did not show any obvious sequence homology to those from bacteria, chloroplast, or bovine mitochondria [17]. The sequence, however, was homologous to the amino acid residues 56–73 of tobacco F_1 β-subunit precursor sequence deduced from the DNA sequence by Boutry and Chua [14] (fig.3). The precursors for tobacco and sweet potato F_1 β-subunits are reported to be larger than the respective mature forms of about 8800 [14] and 6500–7500 Da [7], respectively. These results suggest that the N-terminal 55 residues of the tobacco precursor constitute the presequence. The apparent discrepancy between the size of the presequence estimated from SDS-polyacrylamide gel electrophoresis and that predicted from the se-

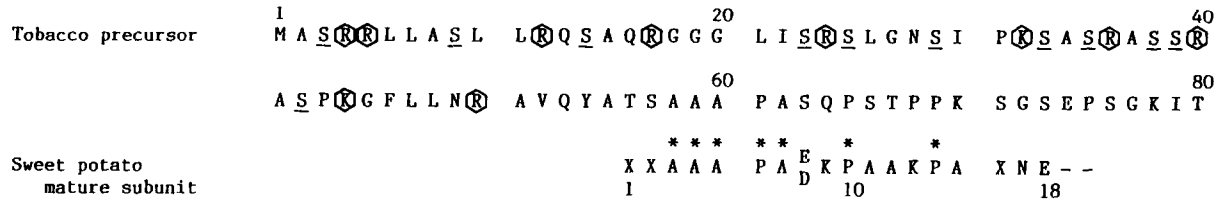

Fig.4. Amino-terminal amino acid sequence of sweet potato mitochondrial F_1ATPase β-subunit and its comparison to the tobacco precursor. The amino acid sequence of the tobacco precursor deduced from the DNA sequence is from [14]. Amino acid residues at positions 1, 2 and 16 (indicated by X) of the sweet potato mature subunit could not be determined (see section 3). Homologies between two amino acid sequences are indicated by asterisks. Basic amino acids (○) and serine (▬) in the putative presequence of the tobacco precursor are indicated.

quence could be due to the basic nature of the presequence.

The putative presequence for tobacco F_1 β-subunit does not show any sequence homology with that of human F_1 β-subunit recently reported by Ohta and Kagawa [19]. Both presequences, however, are similar to each other with respect to their length and characteristic features that they are very poor in acidic amino acids and enriched in basic amino acids, serine and threonine. These structural characteristics of presequences are similar to those of many imported mitochondrial proteins [1]. The transit peptides of nuclear-encoded chloroplast proteins, with varying length of 33–57 amino acids, are also rich in basic amino acids, serine and threonine [13]. Unlike mitochondrial presequences, sequence homologies at three locations of these transit peptides are observed [13]. The putative presequence for tobacco F_1 β-subunit does not show homology to any one of these framework sequences shared by transit peptides.

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