Immunological comparisons among energy-transducing adenosine triphosphatases from higher plants with respect to the structures of their subunits

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Anti-sweet potato mitchondrial F_1-ATPase antibody that recognized the α- and β-subunits of its antigen reacted with β-, but not α-, subunits of F_1-ATPases from guinea pig liver mitochondria and sweet potato, pea and spinach chloroplasts. The peptide maps and their immunoblots corresponded closely with the antibody of the β-subunits from various sources.

Mitochondrion *Chloroplast* *F_1-ATPase* *Peptide mapping* *Immunological comparison*

1. INTRODUCTION

Higher plants possess two types of F_1F_0-ATPases; one in the mitochondria and the other in the chloroplasts. The two enzyme complexes resemble each other in subunit composition of the F_1 portion as well as in function [1,2], but differ from each other in genetic control. Considering the genetic control of mitochondrial F_1-ATPase in other organisms [3,4], all the subunits of the plant enzyme complex are probably coded by the nuclear genome, although the α-subunit may be exceptional: a recent paper reported its coding by mitochondrial DNA [5,15] whereas another showed its in vitro translation with a wheat germ translation system and plant poly(A)-RNA [6]. In contrast, only γ- and δ-subunits of chloroplast F_1-ATPase are coded by nuclear genome and the others (α, β and ϵ) by chloroplast DNA [7]. This work compared plant mitochondrial and chloroplast F_1-ATPases with respect to the structures of their subunits using an antibody against sweet potato mitochondrial F_1-ATPase.

2. MATERIALS AND METHODS

Sweet potato (*Ipomoea batatas*, Kokei No.14) roots and pea (*Pisum sativum* var. Alaska) seeds were surface-sterilized with 1% sodium hypochlorite for 10 min, followed by thorough washing with tap water. They were cultivated at 25°C in vermiculite for 2 months and 3 weeks, respectively, under illumination with light of about 4000 lux (pea seeds were allowed to soak in tap water in the dark at 25°C for 1 day before cultivation). Spinach (*Spinacia oleracea* L.) leaves were obtained from a local market. Submitochondrial particles and chloroplasts were isolated as in [9,10], except that the homogenizing medium for sweet potato chloroplasts contained 1% (w/v) BSA. Thylakoid membranes were obtained by washing the chloroplast suspensions (1 ml each, 0.1 mg chlorophyll·ml^{-1}) 3 times with 20 ml each of 10 mM

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Abbreviations: BSA, bovine serum albumin; F_1-ATPase, soluble form of energy-transducing adenosine triphosphatase; F_1F_0-ATPase, energy-transducing adenosine triphosphatase

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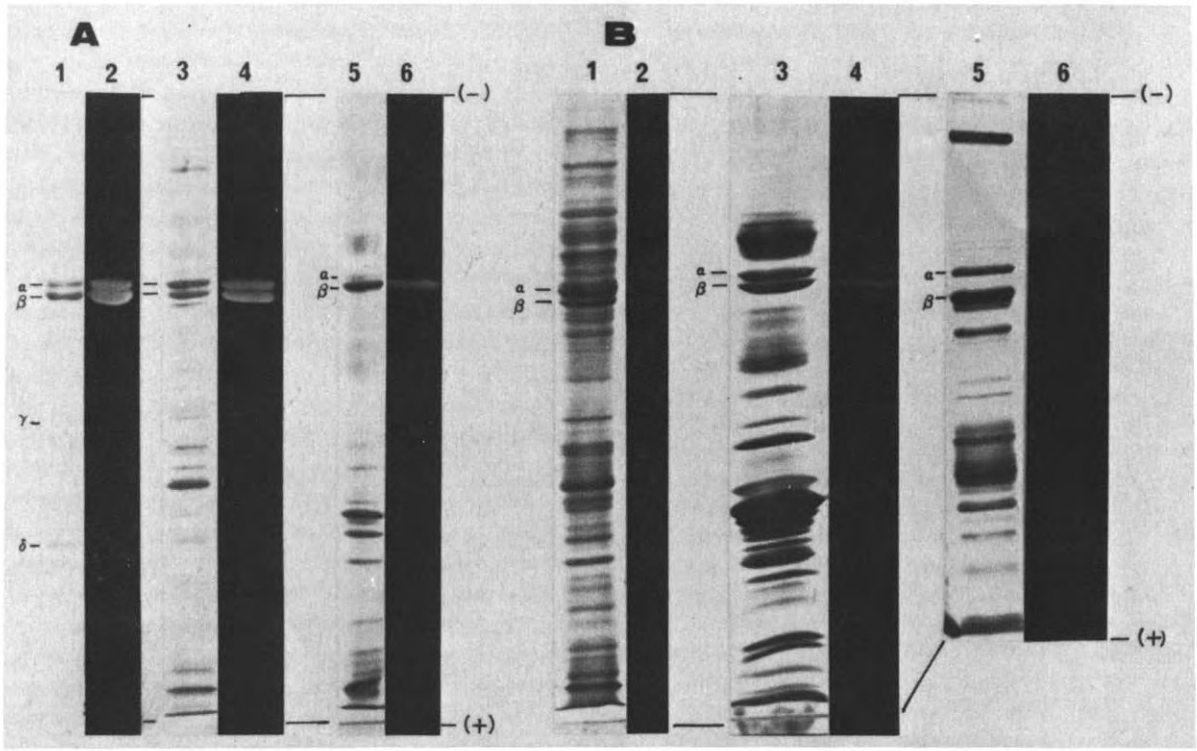


Fig.1. Immunoblotting with anti-sweet potato mitochondrial F_1-ATPase antibody of SDS-polyacrylamide gels applied with submitochondrial particles and thylakoid membranes. The particles and membranes were subjected to SDS-polyacrylamide gel electrophoresis (SDS–PAGE), then the gels were stained with Coomassie brilliant blue (odd number lanes) or analyzed by immunoblotting (even number lanes) as described in section 2. (A) Lanes 1,2, purified sweet potato mitochondrial F_1-ATPase (3 μg protein); lanes 3,4, sweet potato root submitochondrial particles (25 μg protein); lanes 5,6, sweet potato thylakoid membrane (25 μg protein). (B) Lanes 1,2, guinea pig liver submitochondrial particles (105 μg protein); lanes 3,4, pea thylakoid membrane (200 μg protein); lanes 5,6, spinach thylakoid membrane (50 μg protein).

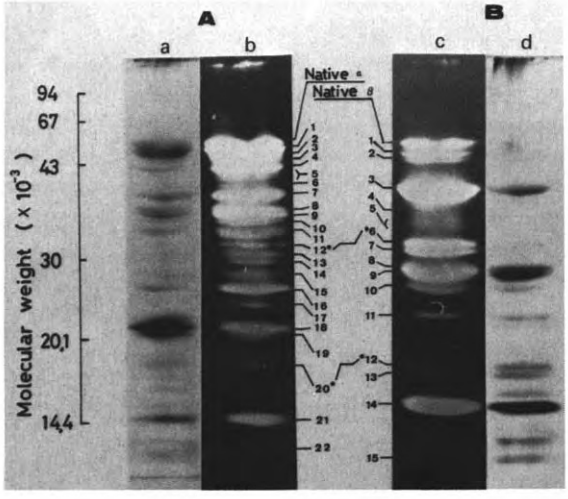
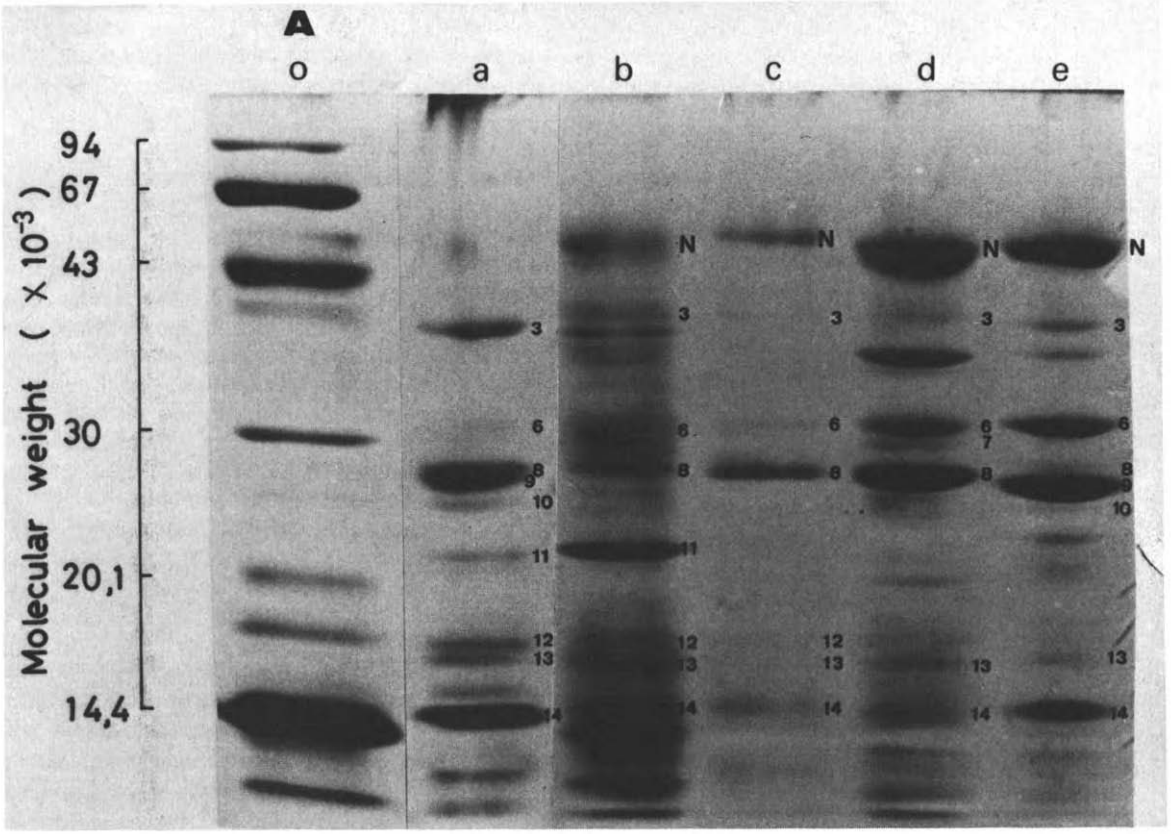


Fig.2. Peptide maps of the α (A)- and β (B)-subunits of sweet potato mitochondrial F_1-ATPase. After SDS–PAGE of sweet potato root submitochondrial particles (fig.1A, lane 3), the band of the α- or β-subunit was cut from the gel and again subjected to SDS–PAGE after addition of 2 μl of 5 ng·ml^{-1} *Staphylococcus* V8 protease. Then the gel was stained with the Bio-rad silver staining kit (lanes a,d; submitochondrial particles with 25 μg protein were used) or subjected to immunoblotting with anti-sweet potato mitochondrial F_1-ATPase antibody (lanes b,c; submitochondrial particles with 60 μg protein were used). The blots detected are numbered in the M_r order of the corresponding peptide, and the two pairs of blots with asterisked numbers connected with a line (12 for A and 6 for B, 20 for A and 12 for B) are of peptides with the same M_r.

NaCl. Electrophoresis on a 12.5% polyacrylamide gel (slab gel, acrylamide:bisacrylamide = 30:0.8) containing 0.1% SDS and peptide mapping with *Staphylococcus* V8 protease were performed as in [11]. The gels were stained with Coomassie brilliant blue or a Bio-rad silver staining kit. Antiserum against purified sweet potato mitochondrial F_1-ATPase was prepared as in [1]. Immunoblotting was performed as in [12], except that 1% Triton X-100 was added to the washing solution. Protein was determined as in [13] after precipitation with trichloroacetic acid.



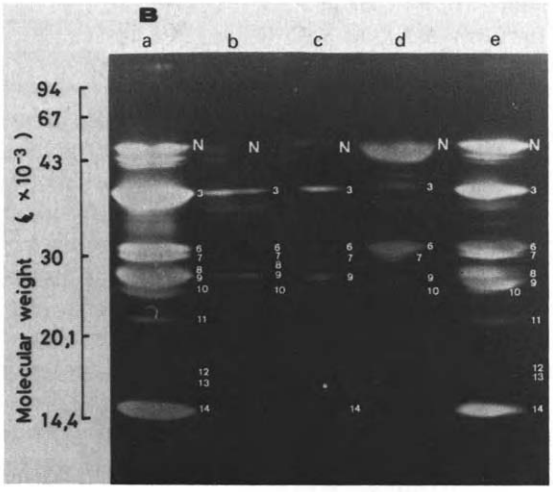


Fig.3. Peptide maps of β-subunits of F_1-ATPases from various sources. The bands of β-subunits from various submitochondrial particles and thylakoid membranes were subjected to peptide mapping as in fig.2. (A) Stained with Bio-rad silver staining kit; (B) immunoblotting. Lane a, from sweet potato root submitochondrial particles with 20 and 60 μg protein for A and B, respectively; lane b, from guinea pig liver submitochondrial particles with 200 μg protein each; lane c, from sweet potato thylakoid membrane with 100 μg protein each; lane d, from pea thylakoid membrane with 400 μg protein each; lane 3, from spinach thylakoid membrane with 400 μg protein each. The numbers correspond to those shown in fig.2, and N indicates each native subunit.

3. RESULTS AND DISCUSSION

The antibody against purified sweet potato mitochondrial F_1-ATPase reacted vigorously with the α- and β-subunits of the antigen in immunoblotting (fig.1A, lanes 1,2). It also reacted with the γ- and δ-subunits, but the reactions were too weak to be utilized for comparing either γ- or δ-subunits of F_1-ATPases from various sources with respect to the immunological properties. No blots other than those for the α- and β-subunits were detected with the submitochondrial particles from sweet potato root tissue (fig.1A, lanes 3,4), indicating that the antibody was very specific for the F_1-ATPase.

The antibody also reacted with β-, but not α-, subunits of F_1-ATPases from various other sources including guinea pig liver mitochondria, and sweet potato, pea and spinach chloroplasts (fig.1A, lanes 5,6, fig.1B). The results indicate that β-subunits of F_1-ATPases from various sources resemble one another in immunological properties, whereas the α-subunit of sweet potato mitochondrial F_1-ATPase differs from the corresponding subunits from other sources, even from chloroplasts in the same plant species. Immunological homology among the β-subunits of F_1-ATPases from *E. coli*, yeast mitochondria, and Swiss chard chloroplasts has been reported [14].

The anti-sweet potato mitochondrial F_1-ATPase antibody also reacted with almost all peptides produced from either the α- or β-subunit of its antigen by digestion with *Staphylococcus* V8 protease in immunoblotting, although there were differences in the reactivity among the peptides (fig.2). Marked differences were observed in both the one-dimensional peptide map and its immunoblotting profile between the α- and β-subunits. There were similarities, however, among the maps and profiles of β-subunits of F_1-ATPases from various sources (fig.3). In the map and the profile for any β-subunit, peptide bands or blots corresponding to almost all of those for the β-subunit of sweet potato mitochondrial F_1-ATPase were detected although there were some differences in the densities of the peptide bands or blots. The results indicate remarkable similarities among the structures of the β-subunits from various sources. Thus our work confirms the concept that the β-subunit of F_1-ATPase has been very conservative during its evolution [8,14]. We emphasize that in a plant species, there is close correspondence among the structures of the β-subunits, but striking differences in the structures of the α-subunits between mitochondrial and chloroplast F_1-ATPases.

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