THE ROLE OF δ SUBUNIT IN THE COUPLING ACTIVITY OF CHLOROPLAST COUPLING FACTOR 1

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1. Introduction

Chloroplast coupling factor 1 (CF_1) is composed of five distinct polypeptides that were designated as α, β, γ, δ and ε subunits [1,2]. The individual subunits have been isolated following denaturation by pyridine and urea treatments [3]. Among the five isolated subunits only the ε has been shown to preserve its alleged in situ activity [2]. Purified ε subunit inhibits the ATPase activity of heat activated CF_1. Recently we reported about experimental results that suggest that the δ subunit is involved in the binding of CF_1 to the membrane [4].

Purification of *E. coli* ATPase yielded an enzyme containing only 4 polypeptides with no detectable δ subunit [5–7]. This preparation possesses high ATPase activity but was not capable to restore energy coupling of depleted membranes. Recently Smith and Sternweis [8] reported on restoration of coupling factor activity by a preparation that was enriched with δ and ε subunits.

It is the purpose of this communication to report on purification of δ subunit of CF_1 that can restore coupling activity of CF_1 that was depleted of this subunit.

2. Materials and methods

Lettuce chloroplasts were prepared as previously described [9]. Stable EDTA-treated chloroplasts were prepared by a modification of previously described procedure [10]. This was based on the observation that the conditions for the removal of CF_1 from the chloroplast membranes vary from one plant species to another. Therefore, the concentration of EDTA and the duration of the treatment should be adjusted prior to the large scale preparation of EDTA-particles. Chloroplast suspension in a medium containing 0.4 M sucrose, 10 mM NaCl, 10 mM Tricine (pH 8) and 10 mg/ml bovine albumin at chlorophyll concentration of about 1.5 mg/ml were diluted 10 times in 2 mM EDTA solution at 0°C. Immediately after the dilution the suspension was centrifuged at 20 000 × *g* for 10 min and the pellet was suspended in a medium containing 0.4 M sucrose, 10 mM NaCl, 10 mM Tricine (pH 8) and 10 mg/ml bovine albumin at a chlorophyll concentration of about 1.5 mg/ml. The CF_1 depleted particles were divided to 0.5 ml portions, frozen immediately at –70°C and thawed just before use.

The reconstitution of EDTA-particles was performed at 0°C for 20 min. The reaction mixture contained in a total volume of 0.5 ml; 20 μmol of Tricine (pH 8), 20 μmol of NaCl, CF_1 as specified, 10 μl of EDTA-particles and 5 μmol of $MgCl_2$. After the incubation 1 ml of photophosphorylation reaction mixture containing; 33 μmol of Tricine (pH 8), 33 μmol of NaCl, 13 μmol of $MgCl_2$, 6.6 μmol of sodium P_i (pH 8), 2 μmol of ADP and about 10^6 cpm of $^{32}P_i$ as well as 0.3 ml water and 0.2 ml of 0.3 mM *N*-methylphenazonium methosulfate were added. After illumination by white light (3×10^5 ergs/cm^2/s) for 2 min the reaction was terminated by the addition of 0.2 ml of 30% trichloracetic acid. The photophosphorylation was assayed according to Avron [11].

Chloroplast CF_1 was prepared by the method of Lien and Racker [12] and was stored at 4°C in 50% saturated ammonium sulfate. Aliquots were precipitated, dissolved in a solution containing 10 mM Tricine

(pH 8) and 1 mM EDTA and desalted by passing through Sephadex G-50 column (1 X 20 cm) that was equilibrated with the same buffer. The protein concentration was determined spectrophotometrically [12] and ATP at 1 mM final concentration was added to the CF_1 containing fractions. In the presence of ATP the CF_1 can be kept frozen at −20°C without loss of activity.

The chlorophyll concentration [13], protein concentration [14] and SDS–gel electrophoresis [3,15] were assayed by the published procedures.

3. Results

The δ subunit of CF_1 was purified by a modified procedure for the purification of the ε subunit [2]. About 35 mg of purified CF_1 in 50% saturated ammonium sulfate were precipitated and dissolved in 20 ml of a medium containing 10 mM Tricine (pH 8) and 2 mM EDTA. Twenty ml of pyridine were added with vigorous stirring and the solution was incubated for 10 min at room temperature. Sixty ml of distilled water were added and after incubation at 0°C for 30 min the turbid solution was centrifuged at 10 000 X *g* for 10 min. The supernatant solution was put under reduced pressure in a rotary evaporator until the solution became turbid. Most of the residual α, β, γ and ε subunits were removed by centrifugation at 10 000 X *g* for 10 min. The supernatant was

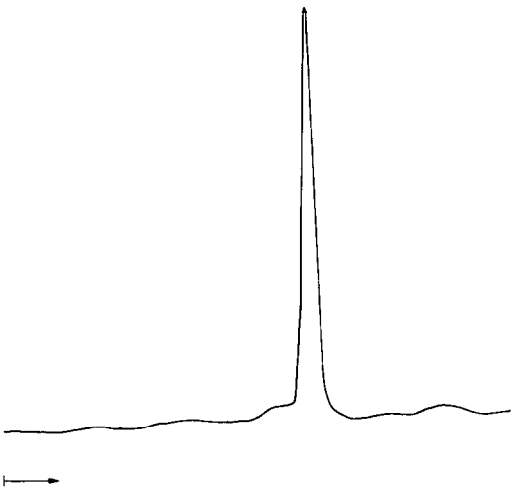

Fig.1. The SDS–gel electrophoresis pattern of the purified δ subunit of CF_1. A sample containing about 10 μg protein was incubated for 2 h at room temperature in a medium containing 2% SDS, 2% mercaptoethanol and about 10% sucrose. The electrophoresis was performed as described in Materials and methods.

Table 1
Amino acid analysis of the δ subunit of CF_1

Amino acid	Amount per 100 μmoles of amino acid	Residues per molecule (mol. wt 17 500)
Lysine	5.48	9
Histidine	1.63	3
Arginine	3.08	5
Aspartic acid	9.98	17
Threonine	5.30	9
Serine	9.96	17
Glutamic acid	13.82	23
Proline	2.39	4
Glycine	13.13	22
Alanine	8.39	14
Valine	8.03	13
Methionine	0.60	1
Isoleucine	5.29	9
Leucine	6.95	11
Tyrosine	2.87	5
Phenylalanine	3.08	5

Molecular weight of 17 500 was used to determine the number of residues per peptide unit.

lyophylized and the dry material washed with 2 ml of water and then dissolved in 1 ml of 10 M urea. The mixture was applied on DEAE–cellulose column (1.0 × 6.5 cm) that was equilibrated with a solution containing 10 mM Tris–Cl (pH 8) and 9 M urea. The elution was carried out with 1 ml of the equilibration buffer, then 4 ml of the same buffer containing 0.5 M NaCl, then 4 ml of the same buffer containing 1 M NaCl and finally 8 ml of the same buffer containing 2 M NaCl. One ml fractions were collected and the δ subunit appeared in the second protein peak around the tenth fraction.

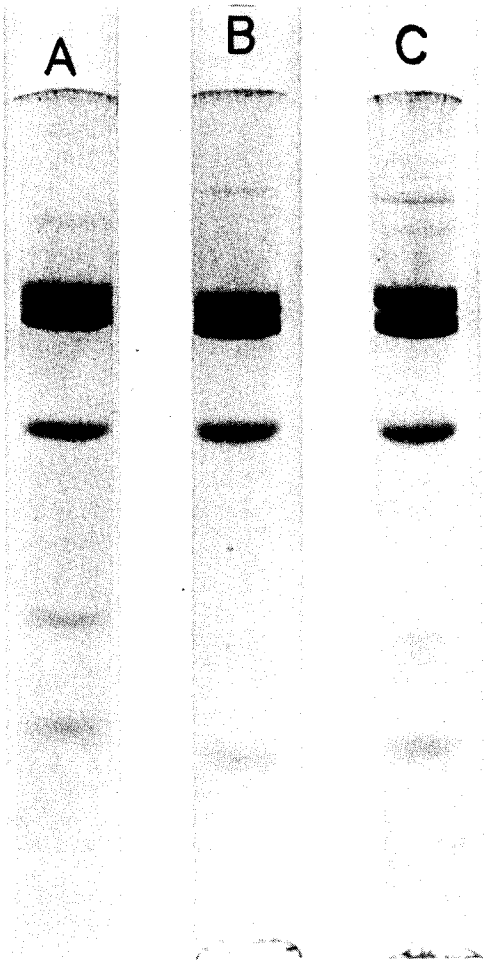

Fig.2. The SDS–gel electrophoresis pattern of CF_1 prepared from spinach, lettuce, and pea chloroplasts. About 35 μg of pea (A), lettuce (B) and spinach (C) CF_1 were incubated with SDS and electrophorised as described in fig.1.

Figure 1 shows the SDS–gel electrophoresis pattern of the fraction containing the purified δ subunit. The preparation appeared to be nearly homogeneous and other polypeptides seemed not to exceed 5% of the total protein.

Table 1 reveals the amino acid composition of the δ subunit of CF_1. It does not resemble those of the other 4 subunits [2,3]. The one methionine residue per molecule of δ seems to be suitable for the initiation of chemical studies with this polypeptide.

In order to study the activity of the purified δ subunit we looked for a CF_1 preparation that lacks this subunit. It appeared that in CF_1 from different plant species the firmness of the δ subunit binding is variable. Figure 2 shows the SDS–gel electrophoresis pattern of CF_1 prepared from lettuce, spinach and pea chloroplasts. It is apparent that lettuce CF_1 is depleted of the δ subunit. Figure 3 depicts the coupling activity of the various CF_1 preparations. The most effective CF_1 in the reconstitution experiments was the pea enzyme while the lettuce was much less effective. These studies seem to show that the amount of δ subunit in CF_1 determines the coupling activity of the enzyme. When the purified δ subunit was added during reconstitution of EDTA-particles with lettuce CF_1 its coupling activity was increased (fig.4). The reaction was saturated with amounts of

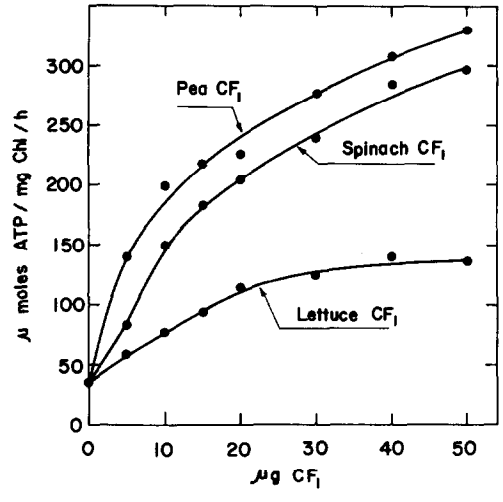

Fig.3. Reconstitution of EDTA-particles by CF_1 prepared from spinach, lettuce and pea chloroplasts. The reconstitution was carried out as described in Materials and methods. The EDTA-particles contained 1.5 mg chlorophyll per ml.

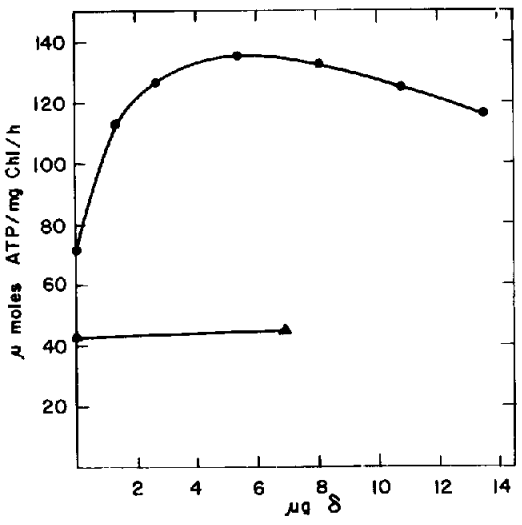

Fig.4. The effect of purified δ subunit on coupling activity of lettuce CF_1. The reconstitution experiment was carried out as described in fig.3. (●——●) 10 μg of lettuce CF_1 were present during reconstitution (▲——▲) without the addition of CF_1. The control value for reconstitution with 10 μg of pea CF_1 was 180 μmol ATP/mg Chl/h.

δ subunit approximating those of the depleted CF_1. Addition of δ subunit by itself or with pea CF_1 did not affect the phosphorylation activity of reconstituted EDTA-particles. Preincubation of the purified δ subunit with lettuce CF_1 was not beneficial.

4. Discussion

Previous studies of the mechanism of action of *E. coli* ATPase and CF_1 indicated that the δ subunit might be involved in the binding of the enzyme to the membrane [5–7]. The work of Smith and Sternweis [8] clearly demonstrated that the addition of a preparation that was enriched in δ and ε subunits renders inactive 4 subunits *E. coli* ATPase to an active coupling factor. They concluded that this activity was contributed by the δ and not the ε subunit. In this communication a purification procedure for the δ subunit of CF_1 is described. By the addition of the purified δ subunit of CF_1 which is depleted of this subunit its coupling activity was restored. It was previously reported that CF_1 which was depleted of δ subunit failed to bind to EDTA-particles. Therefore, it seems that the addition of δ subunit facilitates the binding of CF_1 to the membranes and by doing so renders the enzyme from inactive to active coupling factor.

It was found that in lettuce CF_1 the δ subunit is readily dissociated from the enzyme and that upon addition of any preparation containing the δ subunit the coupling activity of lettuce CF_1 is increased (data not shown). This might suggest that the 'second coupling factor' that was reported by Shoshan and Shavit [16] was in fact the free δ subunit in their preparation.

It is noteworthy that the δ subunit was added in a completely denatured state and during the reconstitution period should undergo renaturation. It is likely that this renaturation is facilitated by the depleted CF_1 or by the chloroplast membrane serving as a template for this process. Similar phenomenon was first described for the ε subunit of CF_1 which was shown to act as an ATPase inhibitor [2]. However, the δ subunit might fulfill a more complex role than the ε subunit. If indeed the δ subunit makes the connection between CF_1 and the membrane, the protons might be channeled through this subunit during energy transduction.

Acknowledgement

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