RELEASE OF THE α SUBUNIT OF COUPLING FACTOR F_1 ATPase FROM MEMBRANES OF AN UNCOUPLED MUTANT OF *ESCHERICHIA COLI*

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1. Introduction

The coupling factor F_1-ATPase of *Escherichia coli*, consisting of 5 subunits, α, β, γ, δ and ε, is bound to an integral part (F_0) of the membranes. The entire complex F_1–F_0 is active in synthesis of ATP coupled to a protonmotive force, as shown in mitochondria and chloroplasts (reviewed [1–3]). Differences have been found in the ionophoric properties of F_0 from cells of F_1 mutants of *E. coli* grown in different culture conditions [4–7]. Membranes from an uncoupled mutant DL54 [8] (grown in rich medium) did not become leaky to protons when washed with EDTA, whereas membranes from the same strain grown in a synthetic medium were leaky to protons even before washing with EDTA [7]. This treatment removes F_1 from wild-type membranes and protons can then pass freely through the F_0 portion [9].

Here we analyzed the EDTA extract of the mutant membranes and detected the active α subunit in it. The β and γ subunits were not detectable in the extract, but were found immunochemically in the residual membranes. The α subunit could be partially purified from the extract. These results suggest that the mutation, possibly in 1 subunit of F_1 (other than α), altered the assembly properties of the entire F_1 complex.

2. Materials and methods

The uncoupled mutant DL54 [8] and its parent ML308-225 (i^-, z^-, y^+, a^+) were grown at 37°C to the late logarithmic phase in a rich medium [10] containing beef extract and peptone or in a synthetic medium containing 0.5% glucose as the sole carbon source. Inverted membrane vesicles were prepared as in [11] from cells disrupted in a French pressure cell (400 kg/cm^2). They were suspended in 1.0 mM Tris–HCl (pH 8.0) containing 0.5 mM EDTA and 10% glycerol (0.5 mg protein/ml) and centrifuged and the supernatant fraction (EDTA-extract) was concentrated to final conc. 1–5 mg protein/ml by ultrafiltration (Amicon filter, UM10). The precipitate (washed membranes) was suspended at 10 mg/ml in a buffer (10 mM Tris–HCl (pH 8.0), 0.14 M KCl, 2 mM β-mercaptoethanol, 10 mM $MgCl_2$ and 10% glycerol). Inverted membrane vesicles were also suspended in the same buffer without washing and used as 'unwashed membranes'. All the above procedures were carried out at 4°C. ATPase was assayed as in [12]; 1 unit of the enzyme was defined as the amount hydrolyzing 1 μmol ATP/min under standard conditions. Antibodies against individual subunits of F_1 were gifts from Dr L. A. Heppel. Other procedures and materials were as in [10–12].

Abbreviations: F_1–F_0, coupling factor proton-translocating ATPase; EDTA, ethylene diamine tetraacetate; SDS, sodium dodecyl sulfate

3. Results

3.1. *Release of α subunits from membranes of DL54*

At least part of the F_1 molecule can be solubilized by washing the membranes of an uncoupled mutant DL54 with dilute buffer containing EDTA [13,14]. On SDS–polyacrylamide gel electrophoresis, the solubilized material (EDTA extract) from membranes of DL54 (grown in the rich medium) gave a protein band in the position of the α subunit, but no band in the position of the β subunit of F_1 (fig.1). This result is in agreement with our preliminary finding that high ATPase activity could be reconstituted when

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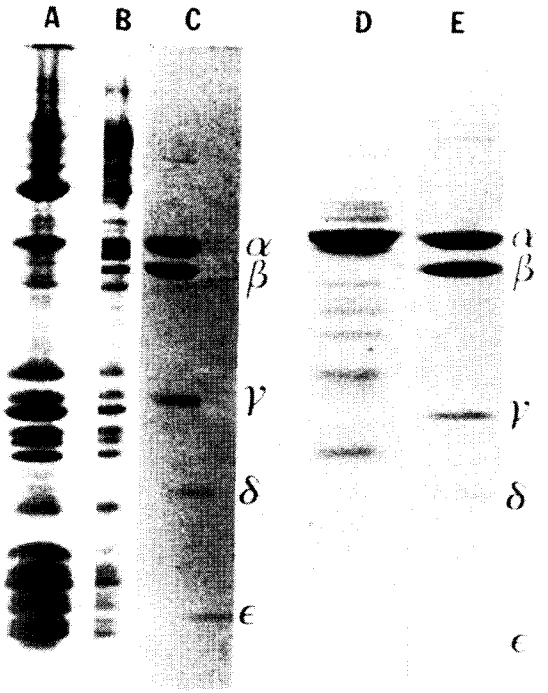

Fig.1. Polyacrylamide gel electrophoresis of the EDTA extract and partially purified α subunit from *Escherichia coli* strain DL54. Samples were incubated in 1.0% SDS and 2.0% β-mercaptoethanol for 5 min a boiling water bath. The EDTA extract from DL54 (A, 65 μg) and the partially purified α subunit (D, 16 μg) were applied to a gradient slab gel (12–25% polyacrylamide gel in the presence of 0.1% SDS [10]). Gels were stained with Coomassie brilliant blue. For comparison, electrophoregrams of active F_1 (C and E, 15 μg) and an EDTA extract (B, 38 μg) of strain ML308-225 are also shown. About 15 mg partially purified α subunit was obtained from DL54 (74 g (wet wt) of cells).

wild-type β and γ subunits were added to the EDTA extract from DL54 [14], suggesting that the extract contained only the active α subunit. Mutant membranes after washing with EDTA contained materials that crossreacted with antibodies to β and γ subunits (fig.2), and did not bind an appreciable amount of wild-type F_1 (fig.3). These findings suggest that part of the inactive F_1 molecule is still present in DL54 membranes after they have been washed with EDTA. Essentially the same results were obtained with mutant cells grown in a synthetic medium containing glucose.

3.2. *Partial purification of the α subunit*

Material crossreacting with antibodies to wild-type F_1 was purified from the EDTA extract of DL54,

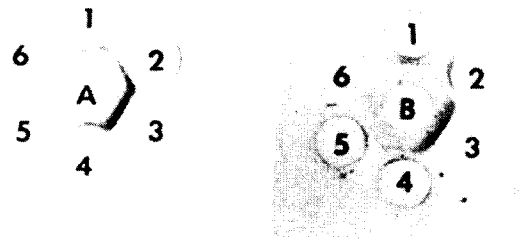

Fig.2. Interaction between membranes of DL54 and ML308-225 and antibodies against individual subunits of *Escherichia coli* on immunodiffusion. Unwashed membranes of ML308-225 (A, 130 μg protein/well) and washed membranes of DL54 (B, 130 μg protein/well) in 0.2% SDS were placed in the center wells and 15 μl of the following sera in the peripheral wells: (1) anti F_1; (2) anti-α; (3) anti-β; (4) anti-γ; (5) anti-ε; (6) control serum. Immunodiffusion was carried out as in [16]. Other conditions were as described in the text.

following the purification by the double immunodiffusion test. The procedure used was essentially the same as that used for wild-type F_1 [12]. The antibodies used in this study reacted with purified α and β subunits. The crossreacting material was recovered in a single peak by chromatography on DEAE–Sepharose (CL6B) (Pharmacia Co.) or Bio-Gel A0.5m (Biorad. Co.). The final fraction was analyzed by SDS–gel electrophoresis and a protein band was detected in the same position as the α subunit from wild-type F_1 (fig.1D). No bands were detected in the positions of other subunits of F_1.

ATPase activity was reconstituted by mixing the β and γ subunits from the wild-type strain with partially purified α subunit of the mutant and the mixture was dialyzed against buffer containing $MgCl_2$ and ATP [11]; ATPase activity (15 units/mg protein) was reconstituted when partially purified α (46 μg) of DL54 was mixed with β (10 μg) and γ (2.0 μg). Addition of any of the subunits alone or in other combinations did not result in significant activity. It is noteworthy that reconstitution of ATPase was observed without treating the partially purified fraction with a high concentration of salt in the cold, a treatment that is essential for dissociating *E. coli* F_1 into subunits [11]. This fact indicates that partially purified α subunits were active, and were not present as complexes with other polypeptides.

4. Discussion

In this work we found that the crossreacting

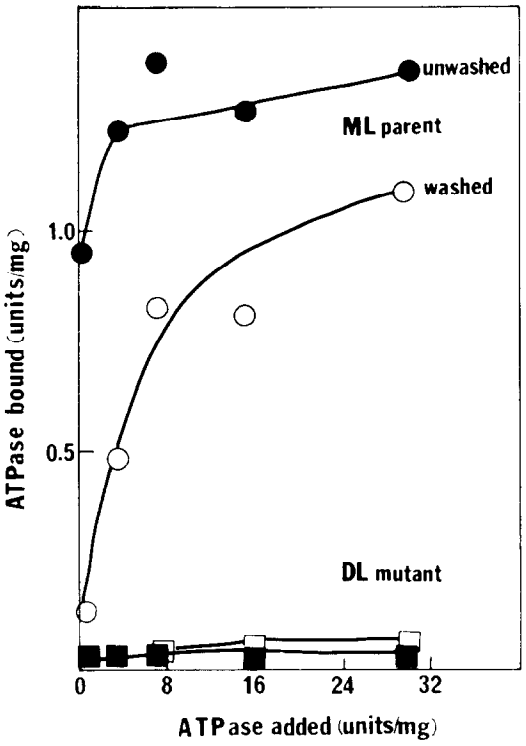

Fig.3. Binding of purified F_1 ATPase (wild-type) to depleted membranes of the wild-type and mutant. Unwashed and washed membranes were obtained from the wild-type and mutant strain (grown in rich medium). Samples of 250 μg washed membranes (○—○, from strain ML308-225; □—□, from DL54) or unwashed membranes (●—●, from ML308-225; ■—■, from DL54) were incubated with various amounts of purified F_1 ATPase in 0.05 M Tris–HCl (pH 7.0) containing 10 mM $MgCl_2$. After incubation at 22°C for 10 min, the membranes were precipitated (100 000 × *g*, 60 min) and their ATPase activity was assayed. Other conditions were as described in the text or in [10,12].

material released from the membranes of an F_1 mutant of *E. coli* DL54 by treatment with EDTA was mostly the α subunit, whereas it is well known that the entire F_1 complex is released from wild-type membranes by the same procedure [12]. Furthermore partially purified α subunit of the mutant obtained from the EDTA extract did not contain other subunits, suggesting that other subunits, if present in small amount in the EDTA extract, dissociated from the α subunit during purification. The present results suggest that this mutant has alterations in the subunit interactions of the F_1 complex itself and in the binding properties of the complex to the membrane sector (F_0). It is of interest that this mutation, possibly in only one subunit of F_1, altered the assembly properties of the entire F_1–F_0 complex and resulted in loss of catalytic activity. As part of the F_1 molecule is still bound to membranes of DL54 grown in rich or synthetic medium, the physiological change of the proton pathway in this strain [7] (discussed in section 1) does not seem to be due to loss of the whole F_1 molecule from the F_0 portion. This mutant may be useful for obtaining the α subunit by a simpler procedure than those used in [11,15].

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