ATP-SYNTHETASE COMPLEX (F_1F_0) FROM *ESCHERICHIA COLI*

Purification and characterization of subunits A and B of the F_0 part

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1. Introduction

The ATP synthetase plays an important role in bacterial energy transduction [1]. The enzyme is composed of two entities: the F_1 part is a peripheric membrane protein that catalyzes the hydrolysis of ATP, whereas the F_0 part is involved in H^+ translocation across the cytoplasmic membrane. The F_0 part of *E. coli* is composed of three different kinds of subunits (a, b and c). The molecular weights of proteins a and b have been determined by SDS gel electrophoresis as 24 000 and 19 000 respectively [2], whereas the smallest subunit (c) has a M_r of 8288 (as calculated from the primary structure [3]).

The latter protein was shown to provide the DCCD binding site and might play a crucial role in F_0-mediated H^+ conduction. This view is supported by two lines of experimental evidence:

(i) DCCD blocks H^+ translocation through F_0 by modifying a single aspartic acid residue [3];
(ii) In certain mutant strains lacking H^+ translocation through F_0 this particular aspartic acid residue is replaced by glycine [4,5].

The function of the other two subunits in the F_0 part still remains to be established. It has been found [6,7] that in two mutant strains lacking subunit b the translocation of H^+ through F_0 is impaired. However, this observation does not necessarily imply that subunit b is directly involved in H^+ translocation: it may play an indirect structural role.

Abbreviations: F_1 and F_0, peripheral and integral membrane portions of the H^+-translocating ATPase; DCCD, dicyclohexylcarbodiimide; SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonylfluoride; EGTA, ethyleneglycol-bis (2-aminoethylether) *N,N'*-tetraacetic acid

To study the structure and function of the individual subunits it is necessary to isolate the proteins in a homogeneous form. This report describes the purification of subunits a and b of the F_0 part from *E. coli* by column chromatography in the presence of SDS. After removal of detergent the amino acid compositions of the polypeptides were determined.

2. Materials and methods

Escherichia coli K12 Y_{mel} (γ) was grown in the minimal medium of [8] with 0.2% glucose as a carbon source.

ATP synthetase was prepared as in [9]. F_0 was prepared by hydrophobic affinity chromatography as in [10,11]. SDS gel electrophoresis was done as in [12] on 13% acrylamide gels. To isolate subunit b by column chromatography F_1F_0 was pretreated in the following way: 8–10 mg of purified F_1F_0 in 3 ml 50 mM Tris–HCl (pH 8.0), 10 mM taurodeoxycholate, 1 mM $MgCl_2$, 0.2 mM dithiothreitol, 0.2 mM EGTA, 1 mM PMSF, 6 mM *p*-aminobenzamidine, 20% (v/v) methanol and 50 μg/ml soybean phospholipids were centrifuged for 14 h at 250 000 × *g*. The precipitate was redissolved in 1 ml 5% (w/v) SDS, 0.2% (v/v) mercaptoethanol, 0.1 mM PMSF and incubated with gentle stirring overnight at room temperature. The solution was layered onto a Bio-Gel P-30 column (5 × 30 cm) equilibrated with 3% SDS and 0.1 mM PMSF. The eluate was analyzed by SDS–polyacrylamide gel electrophoresis. Fractions containing subunit b were pooled and SDS was removed by precipitation of the protein using solvent system A as in [13]. The precipitate was dried under a stream of

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nitrogen, samples were hydrolyzed in 6 M HCl under vacuum at 110°C and subjected to amino acid analysis, which was carried out on a Durrum resin DC6A in a modified BC 200 (BioCal, Munich) analyzer.

For the isolation of subunit a, purified F_0 (1.7 mg) in 10 mM Tris–HCl (pH 8.0), 150 mM NaCl and 10 mM taurodeoxycholate was diluted to 5 ml by adding 5% (w/v) SDS, 0.2% (v/v) mercaptoethanol, 0.1 mM PMSF and incubated for several hours at room temperature. The further procedure was identical to that described for subunit b.

Protein was determined as in [9]. Acrylamide (4 × recrystallized) and *N',N*-methylenbisacrylamide (1 × recrystallized) were obtained from Serva (Heidelberg). All other chemicals were of analytical grade and were used without further purification.

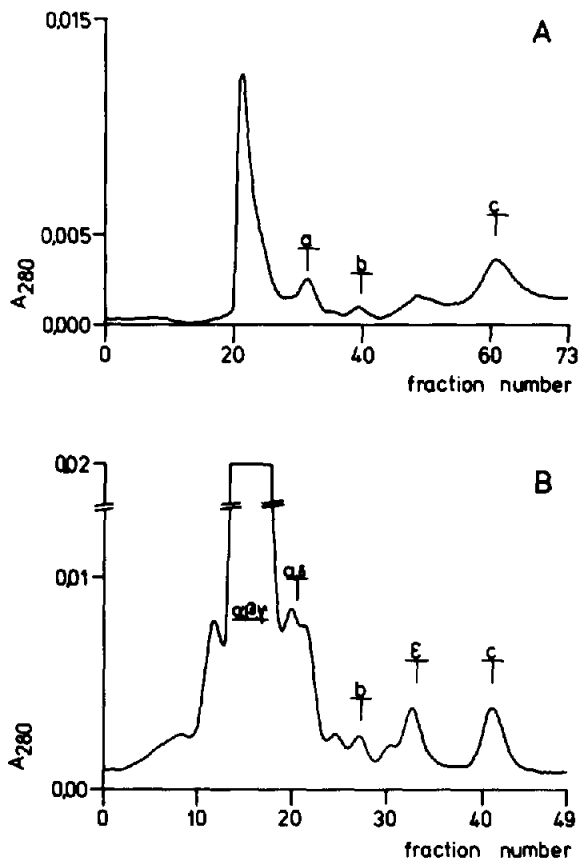

Fig.1. Chromatography of F_1F_0 complex (B) and F_0 (A), respectively on Bio-Gel P-30 in the presence of SDS. The flow rate was adjusted to 7 ml/h by hydrostatic pressure. Samples of 5 ml were collected. Subunits of the F_1 part are designated α, β, γ, δ, and ε, whereas a, b and c are subunits of the F_0 part.

3. Results

To purify the subunits of the F_0 part we used the method originally developed for the fractionation of subunits from mitochondrial cytochrome *c* oxydase [14]. The elution pattern obtained after the application of F_1F_0 onto the column is shown in fig.1B. As determined by SDS–polyacrylamide gel electrophoresis, only three of the eight subunits of the F_1F_0 complex, namely b, c and ε, were eluted in a homogeneous form. The homogeneity of subunit b is shown in fig.2, lane D. About 330 μg of subunit b as determined by amino acid analysis were yielded by 10 mg of F_1F_0 protein.

Since subunit a coeluted with δ (fig.1B) we had to take isolated F_0 as the starting material for the purification of a. The elution profile of F_0 (1.7 mg) on the Bio-Gel column is shown in fig.1A. As demonstrated by SDS gel electrophoresis all these subunits were clearly separated by this procedure. This is shown

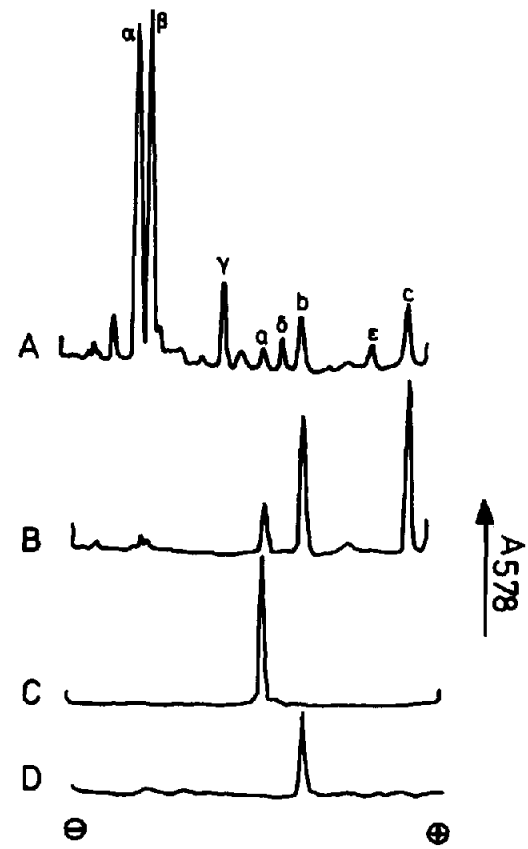

Fig.2. SDS–polyacrylamide gel electrophoresis of F_1F_0, F_0 and subunits a and b. Lane A: 14 μg of F_1F_0; lane B: 14 μg of F_0; lane C: 19 μg of subunit a; lane D: 4 μg of subunit b.

Table 1
Amino acid composition of subunit a and b from *E. coli* F_0

Amino acid	Molar ratios[a]	
	Subunit a	Subunit b
Asx	20.4 (20)	12.0 (12)
Thr	13.4 (13)	4.2 (4)
Ser	17.2 (17)	6.0 (6)
Glx	19.0 (19)	27.2 (27)
Pro	13.8 (14)	3.6 (4)
Gly	23.5 (24)	6.4 (6)
Ala	22.4 (22)	27.2 (27)
Cys	0.6 (1)[b]	1.0 (1)[b]
Val	21.3 (21)	9.7 (10)
Met	7.9 (8)	2.9 (3)
Ile	19.0 (19)	11.2 (11)
Leu	36.6 (37)	14.4 (14)
Tyr	6.3 (6)	1.6 (2)
Phe	17.2 (17)	3.9 (4)
Trp	n.d.	n.d.
His	5.7 (6)	1.3 (1)
Lys	10.0 (10)	13.3 (13)
Arg	9.7 (10)	9.5 (10)
Total	264	155
Polarity index[c]	36.0%	47.1%

[a] Values were extrapolated from 24, 48 and 90 h hydrolysis
[b] Determined after performic acid oxidation [23]
[c] Calculated by the method of [15]

n.d., not determined

for subunit a in fig.2, lane C. The yield in subunit a was 83 μg protein as calculated from the amino acid analysis.

After removal of SDS the purified subunits a and b were subjected to amino acid analysis. The data obtained are listed in table 1. Strikingly, subunit a is enriched in hydrophobic amino acid residues whereas subunit b exhibits a high content in Glx, Asx, Lys, Arg, Ala and Leu. The polarity index, calculated according to [15] was 36.1% for a and 47.1% for b.

4. Discussion

For studies on the structure and function of F_0 it is necessary to isolate the single polypeptides. This has been achieved so far only for subunit c, the DCCD-reactive protein [16,17]. Starting from a purified F_1F_0 complex we have now succeeded in isolating subunit b in a homogeneous form by the procedure described in section (2). Unfortunately, subunit a coeluted with the δ subunit of F_1. We have circumvented this problem by applying purified F_0 to the Bio-Gel column. This latter procedure has only become feasible since we have developed a new method to isolate F_0 in high yield [11].

Our results clearly indicate that chromatography in the presence of SDS is a useful tool to isolate individual subunits from complex oligomeric membrane proteins [14]. The amino acid composition of both a and b subunits compare well with those deduced from the corresponding DNA sequences [18–20]. Although also a and b are intrinsic membrane proteins, their polarity indices (36% and 47%) are significantly higher than that of subunit c which exhibits the extremely low value of 16% [16]. Especially the index of b is similar to that of many water-soluble proteins. This surprising result can be explained by extended hydrophilic and hydrophobic domains within the primary structure [18]. The amphiphatic nature of the b protein gives rise to the speculation that it might be involved in the binding of the hydrophilic F_1 part to the membrane. This idea is supported by data on the band 6 protein of the thermophilic bacterium PS 3 [21]. This protein exhibits some striking similarities to that of *E. coli* subunit b, especially in the contents of Glx, Ala and Leu [22] and has been shown to carry the binding site for TF_1 [21]. Based on the purification procedure for a and b reported here the analyses of mutant proteins and chemically-modified subunits are now possible. These studies should help in further elucidating the functions of those subunits.

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