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Exchangeability of the b subunit of the Cl^--translocating ATPase of *Acetabularia acetabulum* with the β subunit of *E. coli* F_1-ATPase: Construction of the chimeric β subunits and complementation studies

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Abstract

The gene encoding the b subunit of the Cl^--translocating ATPase (*aclB*) was isolated from total RNA and poly(A)$^+$ RNA of *Acetabularia acetabulum* and sequenced (total nucleotides of 3038 bp and an open reading frame with 478 amino acids). The deduced amino acid sequence showed high similarity to the β subunit of the F type ATPases, but was different in the N-terminal 120 amino acids. The role of the N-terminal region was investigated using an F_1-ATPase β-less mutant of *E. coli*, JP17. The JP17 strain expressing the *aclB* could not grow under conditions permitting oxidative phosphorylation, although ACLB was detected in the membrane fraction. The β subunit was divided into three portions: amino acid position from 1 to 95 (portion A), 96 to 161 (portion B) and 162 to the C-terminus (portion C). The corresponding regions of ACLB were designated as portions A′ (from 1 to 106), B′ (from 107 to 172) and C′ (from 173 to 478). Chimeric proteins with combinations of A–B′–C′, A–B–C′ and A′–B–C restored the function as the β subunit in *E. coli* F_0F_1-complex, but those with combinations of A′–B′–C and A–B′–C had no function as the β subunit. These findings suggested that portion B plays an important role in the assembly and function of the β subunit in the F_0F_1-complex, while portion B′ of ACLB exhibited inhibitory effects on assembly and function. In addition, portion A was also important for interaction of the β subunit with the α subunit in *E. coli* F_0F_1-complex. These findings also suggested that the b subunit of the Cl^--translocating ATPase of *A. acetabulum* has a different function in the Cl^--translocating ATPase complex, although the primary structure resembled to the β subunit of the F_1–ATPase. © 1997 Elsevier Science B.V.

Keywords: Expression; Cl-b subunit; Chimeric plasmids; EF_1-β less mutant; Complementation; *Acetabularia acetabulum*

1. Introduction

The F type ATPases synthesize ATP at the expense of proton motive force and are found in bacteria, mitochondria and chloroplasts [1–5]. They consist of the F_0 portion, the proton channel, and the extrinsic F_1 portion, the water-soluble region which carries the catalytic site [1–5]. The CF_1 type is com-

Abbreviations: ATPase: Adenosinetriphosphatase; ATP: Adenosine 5′-triphosphate; Cl-b: Cl^--translocating ATPase b subunit; CF_1: Chloroplast F_1; CF_0: Chloroplast F_0; EF_1: *E. coli* F_1; EF_0, *E. coli* F_0; pBS: pBluescript II SK (+); SDS–PAGE: Sodium dodecylsulfatepolyacrylamide gel electrophoresis; PBS: Phosphate-buffered saline; TKMG buffer: Tris–KCl–$MgCl_2$–glycerol buffer (pH 8.0)

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posed of α (56 kDa), β (54 kDa), γ (31–36 kDa), δ (21 kDa) and ϵ (15 kDa) subunits, and the stoichiometry is always $\alpha_3\beta_3\gamma\delta\epsilon$. The mitochondrial α, β and a portion of the γ subunits of bovine F_1 have been crystallized and analyzed [6]. Since the β subunit of various species has the catalytic site with the most highly conserved sequence, its expression was examined in *E. coli uncD* mutant strains [7–10].

Among the F type ATPases, the ATPase in *Propiogenium modestum* shows ion specificity for Na^+ ion [11]. A hybrid consisting of the F_1 moiety of the *E. coli* ATPase and F_0 of that from *P. modestum* performed Na^+ or H^+ transport in a reconstituted system [12]. This is the first evidence that the F_0 portion determines ion specificity.

In previous reports [13–15], the isolation, characterization, and reconstitution into liposomes of a novel and unique type of ATPase from *Acetabularia acetabulum* were described. The enzyme consisted of two subunits, a (54 kDa) and b (50 kDa) with a minor band around 40 kDa (c subunit) and has been demonstrated to be an ATP-driven chloride translocator of electrogenic nature by reconstitution studies [14,15]. We further reported the complete nucleotide sequence of a cDNA clone and partial sequences of two cDNA fragments coding for subunits in *A. acetabulum* resembling F_1-β subunits, and its complete cDNA clone encodes the b subunit of the Cl^--translocating ATPase of the organism (Cl-b subunit, gene name designated as *aclB* in the present report): partial amino acid sequences obtained from protein chemical analysis of the b subunit were found on the deduced amino acid sequence from *aclB* [16]. The open reading frame consisted of 1434 bp and 478 amino acids which showed high similarity to the β subunit of CF_1, but the N-terminal 120 amino acids were different and *aclB* possessed $polyA^+$ signals and $polyA^+$ tail. Northern and Southern hybridizations also supported that the three genes were different.

Therefore, the Cl-b subunit may function as the β subunit of the F type ATPase because of the high similarity, but may not be a catalytic subunit (confer the results of chemical modification of the Cl^-–ATPase with adenosine 3′-triphosphopyridoxal in [16]).

We transformed a deletion mutant of *E. coli* strain, JP17 [17] with expression plasmids of Cl-b and chimeric plasmids of EF_1-β and Cl-b subunits. Herein, we report the complementation of these proteins with EF_1-β, and functional domains of the Cl-b subunit.

2. Materials and methods

2.1. Bacterial strain and plasmids

E. coli strain, JP17 [17] contains a deletion in the *uncD* gene corresponding to amino acids 20 to 332 of the EF_1-β. The genotype of JP17 is Δ*uncD*, *argH*, *entA*, *pyrE*, *recA*::*Tn10*, *tetR*. Plasmid pRM21 contains an internal portion of the *E. coli unc* operon, including *uncD* (β subunit), inserted into pBR322 and is transcribed from a promoter and Shine–Dalgarno sequence of plasmid origin: Briefly, pBR322 was digested with *Eco*RI and *Hin*dIII, treated with a DNA polymerase Klenow fragment, then digested with *Sca*I and *Nhe*I to which a *Sca*I–*Nhe*I digest of pST03β [18] was ligated (pST03βΔ*Hin*dIII). A *Hin*dIII–*Nhe*I digest of pST03β was ligated to a *Hin*dIII–*Nhe*I digest of pST03βΔ*Hin*dIII which was designated as pRM21. Another expression plasmid of the β subunit, pEK05, with basically the same structure as that of pRM21 has two silent mutations at residues Thr252 (position 759 bp) and Asp372 (position 1118 bp) which created two restriction sites for *Kpn*I and *Eco*RV in the β subunit gene, respectively. These mutations were introduced by a two-step polymerase chain reaction (PCR) using mutated oligo DNAs JM7 (GATACTTCGGTTACCGGC) and JM8 (GCGATCATATCTTTCAGT) as the primers and replaced with the corresponding sequences in pRM21. Plasmid pBluescript SK II (+) (Stratagene, La Jolla, CA; abbreviated pBS) was also used as a cloning vector and expression plasmid with the *lac*Z promoter.

2.2. Construction of the entire cDNA encoding the Cl-b subunit

The cDNA clones, pCLB1 and pCLB4 (ca. 0.2 μg, [16]) were used as templates for PCR to obtain the entire cDNA clone, pCLBL. The oligonucleotides used for PCR were B1 (nt 1 to 23) and adaptor [16]. PCR was performed at 94°C for 1 min, 50°C for 2 min, 72°C for 3 min with 20 cycles, and 5 U of *Pfu* polymerase (Gibco BRL) was used as polymerase in

a total volume of 100 μl. The PCR product (around 1.5 kb, *aclB*) was separated in a 0.5% agarose–1.5% Nusieve agarose composite gel, excised and purified over Qiaex DNA Extraction Kit (Diagen, Duesseldorf, FRG). The purified fragment was treated with the Klenow fragment of DNA polymerase and ligated into the multiple cloning site (*Eco*RV site) of pBS. *E. coli* XL1-Blue was transformed with this plasmid (pCLBL).

2.3. Construction of expression plasmid for the chimeric protein

In-frame fusion of the *aclB* in pCLBL obtained above with *lac*Z gene of the pBS was performed. Plasmid pBS was digested with *Not*I, the overhang filled when using the Klenow large fragment as described previously [16], and digested with *Sal*I. The pCLBL was digested with *Sma*I and *Sal*I. The plasmid and gene were ligated, transformed into *E. coli* XLI-Blue and then plated onto LB agar containing X-gal–IPTG–Amp. Recombinant DNA prepared in mini preparations [19] with the desired size was subjected to DNA sequencing.

pEK05 was used as expression plasmid, and the restriction sites *Eco*RI, *Sal*I, *Sac*I and *Bam*HI were used to construct Cl-b, the chimeric EF_1-β–Cl-b and Cl-b–EF_1-β expression plasmids. These restriction sites were introduced into *aclB* through PCR, *Sal*I in position 307 to 329 (5′ GGAGAAAC-AGTCGACAATTTAGG 3′ and its complementary oligonucleotide) and *Sac*I in position 499 to 522 (5′ CTGTTTTAATTATGGAGCTCATT 3′ and its complementary oligonucleotide). The additions at both restriction sites lead to silent mutations. A third restriction site, *Bam*HI was introduced in position 1429 to 1452 (complementary oligonucleotide: 5′ GCTGCCTGGATCCTGCTAAATTG 3′) which is in the 3′-untranslated region. When the *aclB* was inserted into the EF_1-β gene after deletion of pEK05 (*Eco*RI–*Bam*HI digest) and a part of *aclB* was attached to the EF_1-β gene after deletion of the N-terminal region (*Eco*RI–*Sal*I or *Eco*RI–*Sac*I digest of pEK05), 17 amino acids of EF_1-β (ATGKIVQVIGAVVDVEF) and 1 amino acid (aspartate) from pBS were added to the N-terminus of ACLB. The desired fragments were amplified by PCR using pCLBL as a template to obtain modified oligonucleotides: *aclB* with M13 reverse primer and *Bam*HI site introduced, N-terminal *aclB* with M13 reverse primer and *Sal*I or *Sac*I site introduced, and C-terminal *aclB* with *Sal*I or *Sac*I site introduced and *Bam*HI site introduced, respectively. The modified *aclB* fragment was ligated into *Eco*RI–*Bam*HI digest of pEK05 and pBS. Five other fragments were ligated with digests of pEK05 with the *Eco*RI–*Sal*I (pCLEK95: A′–B–C), *Eco*RI–*Sac*I (pCLEK161: A′–B′–C), *Sal*I–*Sac*I (pEK95CL161EK: A–B′–C), *Sal*I–*Bam*HI (pEKCL95: A–B′–C′) and *Sac*I–*Bam*HI (pEKCL161: A–B–C′). pCL95EK161CL (A′–B–C′) was constructed using pCLEK95 and pEKCL161. These replacements were verified by DNA sequencing after ligation of the fragments digested with *Eco*RI–*Bam*HI into pBS digested with the same restriction enzymes and transformation.

2.4. DNA sequencing

Nucleotide sequencing of double stranded templates was performed with a Sequi-Therm Cycle Sequencing Kit (Epicentre Tech., Chicago, IL) and a Li-Cor dNA sequencer, Model 4000L (Li-Cor, Lincoln, NE) according to the manufacturer's instructions.

2.5. Complementation assay

Plasmids were transformed into *E. coli* JP17 by electroporation or by the calcium method and plated onto LB agar containing 100 μg ml^{-1} ampicillin. After growth overnight at 37°C, colonies were streaked onto a Tanaka-succinate plus pyr. mix (TSP) agar plate [17]. To obtain growth curves, 15 μl of a culture having an OD_{650} of 1.0 or 30 μl of a culture having an OD_{650} of 0.5 was transferred to 10 ml of TSP medium containing ampicillin (TSPA) as described above. Cell densities were measured as turbidity at 650 nm.

2.6. Preparation of whole cell extracts and membrane fractions

Whole cell extracts and membrane fractions were prepared according to the method of Kanazawa et al. [20]. Protein content was determined according to the method of Heil and Zillig [21].

2.7. SDS–PAGE and Western blot analysis

After SDS–PAGE [22] on 12.5% acrylamide gels (10 × 10 cm, 1.5 mm thick), the proteins were transferred to nitrocellulose membranes according to the method of Burnette [23] and Towbin et al. [24]. Membranes were blocked in 5 w/v% skim milk–PBS overnight at 4°C. The antibodies were diluted in 5% skim milk–PBS (1:1000 for anti-EF_1-α monoclonal antibody prepared by Kanazawa et al. [25]; 1:500 for anti-EF_1-β antiserum supplied by Altendorf; and 1:10 for anti-EF_1-γ antiserum prepared by Kanazawa et al.) and incubated at room temperature for 2 h with gentle shaking. Anti-rabbit peroxidase-conjugated IgG was diluted 1:5000 and anti-mouse peroxidase conjugated IgG was diluted 1:667 in the case of reaction with anti-EF_1-α. Detection was conducted using an ECL Western Detection Kit from Amersham Life Science (Tokyo, Japan) according to the manufacturer's instructions.

2.8. Assay of ATPase activity

The assay mixture contained 40 mM Tris–HCl buffer (pH 8.0), 8 mM ATP (pH 8.0), 4 mM $MgCl_2$ and enzyme (aliquots diluted with TKMG buffer) in a total volume of 0.1 ml, and incubated at 37°C for 10 min. Inorganic phosphate liberated by ATPase action was colorimetrically determined as described by Lanzetta et al. [26].

3. Results

3.1. Construction of expression plasmids

Fig. 1a and b show a schema of the *A. acetabulum* Cl-b, EF_1-β and their chimeric subunits. The entire gene, *aclB* encoding the Cl-b was obtained by PCR, ligated into pBS and sequenced. DNA sequencing did not cause any frame shift mutation or change in

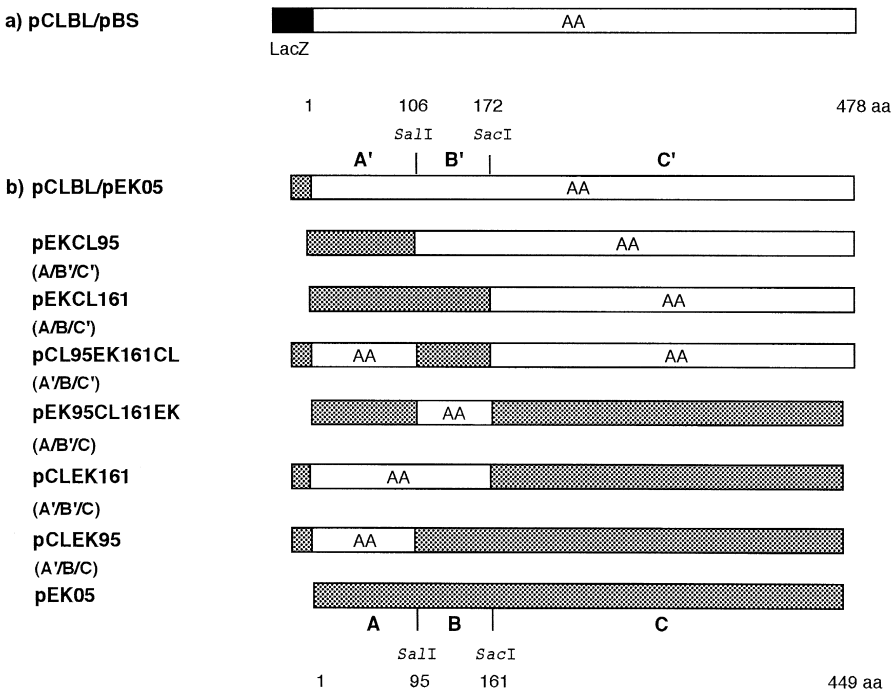

Fig. 1. Chimeric β subunits. (a) Black box represents amino acid residues derived from Lac Z protein in pBS. (b) The location of the *Sal*I and *Sac*I sites in the *E. coli* β gene and incorporated into the *A. acetabulum* Cl-b gene, are indicated at the top of the figure. The designations for the plasmids containing each chimera are shown on the left, and genetic origin of each section is designated by AA (*A. acetabulum*) or shadowed boxes (*E. coli*). The alphabets, A to C and A′ to C′ represent the divided amino acid regions for construction of chimeric proteins expressing plasmids which are indicated at the top of pCLBL–pEK05 and at the bottom of pEK05. Plasmid pCLBL–pEK05 contains the intact *A. acetabulum* Cl-b gene, and plasmid pEK05 contains the intact *E. coli* β gene.

nucleotide sequence (data not shown). The in-frame fused *aclB* to *lacZ* gene of pBS encoded N-terminal 32 amino acids derived from LacZ followed by the Cl-b (designated as pCLBL–pBS). pCLBL–pBS was used for construction of chimeric plasmids with an EF_1-β expression plasmid, pEK05: the *Eco*RI, *Sal*I, *Sac*I and *Bam*HI sites of pEK05 were utilized for construction. The β subunit was divided into three portions: amino acid position from 1 to 95 (portion A), 96 to 161 (portion B) and 162 to the C-terminus (portion C). The corresponding regions of ACLB were designated as portions A′, B′ and C′. The fusion plasmids were designated as pCLBL–pEK05, pCLEK95 (A′–B–C), pCLEK161 (A′–B′–C), pEKCL95 (A–B′–C′), pEKCL161 (A–B–C′), pCL95EK161CL (A′–B–C′) and pEK95CL161EK (A–B′–C); the numbers indicate amino acid residues at which the EF_1-β subunit was substituted and the chimeric proteins were abbreviated as described in the figures.

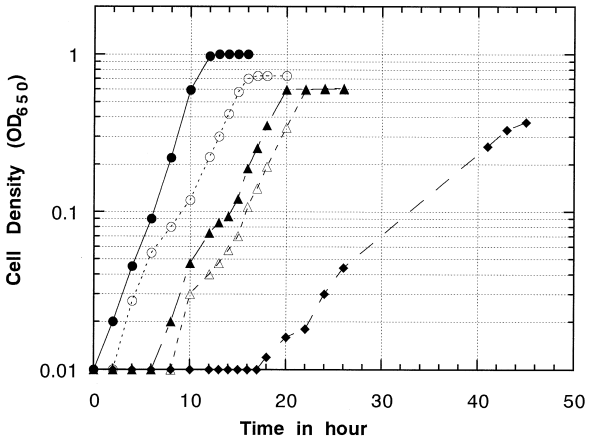

Fig. 2. Growth curves of the transformants with phenotypes suc(+). Growth curves of the respective transformant with phenotype suc(+) were taken at indicated time intervals: ●, pEK05 (wild type); ○, pCLEK95; ▲, pEKCL161; △, pEKCL95; ◆, pCL95EK161CL.

3.2. Genetic and biochemical complementation of E. coli β-less mutant

Each of the above plasmids was transformed into *E. coli* β-less mutant, strain JP17, which contains a chromosomal deletion of *uncD*. The transformants were tested for their ability to grow aerobically on minimal succinate medium. For biochemical assay, cells were grown in TKMG medium [*E. coli* JP17 complemented with (pEK05), (pEKCL95), (pEKCL161), (pCLEK95) and (pCL95EK161CL)] or in LB–Amp medium [*E. coli* complemented with (pCLBL–pBS), (pCLBL–pEK05), (pCLEK161), (pEK95CL161EK) and *E. coli* JP17] to full growth, and membranes were isolated and assayed for ATPase activity. The results are summarized in Table 1. The plasmids pCLEK95, pEKCL95, pEKCL161 and pCL95EK161CL complemented the mutant, while the other chimeric plasmids did not. The chimeric proteins with ATPase activity over 7.5% of that of the wild type restored the growth, while those with less than 5% did not. The ATPase activity, a biochemical index for the transformants, was assayed at pH 8.0, the optimal pH for the EF_0F_1 complex, and also at pH 6.8, a more physiological condition. At pH 6.8, the transformants with phenotype suc(+) showed 20% enzyme activity, while those with phenotype suc(−) 10% of that of the wild type (data not shown). Fig. 2 shows the growth curves of the transformants of the chimeric plasmids with phenotype suc(+). In accordance with the ATPase activity, a lag phase was observed in bacterial growth in TSPA for the respective transformant. Especially, the transformant with the lowest ATPase activity (pCL95EK161CL) among those with phenotypes suc(+) showed a long lag-time before visible growth became in the medium, while the cell density of the transformant with pEK95CL161EK [suc(−)] was below 0.02 even after 45 h of culture.

Table 1
Phenotype, growth and ATPase activity

Plasmids	Phenotype	Growth yield	ATPase activity
pCLBL/pEK05	suc(−)	–	3.6
pEKCL95	suc(+)	0.60	9.5
pEKCL161	suc(+)	0.61	9.0
pCL95EK161CL	suc(+)	0.37	7.5
pEK95CL161EK	suc(−)	–	5.1
pCLEK161	suc(−)	–	5.2
pCLEK95	suc(+)	0.73	8.2
pEK05	suc(+)	1.0	100
E. coli JP17	suc(−)	–	1.5

Growth represents maximum OD_{650} up to 24 h incubation.
ATPase activity: relative activity/mg protein to pEK05.

3.3. Assembly of chimeras with the EF_1-portion in membrane fractions

Membrane fractions were prepared from transformants with all the expression plasmids constructed here, in order to examine the insertion of the chimeric β subunit into the EF_1 portion. Fig. 3 shows the results of SDS–PAGE/silver staining and Western blot analysis using whole cell extracts and membrane fraction from the pCLBL–pBS transformant. A polypeptide around 52 kDa was clearly seen in whole cell extract (upper part of Fig. 3a) and reacted with the anti-EF_1-β antiserum (lower part of Fig. 3a). Immunoreaction of the expressed polypeptide with anti-EF_1-α and -β antibodies was detected in the membrane fraction (Fig. 3b). However, the function was not restored as described above. Western blot analysis was also performed with membrane fractions prepared from JP17 cells after transformation of the chimeric plasmids (Fig. 4). The cross-reactivities with anti-EF_1-α and -γ appeared to reflect the insertion into the membrane fractions which are specific for the respective EF_1 polypeptides. The intensities of cross-reaction were estimated by Bio-Image (Millipore). In transformed JP17 cells showing restored growth under conditions of oxidative phosphorylation, the levels of α and γ subunits in membrane fractions were comparable to those in wild type cells.

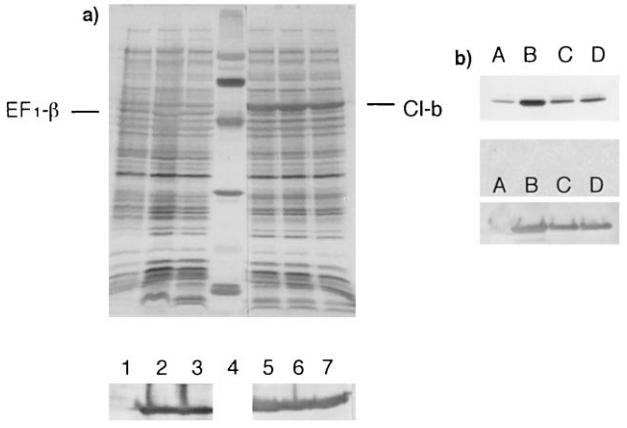

Fig. 3. Expression of the pCLBL–pBS in the JP17 strain. (a) Whole cell extracts: 10 μg protein was subjected to SDS–PAGE followed by silver staining (upper) or Western blot analysis using an anti-EF_1-β antibody (lower). Lane 1, *E. coli* JP17; lanes 2 and 3, pRM21; lane 4, size markers (phosphorylase B, 92.5 K; BSA, 66 K; OVA, 45 K; carbonic anhydrase, 29 K; soybean trypsin inhibitor, 21 K and cytochrome *c*, 14.3 K); lanes 5 to 7, JP17 (pCLBL–pBS) from three separate transformation experiments. (b) Membrane fractions: 3 μg protein was subjected to SDS–PAGE and to Western blot analysis using a monoclonal antibody against EF_1-α (upper lanes) and anti-EF_1-β antiserum (lower lanes). Lane A, *E. coli* JP17; lane B, JP17 (pRM21); lanes C and D, JP17 (pCLBL–pBS).

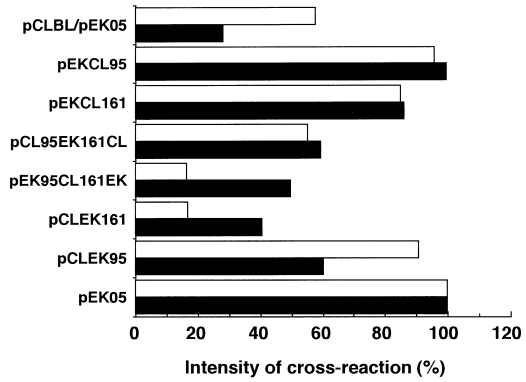

Fig. 4. Assembly of the EF_1-α and -γ subunits within the membrane fractions prepared from JP17 cells after transformation with chimeric plasmids. 2 μg protein was subjected to SDS–PAGE and to Western blot analysis with anti-EF_1-α and -γ antibodies. The intensities of the cross-reactions were estimated by Bio-Image. The values obtained from JP17 (pEK05) are represented as 100. White boxes indicate the intensities of the cross-reactions with anti-EF_1-α and black boxes with anti-EF_1-γ.

4. Discussion

Previous modification experiments with adenosine 3′-triphosphopyridoxal suggested that the catalytic subunit might be the a subunit, although the Cl^--translocating ATPase, b subunit possessed the consensus nucleotide binding fold (G*XXXX*GKT/S) [16]. Using an EF_1-β deletion mutant of *E. coli* strain, JP17, complementation studies were performed and the exchangeability of Cl-b of *A. acetabulum* with F_1-β of *E. coli* was examined. Neither pCLBL–pBS nor pCLBL–pEK05 transformants grew under conditions of oxidative phosphorylation. As described previously [16], the primary structure of Cl-b had the highest similarity to CF_1-β, but Cl-b was different from CF_1-β especially in the N-terminal 120 amino acid residues. Based on this information, the EF_1-β gene (*uncD*) was divided into three portions (A, B and C) and chimeric expression plasmids were con-

structed with the *aclB* and *uncD* genes. Substitution of the N-terminal regions of the *aclB* with the corresponding *uncD* genes restored the growth of the transformants, indicating that the amino acid residues from 1 to 95, from 96 to the C-terminus and 162 to the C-terminus of the ACLB could be exchanged with the EF_1-β subunit. In contrast, transformants with substitution of the EF_1-β gene with the *aclB* (amino acid positions from 1 to 161 and from 95 to 161) did not grow under oxidative phosphorylation which indicated that these regions of the *aclB* gene were not exchangeable with the EF_1-β gene.

Fig. 5 shows the primary structures of EF_1-β and Cl-b. According to Abrahams et al. [6], amino acid positions of 1 to 161 contain β-sheets in the N-terminal β-barrel, a–f, the helices (A and B) and the β-sheets (1–3) in the nucleotide-binding domain. The present findings on pEKCL95 [suc(+)] and pEKCL161 [suc(+)] suggest the importance of the N-terminal regions of EF_1-β in the function and assembly of chimeric proteins attached to the C-terminal regions of Cl-b. In contrast, the replacement of the N-terminal 95 amino acids of EF_1-β with Cl-b [pCLEK95, suc(+)] showed that the β-barrel a–f, and the β-sheets of 1 and 2 were not definitive domains for the function and assembly, when the residual parts consisted of EF_1-β. Identities between the Cl-b and EF_1-β at N-terminal 95 amino acids were about 50%. By further replacement (pCLEK161, pEK95CL161EK and pCL95EK161CL transformants), the chimeric EF_1–ATPase could not function, suggesting that the definitive domain was located between 95 and 161 containing the helices A and B, and that the sheet 3 or tertiary structural changes in the region of 1 to 161 of ACLB could affect the function and assembly of chimeric F_1-β.

The roles of the N-terminal regions of the EF_1-β subunit in the assembly and function of the EF_1 complex have been reported previously [27,28]: βGlu41 (in sheet d) and βArg218 (in helix D). Site-directed mutagenesis on the EF_1-β has been reported and the essential residues for function have been clarified: βLys155, βThr156, βGlu181, βGlu185 [29]. Corresponding amino acid residues of Cl-b were conserved except for βLeu40 [28].

The Cl^--ATPase consisted of the a (54 kDa), b (50 kDa) and c (40 kDa) subunits. Our reconstitution studies suggested that the c subunit is a transmembraneous portion of Cl^--ATPase (unpublished data): The a and b subunits were released from the proteoliposomes by keeping them at 4°C, but the c subunit remained in the proteoliposomes. The a and b subunits are considered to be the catalytic and regulatory subunits of the enzyme complex, respectively. We have already isolated a cDNA clone encoding the Cl^--ATPase, a subunit (*aclA* gene; unpublished data). Coexpression plasmids of the *aclA* and *aclB* genes are now under construction. Molecular cloning of the c subunit is also in progress. Further studies on the line, establishment of a coexpression system of the genes encoding the a, b and c subunits and functional

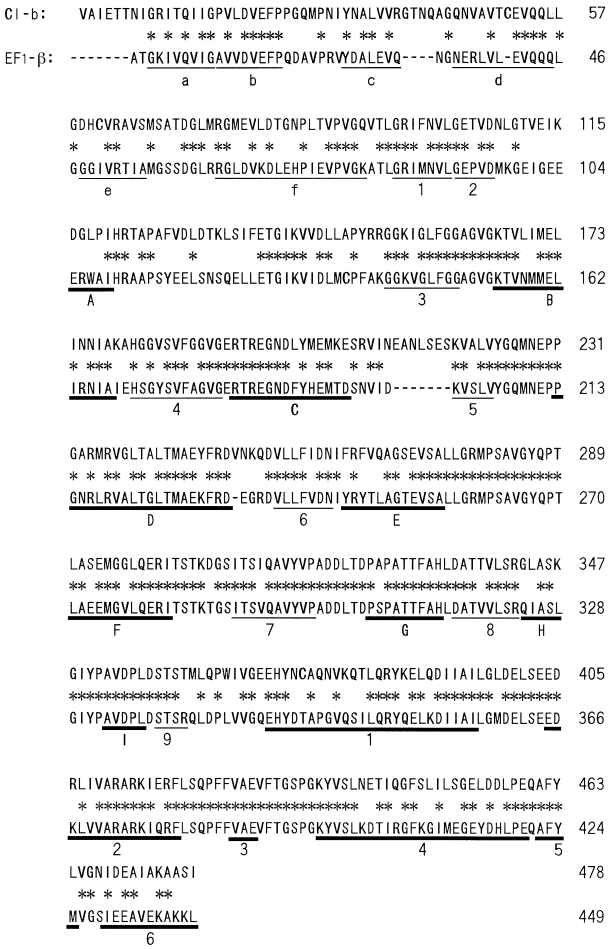

Fig. 5. Alignments of the EF_1-β to Cl-b. Asterisks show identical amino acid residues. Underlined domains (a–f, 1–9) represent the domains forming β-sheets and bold underlined those forming helices which are predicted from the model of Abrahams et al. [6].

analysis of the expressed protein complex are required to elucidate the structural and functional features of the Cl^--ATPase complex.

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