Escherichia coli H^+-ATPase: Role of the δ Subunit in Binding F_1 to the F_o Sector[1]

Masayoshi Jounouchi, Michiyasu Takeyama, Pawinee Chaiprasert, Takato Noumi, Yoshinori Moriyama, Masatomo Maeda, and Masamitsu Futai[2]

Department of Organic Chemistry and Biochemistry, The Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567, Japan

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The roles of the *Escherichia coli* H^+-ATPase (F_oF_1) δ subunit (177 amino acid residues) was studied by analyzing mutants. The membranes of nonsense (Gln-23 → end, Gln-29 → end, Gln-74 → end) and missense (Gly-150 → Asp) mutants had very low ATPase activities, indicating that the δ subunit is essential for the binding of the F_1 portion to F_o. The Gln-176 → end mutant had essentially the same membrane-bound activity as the wild type, whereas in the Val-174 → end mutant most of the ATPase activity was in the cytoplasm. Thus Val-174 (and possibly Leu-175 also) was essential for maintaining the structure of the subunit, whereas the two carboxyl terminal residues Gln-176 and Ser-177 were dispensable. Substitutions were introduced at various residues (Thr-11, Glu-26, Asp-30, Glu-42, Glu-82, Arg-85, Asp-144, Arg-154, Asp-161, Ser-163), including apparently conserved hydrophilic ones. The resulting mutants had essentially the same phenotypes as the wild type, indicating that these residues do not have any significant functional role(s). Analysis of mutations (Gly-150 → Asp, Pro, or Ala) indicated that Gly-150 itself was not essential, but that the mutations might affect the structure of the subunit. These results suggest that the overall structure of the δ subunit is necessary, but that individual residues may not have strict functional roles. © 1992 Academic Press, Inc.

The H^+-translocating ATPase (F_oF_1) of *Escherichia coli* catalyzes ATP synthesis utilizing an electrochemical gradient of protons across the cytoplasmic membrane generated by the electron transfer chain (for review, see Refs. (1–4)). The ATPase consists of two portions, F_1 and F_o. The catalytic sector F_1 extrinsic to the membrane has five subunits, α, β, γ, δ, and ϵ, while the sector F_o in the membrane has three subunits, *a*, *b*, and *c*, and functions as a proton pathway. The δ subunit (177 amino acid residues), coded by the *uncH* gene, is required together with the ϵ subunit for binding of the $\alpha\beta\gamma$ complex to the F_o sector, as shown by *in vitro* reconstitution experiments (5). Thus the δ subunit, a unique polypeptide with a high α helical content (6), seems to be located in the part connecting F_1 and F_o. A close interaction of the δ subunit with the α subunit was suggested by cross-linking between the two subunits (7, 8).

The corresponding subunits from mitochondria (9) and chloroplasts (10, 11) seem to stabilize the linkage between F_1 and F_o: chloroplast F_1 lacking the δ subunit can bind to F_o but the resulting F_oF_1's are not normal (10, 11). It is noteworthy that chloroplast F_oF_1 lacking the δ subunit is capable of photophosphorylation, although its activity is significantly lower than that of the complex with the δ subunit. Thus the chloroplast δ may be functionally different from the δ subunit of *E. coli*, consistent with the low similarity of the amino acid sequences of these two subunits. Detailed genetic analysis of the *E. coli* subunit may provide information on the role of the subunit and its amino acid residues.

In this work, we isolated a series of *E. coli* mutants with altered δ subunits. Studies on the properties of their H^+-ATPases indicated that the δ subunit was required for functional binding of F_1 to F_o. Site-directed mutagenesis studies also suggested that the apparently conserved hydrophilic residues did not have strict functional roles.

EXPERIMENTAL PROCEDURES

Bacterial strains, growth conditions, and identification of base substitutions in mutants. *E. coli* mutants (*uncH, thy, thi*) with defects in the δ subunit (KF5, KF22, KF96, KF136, and KF149) were isolated, and

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[2] To whom correspondence should be addressed.

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strain KF96rA (a *recA* derivative of KF96) was constructed as previously described (12). Minimal medium supplemented with 100 μg/ml thymine, 2 μ/ml thiamine, and a carbon source (0.2% glucose or 0.4% succinate), and a rich medium (L broth) with 50 μg/ml ampicillin or 20 μg/ml tetracycline were used in this study.

The *uncH* mutants were complemented with pRPG57 (13) and their alleles were mapped within the *uncH* gene by recombination assay using pBWF02 (14), pMTH1, and pMTH2 (Fig. 1). The *Bam*H1–*Eco*RI segment (1499 bp) of the *unc* operon was cloned from each mutant, and a smaller segment (*Sau*3AI–*Bst*EII for KF96 and KF136; *Bst*EII–*Acc*I for KF5 and KF149; *Acc*I–*Eco*RI for KF22) was subcloned. Both strands of the entire segment of each subclone were sequenced for identification of base substitutions (15). The absence of mutations in other segments of the *unc* operon was confirmed by genetic recombination.

Construction of plasmids carrying mutations of the uncH gene. A 630-bp *Sau*3AI–*Sph*I fragment carrying the entire *uncH* gene was prepared from a hybrid plasmid pRPG57 (13). pBWH01 was constructed by ligating this fragment into the *Bam*HI–*Sph*I site of pBR322. Recombinant plasmids carrying truncated *uncH* genes were constructed by cassette mutagenesis combined with exonuclease digestion as described by Kuki *et al.* (16): pBMH177e, Ser-177 → end; pBMH176e, Gln-176 → end; pBMH174e, Val-174 → end; pBMH172e, Ala-172 → end; and pBMH170e, Arg-170 → end.

Single-stranded deoxyoligonucleotides synthesized in a Model 381A DNA synthesizer (Applied Biosystems) were used as templates for *in vitro* mutagenesis (17) (Table I). The *Sau*3AI–*Sph*I fragment described above was ligated into the *Bam*HI–*Sph*I site of pUC118 or pUC119 and used for mutagenesis. The *Sau*3AI–*Sph*I fragments of the resulting mutant genes were ligated into pBR322 and plasmids carrying mutant genes (pBMH11F, Tyr-11 → Phe; pBMH26Q, Glu-26 → Gln; pBMH30N, Asp-30 → Asn; pBMH42Q, Glu-42 → Gln; pBMH82Q, Glu-82 → Gln; pBMH85Q, Arg-85 → Gln; pBMH144N, Asp-144 → Asn; pBMH150D, Gly-150 → Asp; pBMH150P, Gly-150 → Pro; pBMH150A, Gly-150 → Ala; pBMH154Q, Arg-154 → Gln; pBMH161N, Asp-161 → Asn; and pBMH163C, Ser-163 → Cys) were isolated.

The mutations introduced were confirmed by DNA sequencing (15). Recombinant plasmids were introduced into strain KF96rA [*uncH* (Gln-29 → end), *recA*] and the properties of the mutant enzymes were studied. Other procedures for DNA manipulation were as described previously (18).

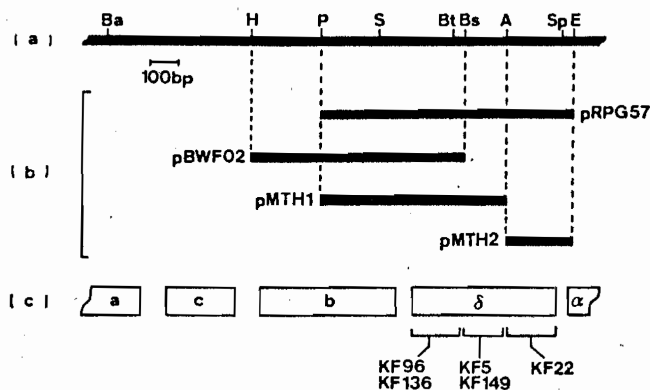

FIG. 1. Mapping of mutations of the δ subunit. DNA segments carried by recombinant plasmids are shown with the results of mapping of mutant alleles. (a) Sites of *E. coli* DNA cleaved by endonucleases: H, *Hin*dIII; P, *Pst*I; Ba, *Bam*HI; A, *Acc*I; E, *Eco*RI; S, *Sau*3AI; Bs, *Bss*HII; Sp, *Sph*I; Bt, *Bst*EII. (b) Segments carried by recombinant plasmids used for mapping the mutations. (c) Reading frames of the *a*, *c*, *b*, δ, and α subunits. The map positions of mutant genes shown were determined by a series of recombination and complementation tests.

TABLE I

Synthetic Oligonucleotides Used for Mutagenesis

Residue replaced	Codon replacement	Deoxyoligonucleotide
Tyr-11 → Phe	TAC → TTC	5′TCGCCCCTtCGCCAAAG 3′
Glu-26 → Gln	GAA → CAA	AAAGTGTAcAACGCTGGC
Asp-30 → Asn	GAC → AAC	GCTGGCAGaACATGCTG
Glu-42 → Gln	GAA → CAA	CCAAAAACcAACAAATG
Glu-82 → Gln	GAA → CAA	TTATGGCTcAAAATGGT
Arg-85 → Gln	CGT → CAA	GAAAATGGTCaaCTTAACGCGC
Asp-144 → Asn	GAT → AAT	ATTGCAAAATCaATAAGTCTGT
Gly-150 → Asp	GGC → GAC	GTAATGGCAGaCGTTATCATC
Gly-150 → Pro	GGC → CCC	TGTAATGGCAccCGTTATCATC
Gly-150 → Ala	GGC → GCC	GTAATGGCAGcCGTTATCATC
Arg-154 → Gln	CGA → CAA	TATCATCCaAGCGGGAG
Asp-161 → Asn	GAT → AAT	TATGGTCATTaATGGCAGCGT
Ser-163 → Cys	AGC → TGC	CATTGATGGCtGCGTACGCGG

Note. Single-stranded deoxyoligonucleotides were used for *in vitro* mutagenesis (17) (altered bases are represented by lowercase letters). The presence of mutations was confirmed by DNA sequencing (15).

Other procedures. Membrane and cytoplasmic fractions were prepared from cells that had been passed through a French press (14). ATPase activity was assayed by measuring the release of inorganic phosphate from ATP: 1 unit of the enzyme was defined as the amount hydrolyzing 1 μmol of ATP per minute under standard conditions (19). Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was carried out as described previously (20). Electrotransfer and immunodecoration with anti-δ subunit antiserum were performed using ^{125}I-labeled protein A (21). The electrochemical gradient of protons (14) and amount of protein (22) were assayed by published methods.

Materials. Antibodies against the δ subunit were prepared by injecting the δ subunit (purified by polyacrylamide gel electrophoresis) into an albino rabbit. [α-^{32}P]dCTP (400 Ci/mmol) for DNA sequencing was from Amersham Corp. and ^{125}I-labeled protein A was from ICN Biomedicals Inc. Restriction endonucleases, the Klenow fragment, *Bal*31 nuclease S, exonuclease III, and T4 DNA ligase were from Takara Shuzo Co., Kyoto, Japan, or Nippon Gene Co., Toyama, Japan. All other reagents used were of the highest grade commercially available.

RESULTS

Properties of mutants of the δ subunit. Mutations were introduced into the *uncH* gene for the δ subunit by random (Fig. 1) or site-directed mutagenesis (Table I). Five *uncH* mutants (strain KF96, Gln-23 → end; KF136, Gln-29 → end; KF5 and KF149, Gln-74 → end; and KF22, Gly-150 → Asp) defective in oxidative phosphorylation were obtained among 170 *unc* strains isolated in this laboratory by hydroxylamine mutagenesis (12) (Fig. 1, Table II). These mutants had low membrane ATPase activities (less than 10% that of the wild type), but higher cytoplasmic activities (about nine times that of the wild type) (Table II), indicating that the δ subunit is required for the binding of F_1 to the F_o sector. The results with three termination mutants (Gln-23 → end, Gln-29 → end, or Gln-74 → end) suggested that the carboxyl half of the δ subunit is essential for its function. Consistent with their negligible ATPase activities, the membranes of these mutants could

TABLE II

Properties of Mutants Defective in the δ Subunit

Strain	Mutation	Growth on succinate	ATPase activity (units/mg protein)	
			Membrane	Cytoplasm
KY7230	Wild	+	1.7	0.10
KF22	Gly-150 → Asp GGC GAC	−	0.02	0.92
KF96	Gln-23 → end CAA TAA	−	0.03	0.95
KF136	Gln-29 → end CAG TAG	−	0.07	0.93
KF149 KF5	Gln-74 → end CAG TAG	−	0.11	1.0

Note. The properties of mutants with defective δ subunits are summarized. Strains KF149 and KF5 had the same mutation, and values for ATPase activities are for the membrane and cytoplasmic fractions of strain KF149. The absence of other mutations was confirmed by genetic recombination assays. Negative growth (−) on succinate indicates no growth (after 2 days) on a minimal agar plate containing 0.4% succinate as the sole carbon source.

not form a proton gradient upon addition of ATP (data not shown).

Role of the carboxyl terminal region of the δ subunit. To determine whether the entire carboxyl terminal region of the δ subunit is essential, we constructed recombinant plasmids carrying truncated *uncH* genes and introduced them into strain KF96rA (Gln-23 → end) (a *recA* derivative of KF96). Strain KF96rA harboring pBWH01 (wild-type *uncH* gene), pBMH177e (Ser-177 → end), or pBMH176e (Gln-176 → end) was able to grow on succinate by oxidative phosphorylation, whereas the same strain harboring pBMH174e (Val-174 → end), pBMH172e (Ala-172 → end), or pBMH170e (Arg-170 → end) could not (Table III). Membranes with the mutant δ subunits Ser-177 → end and Gln-176 → end had ATPase activities similar to that of the wild type (Table III) and formed proton gradients (dependent on ATP) to the same extent as the wild type (data not shown).

Other nonsense mutants (Val-174 → end, Ala-172 → end, and Arg-170 → end) had negligible membrane ATPase activities but had high cytoplasmic activities, confirming that the δ subunit is essential for the binding of F_1 to the F_0 sector. These results indicated that the two carboxyl terminal residues (Ser-177 and Gln-176) of the δ subunit (177 amino acid residues) were dispensable, but that both Leu-175 and Val-174 or only Val-174 was required for forming the functional H^+-ATPase.

Binding of mutant δ subunits to the F_1 portion. As described above, the δ subunit mutants lacking more than four residues (Val-174 → end and other nonsense mutants) had F_1 ATPase activities in the cytoplasm. It was of interest to know whether the mutant δ subunit was attached to the F_1 found in the cytoplasm or to the F_0 sector in the membranes. This question was examined by subjecting membrane and cytoplasmic fractions of strain KF96rA (Gln-23 → end) carrying pBMH174e (Val-174 → end) or pBMH172e (Ala-172 → end) to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and analyzing the mutant subunits immunochemically with antibodies against the δ subunit after electrotransfer of the protein bands to a nitrocellulose filter. As shown in Table IV, the mutant subunits were found in

TABLE III

Effects of Nonsense Mutations of the δ Subunit on Growth Yields and ATPase Activities

Plasmid	*uncH* gene carried by plasmid	Relative growth yield (%)		ATPase activity (units/mg protein)	
		Glucose	Succinate	Membrane	Cytoplasm
pBWH01	None	100	100	1.9	0.22
pBMH177e	Ser-177 → end	97	90	1.7	0.18
pBMH176e	Gln-176 → end	96	92	1.5	0.27
pBMH174e	Val-174 → end	41	1.3	0.06	0.98
pBMH172e	Ala-172 → end	40	1.3	0.05	0.91
pBMH170e	Arg-170 → end	44	1.6	0.04	1.0
pBR322	(No *uncH* gene)	44	1.4	0.03	0.82
None		52	1.7	0.03	0.95

Note. The recombinant plasmid carrying the wild-type or mutant *uncH* gene was introduced into strain KF96rA (Gln-29 → end). Growth yields and membrane ATPase activities are shown. Strain KF96rA carrying each plasmid was grown at 37°C in 70 ml of synthetic medium (pH 7.0) containing ampicillin (50 μg/ml) with glucose (5 mM) or succinate (15 mM) as the sole carbon source. Cell growth was monitored by measuring the optical density at 650 nm and the growth yield was calculated as a percentage of that of KF96rA/pBWH01 (wild-type plasmid) which grew to optical densities at 650 nm of 0.68 and 0.95 in the stationary phase with glucose and succinate, respectively. Membrane vesicles (membrane) and the cytoplasmic fraction (cytoplasm) were prepared from cells (middle of the logarithmic phase) grown in a synthetic medium containing 1% glycerol and ampicillin (50 μg/ml). The ATPase activities of these fractions were assayed.

TABLE IV

Immunochemical Detection of the Mutant δ Subunit

Plasmid	*uncH* gene carried by plasmid	Relative amounts of subunits			
		δ subunit		α and β subunits	
		Membrane	Cytoplasm	Membrane	Cytoplasm
pBWH01	Wild	100	24	100	27
pBMH174e	Val-174 → end	0[a]	113	12	108
pBMH172e	Ala-172 → end	0[a]	89	11	116
pBR322	None	0[a]	0[a]	6	123

Note. Electrotransfer and immunodecoration with the anti-δ subunit and anti-F_1 antibodies were performed. The α, β, and δ subunits were located by autoradiography, the corresponding areas of the nitrocellulose sheets were cut out, and their radioactivities were counted in a γ-counter. Amounts of mutant δ subunits are expressed as percentages of that coded by pBWH01 (wild) (corresponding to 1798 cpm/mg protein). The amounts of the α plus β subunits are also shown as relative values: the radioactivity of membranes of KF96rA harboring pBWH01 was 53,560 cpm/mg protein.

[a] The radioactivities of the corresponding subunits were lower than the background level.

the cytoplasmic fraction, with negligible amounts in the membrane fraction. It is noteworthy that the Val-174 → end and Ala-172 → end mutants had about five times higher cytoplasmic ATPase activities (Table III) and δ subunit levels (Table IV) than those of the wild type. These results suggest that the mutant δ subunits bound to the F_1 sector and did not remain in the F_o sector.

Effects of substitutions of conserved amino acid residues in the δ subunit. Alignment of the primary structures of the δ subunits from eight different species and those of oligomycin-sensitivity conferring proteins from yeast and bovine to obtain maximal homology (23–32) showed that eight residues were apparently conserved (Fig. 2). However, these residues do not form a cluster and may not be

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E. coli                      MS EFITVARPYA KAAFDFAVEH QSVERW-QDM LAFAAEVTKN -EQMAELLSG ALAPETLAES FIAVCGEQLD ENGQ--NLIR VMAENGRLNA LPDVLEQFIH  98
B. megaterium                 M SQPAVAKRYA LALFQLATEK QMIDEMQDQL QIVEEVFAKT PELMDVLTHP KITIE-RKKQ FVSEAFAELS PTVQ--HTVL LLLERHRIQI VSEMVKEYRF  98
PS3                           M NQEVIAKRYA SALFQIALEQ GQLDRIEEDV RAVRQALAEN GEFLSLLSYP KLSL-DQKKA LIREAFAGVS TPVQ--NTLL LLLERHRFGL VPELAGTVSR  98
R. blastica              MSSMKA GVTGVAERYA TALYELAEDR GALDQVSADL RSLKAMLDES GDLRRVIASP VIGRDDQRKA LTALAEKAGF HEIVR-NFLG VVAAKHRSFA VPGMIGAFLE 105
R. rubrum                MAEAAS ISQGIAERYA TALFELSKET GALKTLETDI DALKDVLAGS PDLGAMIASP VISRGDQAKA VAAIAGKMGL SPLMT-NTLA LMSEKRRLFA LPQVLSALAG 105
Synechococcus 6301           MT STSQLFDPYA EALMAIAREQ GLEDRFGEDA ALFRSTLAAS ADLRHLLENP TLFSS-QKKA VLNQVFGSSV HPLVL-NFLN LLVDRNRIAF LDGIADRYQA 100
Anabaena                  MTSKV ANTEVAQPYA QALLSIAKSK SLTEEFGTDA RTLLNLLTEN QQLRNFIDNP FI-AAENKKA LIKQILSEAS -PYLR-NFLL LLVDKRRIFF LPEILQQYLA 102
Spinach                         VDSTASRYA SALADVADVT GTLEATNSDV EKLIRIFSEE PVYY-FFANP VISIDNKRSV LDEIITTSGL QPHTA-NFIN ILIDSERINL VKEILNEFED  97
Bovine OSCP          FAKLVRPPV QIYGIEGRYA TALYSAASKQ NKLEQVEKEL LRVGQILKE- PKMAASLLNP YVKRSVKVKS LSDMTAKEKF SPLTS-NLIN LLAENGRLTN TPAVISAFST 107
S. cerevisiae OSCP  ASKAAAPPPV RLFGVEGTYA TALYQAAAKN SSIDAAFQSL QKVESTVKKN PKLGHLLLNP ALSLKDRNSV IDAIVETHKN LDGYVVNLLK VLSENNRLGC FEKIASDFGV 110

E. coli             LRAVSEATAE VDVISAAALS EQQLAKISAA MEKRLSRK-- --VKLNCKID KSVMAGVIIR AGDM---VID GSVRGRLERL ADVLQS          177
B. megaterium       LANEVRGTAD ATVYSVKPLS ADEKRAISQS FASKVGKHT- --LNISNIVD KTVIGGVKLR IGNR---IYD GSISSKLETI HRGLLAHRS       181
PS3                 PRSTTARGIA KAVAYSGAAS TDEELRALSD VFAQKVGKQT --LEIENIID PELIGGVNVR IGNR---IYD GSVSGQLERI RRQLIG          179
R. blastica         RLAARRGEVT ARIVSATALT SAQKSALTTA LNKATGNT-- --VTIDASVD PALLGGMVVR VGSR---MVD SSLSTKLKRL QLAMKGVG        186
R. rubrum           LIAEEKGEVT AEVTAATKLS AAQAKKLAET LKAKVGKT-- --VKLNTTVD ESLIGGLIVK LGST---MID TSVKSKLASL QNAMKEVG        186
Synechococcus 6301  LLRKLRNTVR ADVSSAVPLT EAQVQVITEK VKQLTGAAG- --VEIESQVD ADLLGGVIIK VGSQ---VLD ASLRGQLKRI SISLAA          180
Anabaena            LLRQLNQTTL AEVTSAVALT EDQQQAVTEK VLALTKARQ- --VELATKVD SDLIGGVIIK VGSQ---VID SSIRGQLRRL SLRLSNS         183
Spinach             VFNKITGTEV AVVTSVVKLE NDHLAQIAKG VQKITGAKN- --VRIKTVID PSLVAGFTIR YGNEGSKLVD MSVKKQLEEI AAQLEMDDVT LAV  187
Bovine OSCP         MMSVHRGEVP CTVTTASALD ETTLTELKTV LKSFLSKGQV --LKLEVKID PSIMGGMIVR IGEK---YVD MSAKTKIQKL SRAMREIL        190
S. cerevisiae OSCP  LNDAHNGELL GTVTSAEPLD PKSFKRIEKA LSASKLVGQG KSLKLENVVK PEIKGGLIVE LGDK---TVD LSISTKIQKL NKVLEDSI        195
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FIG. 2. Alignment of amino acid sequences of δ subunits from various sources. The sequences of the δ subunits of *E. coli* (23), *Bacillus megaterium* (24), thermophilic bacterium PS3 (29), *Rhodopseudomonas blastica* (26), *Rhodospirilum rubrum* (25), *Synechococcus* 6301 (27), *Anabaena* (28), and spinach (31) were aligned together with that of oligomycin-sensitivity conferring protein (OSCP) from bovine (30) and *Saccharomyces cerevisiae* (32). Alignments were made to obtain maximal homology. The amino acid residues of the *E. coli* subunit are numbered from the amino terminus. The sequences for the δ subunit of spinach and OSCP of bovine and *S. cerevisiae* are numbered from the amino termini of the mature proteins. The positions with identical (closed circles) or homologous (33) (open circles) amino acid residues in all eight δ subunits and two OSCPs are shown. Positions with closely similar amino acid residues such as *E. coli* position 26 are also shown (arrowheads).

real functional residues. Of these residues, Gly-150 may be important, because strain KF22 (Gly-150 → Asp) had ATPase activity in the cytoplasm and was defective in oxidative phosphorylation (Table II). Therefore, amino acid substitutions were introduced into this residue by site-directed mutagenesis (Table V): the two mutants (Gly-150 → Asp, Gly-150 → Pro) had the highest cytoplasmic ATPase activities and showed no oxidative phosphorylation activity, whereas in the Gly-150 → Ala mutant 30% of the ATPase was membrane bound and the cells showed active oxidative phosphorylation. Thus Gly-150 itself was not essential, but substitution by more bulky residues (Asp or Pro) than Gly possibly affected the overall structure of the subunit, resulting in loss of its activity.

To identify functionally important amino acid residues, we introduced substitutions into the following hydrophilic residues, which included apparently conserved ones (Fig. 2): Tyr-11 → Phe, Glu-26 → Gln, Asp-30 → Asn, Glu-42 → Gln, Glu-82 → Gln, Arg-85 → Gln, Asp-144 → Asn, Arg-154 → Gln, Asp-161 → Asn, and Ser-163 → Cys. Of these residues, Tyr-11, Arg-85, and Ser-163 are apparently conserved (aligned at the same position) in 10 different species including oligomycin-sensitivity conferring proteins, and Asp-30 and Arg-154 are conserved in most of these proteins (Fig. 2). All the mutants could grow by oxidative phosphorylation and their growth yields were essentially the same as those of the wild type (Table V). Furthermore, all these mutants except the Arg-85 → Gln, Asp-144 → Asn, and Ser-163 → Cys mutants had essentially the same membrane ATPase activities as the wild type. The Arg-85 → Gln, Asp-144 → Asn, and Ser-163 → Cys mutants had about 50% of the membrane ATPase activity of the wild type. Membranes of all the mutants except Arg-85 → Gln showed as high a proton gradient (dependent on ATP) as the wild type cells. Upon addition of F_1, the Arg-85 → Gln membranes formed as high a proton gradient as the wild type, indicating that about half of the F_1 binding sites are exposed. However, all the mutants were capable of oxidative phosphorylation. These results indicate that the hydrophilic residues aligned at identical positions do not play strict functional roles.

DISCUSSION

Mutagenesis studies clearly indicated that the δ subunit is essential for a functional H^+-ATPase. It is striking that the deletion of only four amino acid residues (Val-174 → end) from the carboxyl terminus resulted in the loss of function of the δ subunit defective in mediating the binding of F_1 to the F_o sector. However, the mutant δ subunit did not lose the ability to bind to the F_1 sector. Thus the domain of the δ subunit that interacts with F_o may have been altered by the mutation, whereas the domain that interacts with F_1 remained intact. It is noteworthy that the function of the mutant subunit (Gln-176 → end) lacking two residues from the carboxyl terminus was essentially normal. These results suggest that both the Leu-175 and the Val-174 residues or only the Val-174 is essential for maintaining the proper conformation of the subunit.

TABLE V

Effects of Amino Acid Substitutions of the δ Subunit on Growth Yields and ATPase Activities

Plasmid	*uncH* gene carried by plasmid	Relative growth yield		ATPase activity (units/mg protein)	
		Glucose	Succinate	Membrane	Cytoplasm
pBWH01	Wild	100%	100%	1.9	0.22
pBMH11F	Tyr-11 → Phe	96	95	1.5	0.29
pBMH26Q	Glu-26 → Gln	98	97	1.9	0.20
pBMH30N	Asp-30 → Asn	97	95	1.9	0.20
pBMH42Q	Glu-42 → Gln	98	94	1.9	0.24
pBMH82Q	Glu-82 → Gln	95	96	1.9	0.24
pBMH85Q	Arg-85 → Gln	98	95	0.96	0.32
pBMH144N	Asp-144 → Asn	98	100	1.1	0.31
pBMH150D	Gly-150 → Asp	44	1.3	0.03	0.94
pBMH150P	Gly-150 → Pro	43	1.3	0.03	0.84
pBMH150A	Gly-150 → Ala	90	93	0.56	0.45
pBMH154Q	Arg-154 → Gln	97	97	1.8	0.21
pBMH161N	Asp-161 → Asn	102	104	1.5	0.27
pBMH163C	Ser-163 → Cys	98	93	1.2	0.33
pBR322	No *uncH*	44	1.4	0.03	0.82
None[a]	(None)	52	1.7	0.03	0.95

Note. Plasmids carrying mutant *uncH* genes were constructed and introduced into strain KF96rA (Gln-23 → end). Growth yields and ATPase activities were determined as described in the note to Table III.

[a] No plasmid was introduced.

Alignment of the sequences of the δ subunits and oligomycin-sensitivity conferring proteins from 10 different species suggested the presence of eight conserved residues. However, replacement of the hydrophilic conserved residues caused no apparent impairment of subunit function, indicating that these conserved residues have no specific roles. It is noteworthy that identical residues found by alignment of sequences are not always functionally important. This may be especially true when only limited numbers of *identical* residues are found. Similar findings with other subunits of the ATPase have been reported (34, 35).

The structural requirement(s) of the δ subunit may not be as strict as those of the catalytic subunits. Similarity in higher ordered structure and in the distribution of hydrophilic (or hydrophobic) residues may be sufficient for the function of mutant subunits. In this regard it is noteworthy that 10 different δ subunits showed similar hydropathy profiles (36) and secondary structures estimated by a conventional method (37) (not shown). Although the *E. coli* δ subunit is essential for the binding of F_1 to F_o (5), the corresponding subunits from chloroplasts and mitochondria are not required for binding, but stabilize the binding (9–11). Such differences may be due to slight differences in the higher ordered structures or amino acid sequences of the δ subunits.

Two mutants defective in the δ subunit were isolated previously (38, 39), and their membranes showed no proton conduction. Their phenotypes may be due to the drastic changes of amino acid residues such as that from hydrophilic to hydrophobic or that from a neutral to acidic (or basic) side chain, although precise mutational sites were not determined. It may be also possible that the mutations affected the assembly of the subunits of the F_o portion.

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