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Asymmetry of *Escherichia coli* F_1-ATPase as a Function of the Interaction of α-β Subunit Pairs with the γ and ϵ Subunits*

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The asymmetry of *Escherichia coli* F_1 -ATPase (ECF_1) has been explored in chemical modification experiments involving two mutant enzyme preparations. One mutant contains a cysteine (Cys) at position 149 of the β subunit, along with conversion of a Val to Ala at residue 198 to suppress the deleterious effect of the Cys for Gly at 149 mutation (mutant βG149C:V198A). The second mutant has these mutations and also Cys residues at positions 381 of β and 108 of the ϵ subunit (mutant βG149C:V198A:E381C/ϵS108C). On $CuCl_2$ treatment of this second mutant, there is cross-linking of one copy of the β subunit to γ via the Cys at 381, a second to the ϵ subunit (between βCys^{381} and ϵCys^{108}), while the third β subunit in the ECF_1 complex is mostly free (some cross-linking to δ); thereby distinguishing the three β subunits as β_γ, β_ϵ, and β_{free}, respectively. Both mutants have ATPase activities similar to wild-type enzyme.

Under all nucleotide conditions, including with essentially nucleotide-free enzyme, the three different β subunits were found to react differently with *N*-ethylmaleimide (NEM) which reacts with Cys^{149}, dicyclohexyl carbodiimide (DCCD) which reacts with Glu^{192}, and 7-chloro-4-nitrobenzofurazan (NbfCl) which reacts with Tyr^{297}. Thus, β_γ reacted with DCCD but not NEM or NbfCl; β_{free} was reactive with all three reagents; β_ϵ reacted with NEM, but was poorly reactive to DCCD or NbfCl. There was a strong nucleotide dependence of the reaction of Cys^{149} in β_ϵ (but not in β_{free}) with NEM, indicative of the important role that the ϵ subunit plays in functioning of the enzyme.

An F_1F_0 type ATPase is found in the plasma membrane of bacteria, as well as chloroplast thylakoid and mitochondrial inner membranes, and this enzyme functions to synthesize ATP in response to a light or respiratory chain-driven proton gradient. It is a reversible enzyme, also working as an ATPase, using the hydrolysis of ATP to establish a pH gradient for subsequent use in ion transport processes. The simplest F_1F_0 type ATPases structurally are those from bacteria. The enzyme from *Escherichia coli* (ECF_1F_0)[1] is made up of a total of eight different subunits, five in the ECF_1 part (α, β, γ, δ, and ϵ) and three in the F_0 part (a, b_2, c_{10-12}) (Futai and Kanazawa 1983; Walker *et al.* 1984). The F_1 part of the enzyme from bacteria, mitochondria, and chloroplasts is remarkably similar, particularly with respect to α, β, and γ subunits (Cross, 1988; Senior, 1990; Boyer, 1993). Earlier chemical studies had pointed to an intrinsic asymmetry of F_1, which is expected given a stoichiometry of three copies each of α and β subunits but only one copy of γ, δ, and ϵ subunits. For example, it was shown that one of the β subunits, which contain the catalytic sites, could be cross-linked to the ϵ subunit by the water-soluble carbodiimide EDC (Lötscher *et al.*, 1984a). DCCD was found to react with only two of the three β subunits, excluding the one that is linked to the ϵ subunit (Lötscher *et al.*, 1984a, 1984b; Tommasino and Capaldi, 1985; Stan-Lotter and Bragg, 1986a). Also, it has been shown that NbfCl and DCCD can be reacted with different β subunits (Stan-Lotter and Bragg, 1986b). In addition, clear asymmetry of the F_1 was observed on examination of F_1 by cryoelectron microscopy (Gogol *et al.*, 1989a; Wilkens and Capaldi, 1994), as well as in studies of negatively stained specimens of the enzyme (Boekema and Böttcher, 1992).

The recent high resolution x-ray structure of MF_1 (Abrahams *et al.*, 1994) confirms and provides more details of the asymmetry of the enzyme molecule in relation to the interaction of small subunits and nucleotide occupancy of catalytic sites. In the crystal form examined, one of the β subunits (β_{DP}) contains ADP + Mg^{2+}, and this β is close to and may make contact with the N-terminal α-helical part of the γ subunit. A second β subunit, containing bound AMP-PNP + Mg^{2+} (β_{TP}), is linked via the DELSEED region to a short α-helix (residues 82–99 in the numbering system of ECF_1) in the central part of the γ subunit. The third β subunit, which is empty, (β_E), has several interactions with the C-terminal α-helical part of the γ subunit.

Unanswered, as yet, is whether differences in the structure of α-β subunit pairs such as observed by Abrahams *et al.* (1994) are due to the association of small subunits, nucleotide binding, or both interactions. This is particularly relevant as a crystal form of rat liver F_1 has been reported which is claimed to be symmetrical with respect to features of the α and β subunits and appears to have the small subunits, including γ and ϵ, scrambled (Bianchet *et al.*, 1991; Pedersen *et al.*, 1995).

We are using a combination of molecular biological and biophysical approaches to examine structure-function relationships in ECF_1F_0 (*e.g.* Aggeler and Capaldi, 1992; Turina and Capaldi, 1994a, 1994b) Here, we describe studies in a mutant βG149C:V198A, a functioning enzyme with a Cys residue in the catalytic site region (Iwamoto *et al.*, 1993), thereby allowing probing of the conformation of this region under different nucleotide conditions. We have used this mutant, and a second mutant which includes the above mutations along with a Cys introduced into the DELSEED region of the β subunit (through which different β subunits can be cross-linked to γ and ϵ subunits, respectively) to study the asymmetry of the enzyme in relation to different nucleotide occupancies of catalytic sites.

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[1] The abbreviations used are: ECF_1, soluble portion of the *Escherichia coli* F_1F_0 ATP synthase; ECF_1F_0, *Escherichia coli* F_1F_0 ATP synthase; MOPS, 3-(*N*-morpholino)propanesulfonic acid; NEM, *N*-ethylmaleimide; DCCD, dicyclohexyl carbodiimide; NbfCl, 7-chloro-4-nitrobenzofurazan; AMP-PNP, 5′-adenylyl-β,γ-imidodiphosphate; DTT, dithiothreitol; Me_2SO, dimethyl sulfoxide; kb, kilobase(s); PAGE, polyacrylamide gel electrophoresis.

EXPERIMENTAL PROCEDURES

Materials—[^{14}C]NEM and [^{14}C]DCCD were purchased from NEN DuPont, and Amersham Corp. respectively; Sephadex (G-50 DNA grade, fine, and G-25, medium) was from Pharmacia Biotech Inc.; the BCA protein assay was from Pierce; ATP assay kit was from BioOrbit (Finland); restriction enzymes were from Boehringer Mannheim and New England Biolabs; and NEM, Nbf-Cl (NBD-Cl), AMP-PNP, and other chemicals were from Sigma.

Strains and Construction of Plasmids Containing Mutations in the uncD, uncC, and uncG Genes—A 1.44-kb *Rsr*II/*Eag*I fragment containing the *uncD* mutation βG149C:V198A was isolated from pBMUD14 (a generous gift from Dr. M. Futai, Osaka University, Japan) (Iwamoto *et al.*, 1993) and inserted into the 11.23-kb *Rsr*II/*Eag*I fragment of the plasmid pRA100 coding for wild-type (derivitized pAN45 vector described in Aggeler *et al.*, 1992), or pRA112 containing the *uncG* mutation γS8C (Aggeler and Capaldi, 1992). The resulting plasmids pRA123 and pRA124 contain the mutations βG149C:V198A and βG149C:V198A/γS8C, respectively, and all of the genes encoding the ECF_1F_0 ATPase. Experiments showed that the presence of a Cys at position 8 of the γ subunit (for subsequent studies in which this site is reacted with a fluorophore) had no effect on modification by any of the reagents used here. Therefore, in some experiments, enzyme including this mutation was used. The plasmid pRA137, containing the mutation βG149C:V198A:E381C/εS108C, was constructed by ligation of the 6.7-kb *Eag*I/*Eag*I fragment of pRA123 (containing βG149C:V198A) to the 5.9-kb fragment of pRA134 (containing βE381C/εS108C, Aggeler *et al.*, 1995). The *unc*$^-$ *E. coli* strain AN888 (*uncB*$^+$ Mu::416 *argH pyrE entA nalA recA*) (Aggeler *et al.*, 1992) was transformed with the plasmids pRA134 (Aggeler *et al.*, 1995), pRA123, pRA124, and pRA137 (this work) for expression of mutant ECF_1. The wild-type *E. coli* strain AN1460 (pAN45/*unc*413::Mu *argH pyrE entA nalA recA*) is described in Aggeler *et al.* (1992). The plasmid pAN45, and strains AN1460 and AN888, were the generous gift of Graeme B. Cox, The Australian National University, Canberra). The *E. coli* strain XL1 Blue (Stratagene) was used for routine subcloning procedures (Davis *et al.*, 1986; Sambrook *et al.*, 1989).

Preparation of ECF_1 and ECF_1F_0—ECF_1 and ECF_1F_0 were isolated by a modification of the methods of Wise *et al.*, (1981), Foster and Fillingame, (1979), described in Aggeler *et al.* (1987) and Gogol *et al.* (1989b). ECF_1, either wild-type or mutant, was precipitated in 70% $(NH_4)_2SO_4$ for 1 h at 4 °C, then pelleted by centrifugation at 10,000 × *g* for 20 min. The protein was dissolved in 50 mM MOPS, pH 7.0, 0.5 mM EDTA, and 10% glycerol (v/v) (MOPS pH 7.0 buffer) then passed through two consecutive centrifuge columns (Sephadex G-50, fine, 0.5 × 5.5 cm) (Penefsky, 1977), equilibrated in the same buffer at a concentration of 8–26 μM (3–10 mg/ml) in order to remove loosely bound nucleotides, as described in Aggeler *et al.* (1992).

Removal of Endogenous Nucleotides and Analysis of Bound ATP and ADP—ECF_1 (pRA124) was freed of endogenous nucleotides as described by Senior *et al.* (1992). Briefly, protein (10 mg) was precipitated twice in 70% saturated $(NH_4)_2SO_4$ then passed through a column of Sephadex G-25, medium, (1 cm × 100 cm, flow rate 0.8 ml/h, 23 °C) equilibrated in 100 mM Tris-H_2SO_4, pH 8.0, 4 mM EDTA, and 50% (v/v) glycerol (Tris pH 8.0 buffer). Peak fractions were precipitated in 70% saturated $(NH_4)_2SO_4$, centrifuged, dissolved in 100 mM MOPS, pH 7.0, 1 mM EDTA, and 50% (v/v) glycerol, and stored in liquid N_2. The concentration of endogenous nucleotide (ATP + ADP) in denatured F_1 plus standard ATP was determined by the luciferin/luciferase technique as described in Fromme and Gräber (1990), using the BioOrbit ATP assay kit and a FlowTech Engineering Luminometer model 1030. Total ATP content was estimated following incubation of denatured protein with phosphoenol pyruvate and pyruvate kinase (Fromme and Gräber, 1990; Senior *et al.*, 1992). Mutant ECF_1 was found to contain 1.1–1.4 mol of nucleotide (ATP + ADP) *cf.* 1.3–1.5 mol of nucleotide/mol in wild-type enzyme after two gel centrifugation columns (and initial precipitation with $(NH_4)_2SO_4$). Enzyme treated to remove all nucleotides was found to contain 0.2–0.3 mol of (ATP + ADP)/mol.

Maleimide Reaction of ECF_1 and ECF_1F_0—For modification by maleimides, mutant ECF_1, either βG149C:V198A or βG149C:V198A:γS8C (1–2 μM) from which loosely bound nucleotides had been removed (as above), was equilibrated for 30 min-1 h at room temperature in a buffer of 50 mM MOPS, pH 7.0, 0.5 mM EDTA, and 10% glycerol, with or without the addition of nucleotide. As specified in individual experiments, nucleotide was added to give aliquots containing 0.5 mM EDTA (EDTA), 5 mM ATP + 0.5 mM EDTA (ATP + EDTA), 5 mM ADP + 5.5 mM $MgCl_2$ + 5 mM NaH_2PO_4 (ADP + Mg^{2+} + P_i), or 5 mM ATP + 5.5 mM $MgCl_2$ (ATP + Mg^{2+}) (equivalent to ADP + Mg^{2+} + P_i after catalysis). [^{14}C]NEM was incorporated by the addition of 25 μM [^{14}C]NEM (2.6 mM stock in Me_2SO, specific activity 40.0 mCi/mmol) or 200 μM [^{14}C]NEM (10 mM stock in Me_2SO, specific activity 8.3 mCi/mmol) to 100–200-μl aliquots of F_1, where the final concentration of Me_2SO did not exceed 2–4% (v/v). At specific time intervals (1–120 min), aliquots were withdrawn, and the reaction quenched by the addition of 10 mM L-cysteine or 10 mM NEM. 20–50 μg of samples were dissociated in a DTT-containing buffer, pH 6.2, and subjected to 10–18% linear gradient SDS-PAGE (see below). For the analysis of cross-linked products, DTT was omitted from the dissociation buffer. The radioactivity in β subunits was determined from gel slices and expressed as moles of [^{14}C]NEM incorporated per mole of enzyme (as described in Aggeler *et al.*, 1987). The stoichiometry of incorporation of [^{14}C]NEM into the β subunits was determined from an aliquot which was denatured in 2% SDS prior to reaction with 200 μM [^{14}C]NEM.

$CuCl_2$-induced Cross-link Formation—ECF_1 (7.5–12.5 μM) from which loosely bound nucleotides had been removed (as above) was passed through two consecutive centrifuge columns equilibrated in 50 mM MOPS, pH 7.0, 30 μM $CuCl_2$, and 10% glycerol to induce disulfide bond formation between β-γ, β-ε, β-δ, and α-δ (Aggeler *et al.*, 1995). After incubation at room temperature for a further 30 min, the cross-linking reaction was stopped with 1 mM EDTA. For the analysis of cross-linked products, samples were dissolved in DTT-free dissociation buffer prior to 10–18% linear gradient SDS-PAGE.

Modification by DCCD and NbfCl—ECF_1 (2 μM) was reacted with 200 μM DCCD (10 mM ethanolic stock), or 200 μM [^{14}C]DCCD (10 mM ethanolic stock, 26.6 mCi/mmol) in MOPS pH 7.0 buffer (50 mM MOPS, 0.5 mM EDTA, and 10% (v/v) glycerol) for up to 3 h. For determination of ATPase activity, aliquots were withdrawn at time intervals of 30 s, 2, 5, 10, 15, 30, 60, 120, and 180 min and assayed immediately (5 μg/ml). In a parallel experiment, the rate of incorporation of [^{14}C]DCCD into βGlu^{192} was determined from incorporation of ^{14}C into gel slices (as described above), at the same time intervals. Where indicated in figure legends, ECF_1 was equilibrated with nucleotides ATP, or ADP + P_i + Mg, prior to reaction with DCCD, or [^{14}C]DCCD. Unreacted [^{14}C]DCCD was removed by passage through a centrifuge column of Sephadex G-50, fine, equilibrated in MOPS pH 7.0 buffer when subsequent chemical modification was required, or by addition of 10 mM NaOAc, pH 5.2 if loaded directly on SDS-polyacrylamide gels. Formation of cross-links between β-γ, β-ε, β-δ, and α-δ (as described above) was induced both prior to, and subsequent to, incorporation of [^{14}C]DCCD.

Prior to modification by NbfCl, ECF_1 was reacted with $CuCl_2$ to induce formation of cross-links and the reaction stopped by addition of 1 mM EDTA. NEM (200 μM) was then added (for 1 h) to modify accessible Cys residues prior to reaction with NbfCl. Excess NEM was removed by transfer to a buffer containing 50 mM Tris-H_2SO_4, 1 mM EDTA, and 10% (v/v) glycerol (Tris pH 7.5 buffer) by column centrifugation. Cross-linked ECF_1 (2–5 μM) was reacted with 500 μM NbfCl (10 mM ethanolic stock) in Tris pH 7.5 buffer for 1 h at 30 °C then unreacted NbfCl was removed by passage through a centrifuge column equilibrated in MOPS pH 7.0 buffer. The Nbf-modified F_1 was split into two aliquots, one of which was further modified by reaction with [^{14}C]DCCD (200 μM) for 3 h at room temperature, after which time unreacted [^{14}C]DCCD was quenched by addition of 10 mM NaOAc, pH 5.2. Aliquots were subjected to modified SDS-PAGE where the pH of the separating gel was lowered from 8.6 to 8.0 in order to increase the stability of the Tyr^{297}-Nbf adduct.

Calculation of Kinetic Constants—Kinetic constants describing the incorporation of [^{14}C]NEM and [^{14}C]DCCD were determined assuming pseudo-first-order reactions of one, two, or three independently reacting cysteinyl residues on ECF_1 using KaleidaGraph™ data analysis and graphics program for personal computer. For example, the following equation describes the reaction of two cysteinyl residues with NEM,

$$P_1 + P_2 = 2C - \exp(-k_1Bt) - \exp(-k_2Bt) \qquad \text{(Eq. 1)}$$

where P_1 = Cys-1-[^{14}C]NEM adduct, P_2 = Cys-2-[^{14}C]NEM adduct, C = concentration of protein (ECF_1), B = initial concentration of ligand ([^{14}C]NEM), k_1, k_2 = second-order reaction constants for Cys-1, Cys-2, respectively, and t = time.

Other Methods—ATPase activity was measured with a regenerating system described by Lötscher *et al.* (1984b). Protein concentrations were determined using the BCA protein assay (Pierce). Trypsin cleavage (1:50 w/w, protease/protein) of wild-type and mutant (βG149C:V198A) ECF_1 (3 μM) was carried out in 50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, and 5 mM ATP, for 1 h at room temperature. SDS-polyacrylamide gel electrophoresis was routinely performed with a 4% stacking gel and 10–18% linear gradient separating gel (Laemmli, 1970) stained

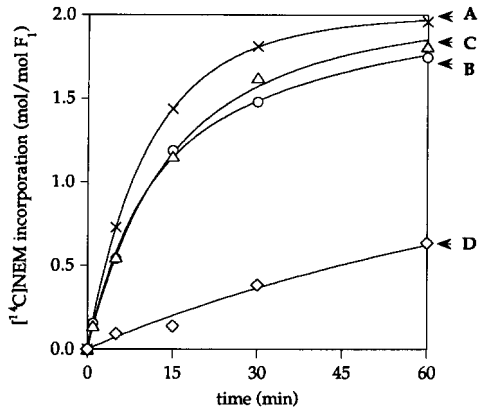

FIG. 1. **Kinetics of incorporation of [^{14}C]NEM into βCys^{149} of mutant ECF$_1$ (βG149C:V198A) under different nucleotide conditions.** ECF$_1$ (2 μM) in MOPS pH 7.0 buffer containing EDTA (*A, crosses*), ATP + EDTA (*B, circles*), AMP-PNP + Mg^{2+} (*C, triangles*), or ADP + Mg^{2+} + P_i (*D, diamonds*) was incubated with 25 μM [^{14}C]NEM for varying times up to 2 h. Aliquots were withdrawn at the times indicated, quenched with 10 mM L-Cys, and electrophoresed on a 10–18% linear gradient SDS-polyacrylamide gel. The incorporation of radioactivity into βCys^{149} (mol [^{14}C]NEM/mol F$_1$) was determined in gel slices of β subunits (described under "Experimental Procedures"). The curve fits represent modification of 2 Cys residues according to Equation 1.

with Coomassie Brilliant Blue R (Downer *et al.*, 1976). Samples were dissociated in 0.12 M Tris-HCl buffer, pH 6.2, 2% SDS, 5% glycerol, and 50 mM DTT prior to electrophoresis. For quantitation of yield of cross-linked products, Coomassie Brilliant Blue-stained gels were scanned with a Microtek™ flat-bed scanner and the intensity of protein bands digitized by the NIH *Image 1.53* processing and analysis program for Macintosh™. For identification of subunit composition of cross-linked products, gels were electroblotted to Immobilon™ polyvinylidene difluoride membranes (Millipore), incubated with monoclonal antibodies, and visualized as described previously (Gogol *et al.*, 1989a; Aggeler *et al.*, 1990; Mendel-Hartvig and Capaldi, 1991).

RESULTS

The double mutant βG149C:V198A was obtained by Futai and colleagues as an enzymatically active revertant of the deleterious mutation βG149C (Iwamoto *et al.*, 1993). For our purposes, these two mutations were inserted into the plasmid pRA100 as described under "Experimental Procedures." The ATPase activity of ECF$_1$ isolated from the mutant was 8–14 μmol of ATP hydrolyzed/min/mg as assayed at pH 7.5 in the presence of 2 mM ATP and 5 mM $MgCl_2$, an activity in the same range as that of the wild-type enzyme when measured under the same conditions. After removal of the ϵ subunit by trypsin cleavage, the activity of the mutant βG149C:V198A increased to 40–55 μmol of ATP hydrolyzed/min/mg, again the same value obtained with wild-type ECF$_1$.

The Different Reactivities of the Three βCys149 Residues Indicates Asymmetry of ECF$_1$ under All Nucleotide Conditions—The presence of Cys^{149} allows probing of the catalytic site region of each of the three β subunits under various nucleotide conditions. For most experiments, enzyme was subjected to two column centrifugation steps to remove loosely bound nucleotide, and then reacted with [^{14}C]NEM, either 25 or 200 μM, for varying lengths of time. Such preparations were found to retain 1.1–1.4 mol of tightly bound nucleotide which, based on other studies, were assumed to be in non-catalytic sites. The time courses of labeling of the enzyme prepared under these conditions showed incorporation of around 2 mol of [^{14}C]NEM. This involves modification of only two of three β subunits (see later) which react with rate constants differing by a factor of around 2 (Fig. 1, *trace A*). In the experiment in Fig. 1, the rate of incorporation of the first mole of NEM was calculated to be 88 M^{-1} s^{-1} and that of the second mole 38 M^{-1} s^{-1}. As the endogenous Cys in the β subunits is buried, based on the lack of reactivity of this site in the wild-type enzyme, this labeling is in the introduced Cys^{149}. Some modification of the third copy of Cys^{149} could be achieved by prolonged incubation with high concentration of [^{14}C]NEM (500 μM), when incorporation of as much as 2.6 mol of maleimide/mol ECF$_1$ was obtained. However, the rate constant for reaction of this third β subunit (0.2 M^{-1} s^{-1}) is negligible under the conditions used in the experiments reported here.

One possible explanation of the observed asymmetry described above is that 1 mol of nucleotide is tightly bound in catalytic sites rather than in non-catalytic sites after two passages through centrifuge columns. To avoid this ambiguity, labeling studies were also conducted with ECF$_1$ freed of nucleotide according to Senior *et al.* (1992). In this procedure, the enzyme is twice precipitated from solution in $(NH_4)_2SO_4$ and then subjected to gel filtration in a buffer containing 50% glycerol, 4 mM EDTA. After such treatment, the βG149C:V198A mutant was found to retain less than 0.3 mol of nucleotide/mol of enzyme. Reaction of this essentially nucleotide-free enzyme with [^{14}C]NEM still gave the same labeling profiles as that obtained with enzyme retaining from 1 to 2 mol of tightly bound nucleotide (result not shown).

The profile of [^{14}C]NEM reaction with Cys^{149} of ECF$_1$ in the presence of 5 mM ATP (*trace B*) or 5 mM AMP-PNP + 5 mM $MgCl_2$ (*trace C*), conditions in which all three catalytic sites would be occupied by nucleotide, are also shown in Fig. 1. The rates of incorporation of NEM under these conditions were 84 M^{-1} s^{-1} and 16 M^{-1} s^{-1} in ATP, and 81 M^{-1} s^{-1} and 17 M^{-1} s^{-1} in AMP-PNP, respectively in Fig. 1. The data are similar to those obtained for ECF$_1$ without added nucleotides (*trace A*), except for an approximately 2-fold lower rate of incorporation of the second mole of NEM.

[^{14}C]NEM modification of ECF$_1$ in the presence of 5 mM ADP + 5 mg Mg^{2+} + 5 mM P_i, both added directly or generated by catalytic turnover of added ATP (Fig. 1, *trace D*), also resulted in incorporation of only 2 mol of reagent. However, with all three catalytic sites occupied by ADP, the rates of NEM incorporation were considerably slower, *i.e.* 10 and 1 M^{-1} s^{-1} leading to incorporation of less that 1 mol of reagent under the conditions described in Fig. 1 (*i.e.* 25 μM, 2 h of incubation). The stoichiometry of incorporation and calculation of kinetic constants (notably k_2), under this nucleotide condition, was therefore determined using a higher concentration of [^{14}C]NEM (200 μM) when 2 mol of reagent could be reacted with the enzyme.

In different experiments, the absolute rates of NEM incorporation varied by a factor of around 20% with the relative differences observed under different nucleotide conditions always maintained. From Fig. 1, it is clear that the conformation of one or both of the NEM reactive, catalytic site regions is significantly different when ADP is bound compared with when ATP is bound or when the catalytic sites are empty.

[^{14}C]NEM Reaction with ECF$_1$F$_0$ from the Mutant βG149C:V198A—Profiles of [^{14}C]NEM incorporation into Cys^{149} in ECF$_1$F$_0$ isolated from the mutant βG149C:V198A are shown in Fig. 2. Again, only 2 mol of NEM were incorporated when experiments were conducted as described. For the two nucleotide conditions, AMP-PNP + Mg^{2+} and ADP + Mg^{2+} + P_i (generated by turnover of the enzyme), the rates of NEM incorporation were similar to that in ECF$_1$. Importantly, the difference in rates of modification of Cys^{149} in ATP compared with ADP is retained in the intact ATP synthase complex (ECF$_1$F$_0$).

The Catalytic Site Asymmetry of ECF$_1$ is Related to Interaction of β Subunits with the γ and ϵ Subunits—We have recently described a mutant, βE381C:ϵS108C, in which $CuCl_2$ treat-

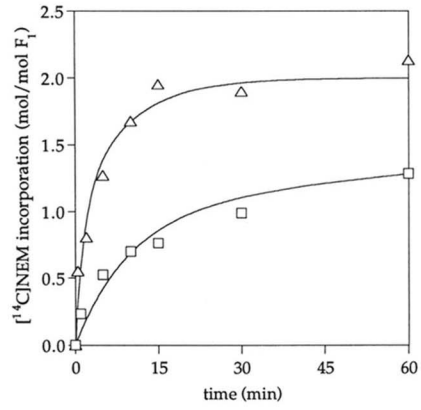

FIG. 2. **Kinetics of incorporation of [^{14}C] NEM into βCys^{149} of mutant ECF$_1$F$_0$ (βG149C:V198A) under different nucleotide conditions.** F_1F_0 (5 μM) in MOPS pH 7.0 buffer containing AMP-PNP + Mg^{2+} (*triangles*), or ATP + Mg^{2+} (ADP + P_i + Mg after catalysis) (*squares*), was incubated with 200 μM [^{14}C]NEM for up to 2 h. At the times indicated, the incorporation of radioactivity into Cys^{149} of β subunits (mol [^{14}C]NEM/mol F_1) was determined as described in Fig. 1.

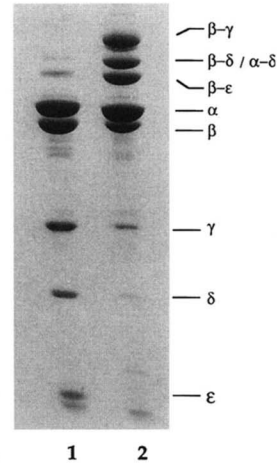

FIG. 3. **Disulfide bond formation between βCys^{381} and γ, ϵ, and δ subunits of mutant ECF$_1$ (βG149C:V198A:E381C/ϵS108C).** Coomassie Brilliant Blue-stained 10–18% linear gradient SDS-PAGE of untreated (*lane 1*) and cross-linked mutant (*lane 2*) ECF_1. Cross-links were induced by passage of ECF_1 (7.5–12.5 μM) through two consecutive $CuCl_2$-containing centrifuge columns then the reaction stopped by the addition of 1 mM EDTA. 40 μg aliquots were incubated in dissociation buffer in the absence of DTT prior to SDS-PAGE.

ment induces essentially quantitative cross-linking between one β subunit and the γ subunit (via the Cys at 381 of β and probably the intrinsic Cys^{87} of the γ subunit) (Aggeler *et al.*, 1995). A second β subunit is cross-linked, again in near 100% yield, to the ϵ subunit (via Cys at 381 of β and Cys^{108} of the ϵ subunit). A portion of the third β subunit becomes cross-linked to the δ subunit, but the majority migrates on gels as free β subunit (Aggeler *et al.*, 1995). With this mutant, it is possible to differentiate the three β subunits by their interactions with small subunits. By combining the mutants βG149C:V198A and βE381C/ϵS108C, a novel mutant was constructed in which the different reactivities of Cys^{149} residues could be related to the association of the different β subunits with the single copy γ and ϵ subunits.

The mutant βG149C:V198A:βE381C/ϵS108C had an ATPase activity of 12–16 μmol of ATP hydrolyzed/min/mg, within the range obtained for wild-type enzyme. ECF_1 for this mutant was reacted first with $CuCl_2$ to generate disulfide bonds between β Cys^{381} residues and the γ and ϵ subunits, respectively (Fig. 3). The cross-linked enzyme was then reacted with [^{14}C]NEM which becomes incorporated into βCys^{149} along with any βCys^{381} not involved in disulfide bond formation with γ or ϵ. The rate of reaction of NEM with the different β subunits was followed by slicing and counting ^{14}C incorporated into the cross-linked products as well as into the non-cross-linked β subunit, as a function of time. As shown in Fig. 4*A*, there was no incorporation of [^{14}C]NEM into the β-γ cross-linked product under any of the nucleotide conditions tested. Therefore, the β subunit, to which the short central α-helix of γ is bound, is the one most shielded from maleimide reaction. There was incorporation of up to 1 mol of ^{14}C into the β-ϵ cross-linked product, the rate of which was nucleotide dependent (Fig. 4*B*). NEM modification of Cys^{149} in this β subunit occurred at the fast rate observed for the reaction of Cys^{149} in the mutant βG149C:V198A when ATP + EDTA was present, but occurred at the slower of the two rates in this mutant when ADP + Mg^{2+} + P_i was present. The nucleotide dependent switching in rates of modification of Cys^{149} in the β subunit linked to ϵ is, therefore, more than 50-fold. In the time course of the labeling experiment, two NEM molecules were bound into the free β subunit, 1 mol into Cys^{149} and the second into the (free) Cys^{381}. There was no significant nucleotide dependence of the rates of modification of these sites in the free β subunit (Fig. 4*C*). Experiments with the mutant βE381C have shown no alteration in the rate of [^{14}C]NEM modification of the Cys at 381 under different nucleotide conditions (results not shown).

Activity Effects of the Modification of Cys^{149} by NEM—Fig. 5 plots the residual ATPase activity of ECF_1 in the mutant βG149C:V198A as a function of incorporation of NEM into the Cys at 149. Reaction of 1 mol of NEM occurring predominantly in the β subunit linked to ϵ when the reaction was done in ATP + EDTA (from Fig. 4) caused only a 60% inhibition of ATPase activity. At 2 mol of NEM incorporated, and therefore with modification of both the β linked to ϵ and free β subunit, ATPase activity was reduced to less than 2% of the untreated enzyme.

DCCD Modification of ECF_1 Examined with the Mutant βE381C:ϵS108C—Reaction of F_1 with DCCD has been shown to lead to incorporation of 1 or 2 mol/mol of enzyme (Satre *et al.*, 1979; Esch *et al.*, 1981; Yoshida *et al.*, 1981; Tommasino and Capaldi, 1985), with evidence presented that the β subunit linked to ϵ (based on cross-linking with EDC) is only poorly reactive with the reagent (Lötscher and Capaldi, 1984). The use of the mutant βE381C/ϵS108C allows the specificity of DCCD for the different β mutants to be examined in more detail than previously possible. Fig. 6*A* shows the time dependence and Fig. 6*B* the molar incorporation of DCCD in relation to inhibition of ATPase activity of the mutant. The results are essentially the same as for wild-type (not shown, but see Tommasino and Capaldi, 1985) and show a Mg^{2+} dependence but no nucleotide dependence of the modification of the enzyme by DCCD.

Table I gives data on incorporation of DCCD into the different β subunits assessed by $CuCl_2$-induced disulfide bond formation in the βE381C/ϵS108C mutant. In these experiments, enzyme was reacted for 1 h in EDTA-containing buffer, by which time between 1.3 and 1.6 mol of [^{14}C]DCCD had become incorporated into the enzyme with more than 90% inhibition of activity. The modification of ECF_1 by the hydrophobic carbodiimide occurred predominantly in the β subunit linked to γ and in the free β subunit, with no major preference between the two. Incorporation of DCCD into the β linked to ϵ was low, as expected from previous studies, and may in part represent background labeling, given that there was a proportionally small labeling of γ and δ subunits in our experiments (result not shown).

Importantly, the same distribution of label, mainly into β-γ

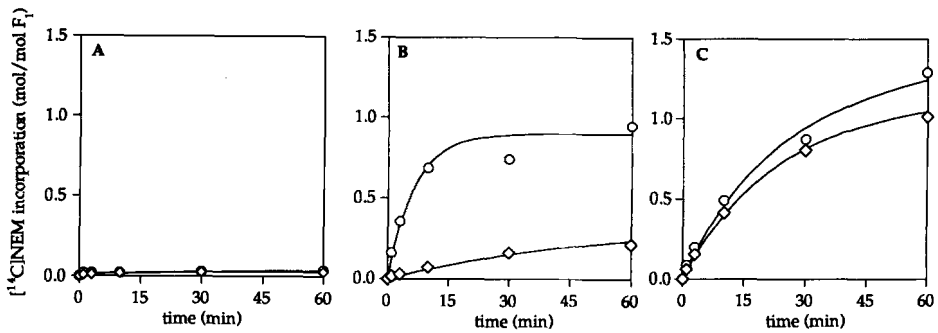

FIG. 4. **Kinetics of incorporation of [^{14}C]NEM into cross-linked β subunits of mutant ECF_1 (βG149C:V198A:E381C/εS108C).** Cross-linked mutant ECF_1 in MOPS pH 7.0 buffer containing ATP + EDTA (*circles*), or ADP + Mg^{2+} + P_i (*diamonds*), was incubated with 200 μM [^{14}C]NEM for varying times up to 2.5 h. At the times indicated, the incorporation of radioactivity into βCys^{149} (mol [^{14}C]NEM/mol F_1) was determined as described in Fig. 1 for β-γ (*A*), β-ε (*B*), and free β subunit (*C*).

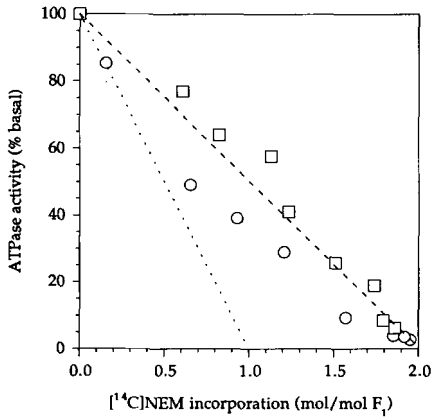

FIG. 5. **Correlation of ATPase activity with incorporation of [^{14}C]NEM into β subunits of mutant ECF_1 (βG149C:V198A).** ECF_1, 1 μM or 2 μM, was equilibrated in MOPS pH 7.0 buffer containing ATP + EDTA (*circles*) or ATP + Mg^{2+} (ADP + P_i + Mg after catalysis) (*squares*), and incubated with 25 μM [^{14}C]NEM (ATP + EDTA) or 200 μM [^{14}C]NEM (ATP + Mg^{2+}) for up to 2 h. The incorporation of radioactivity into β^{Cys}149 (mol [^{14}C]NEM/mol F_1) was determined at various times from gel slices of the β subunit isolated by SDS-PAGE. In a parallel experiment, the residual ATPase activity (% basal activity) was determined at the same time intervals under the same conditions. The *dashed* and *dotted lines* plot for full inhibition at 1 and 2 mol of NEM incorporated, respectively.

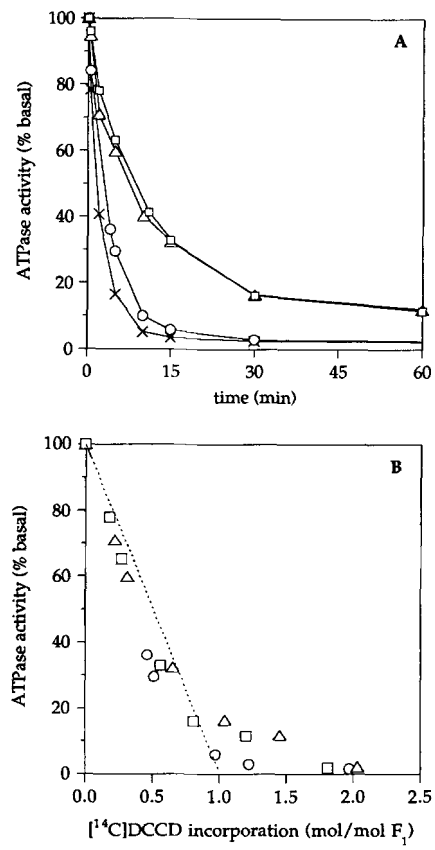

FIG. 6. **DCCD modification of mutant ECF_1 (βE381C/εS108C) under different nucleotide conditions and correlation of ATPase activity with incorporation of [^{14}C]DCCD.** *A*, mutant ECF_1 (2 μM) was equilibrated in MOPS pH 7.0 buffer containing EDTA (*crosses*), ATP + EDTA (*circles*), AMP-PNP + Mg^{2+} (*triangles*), or ATP + Mg^{2+} (ADP + P_i + Mg after catalysis) (*squares*), and incubated with 200 μM DCCD. Aliquots were withdrawn for assay of residual ATPase activity at various time intervals up to 3 h. *B*, in a parallel experiment, aliquots of ECF_1 (2 μM) were equilibrated in MOPS pH 7.0 buffer containing ATP + EDTA (*circles*), AMP-PNP + Mg^{2+} (*triangles*), or ATP + Mg^{2+} (ADP + P_i + Mg after catalysis) (*squares*), and incubated with 200 μM [^{14}C]DCCD. At the times indicated in *A*, and at 2 and 3 h, aliquots were withdrawn and the reaction was quenched by addition of 10mM NaOAc, pH 5.2. The incorporation of radioactivity into βGlu^{192} (mol [^{14}C]DCCD/mol F_1) was determined as described in Fig. 1 and plotted against the corresponding ATPase activity.

and into the free β subunit, was observed whether the DCCD reaction occurred first followed by disulfide bond formation to link β subunits to their partner small subunits, or if cross-linking was performed first, followed by DCCD labeling.

NbfCl Reaction and DCCD Labeling of NbfCl-reacted ECF_1—NbfCl has been found to inhibit F_1 (95%) when reacted under conditions that modify a Tyr in the β subunit, *i.e.* Tyr^{297} on ECF_1 (Andrews *et al.*, 1984). Recently, Weber *et al.* (1994) have shown that the NbfCl effect requires binding of 1 mol of reagent and that the resulting modification causes selective loss of the lowest affinity binding site for nucleotide, *i.e.* that with a K_d for MgATP of around 25 μM.

Fig. 7 shows the labeling of ECF_1 from the mutant βE381C/εS108C with NbfCl. The reagent, detected by its fluorescence, binds predominantly to the free β subunit (seen both in the β subunit band and in the β-δ cross-linked product), with a small amount of reaction in β-ε, but no labeling of that β linked to the γ subunit.

DCCD reaction of NbfCl-labeled ECF_1 led to incorporation of [^{14}C]DCCD into the β subunit linked to γ, and into the free β subunit, to the same extent as with enzyme that had no prior reaction with NbfCl (Table I).

DISCUSSION

The studies reported here exploit the recently described mutant of ECF_1, βE381C/εS108C, in which $CuCl_2$ induces high yield cross-linking between one β subunit and γ, and a second β subunit and the ε subunit. As a consequence, it is possible to distinguish the three β subunits by their interaction with the small subunits. Additionally, a mutant was constructed that contained a Cys introduced into the catalytic site region (β Cys^{149}) as well as Cys at β residue 381 and at ε residue 108. Chemical modification studies were conducted: (i) with NEM to modify Cys^{149}, (ii) with DCCD, which reacts with βGlu^{192}, and (iii) with NbfCl, which reacts at βTyr^{297}.

With DCCD, the modification was introduced both prior to, and following, induction of disulfide bond formation between β

TABLE I

Incorporation of [^{14}C]DCCD and NbfC1 into β subunits of cross-linked mutant ECF$_1$ (βG149C:V198A:E381C/εS108C)

ECF$_1$ was reacted with 200 μM [^{14}C]DCCD in an EDTA-containing buffer (MOPS pH 7.0), for 3 h, prior to (column 1) and following (column 2) induction of cross-links by $CuCl_2$ column centrifugation. In some experiments, aliquots of cross-linked ECF$_1$ were labeled with NbfCl prior to reaction with [^{14}C]DCCD (column 3). The three copies of β subunit are identified as β_{free} (no cross-linked product), βγ (β-γ cross-linked product), and β_ε (β-ε cross-linked product). The incorporation of radioactivity (mol [^{14}C]DCCD/mol F$_1$) was determined from gel slices of the β subunit (β_{free}, β_γ, and β_ε) isolated by SDS-polyacrylamide gel electrophoresis as described under "Experimental Procedures." The stoichiometry of incorporation was determined to be 1.3–1.6 mol [^{14}C]DCCD/mol F$_1$; the results of a typical experiment are given in column 4. Columns 1–3 represent the relative incorporation of radioactivity into β_{free}, and β_γ, and β_ε under the various experimental conditions, expressed as a percentage of the total incorporation into β subunits.

	1. [^{14}C]DCCD 2. X-link	1. X-link 2. [^{14}C]DCCD	1. X-link 2. NbfCl 3. [^{14}C]DCCD	Incorporation of [^{14}C]DCCD (mol/mol)
		%		
β_{free}	44 ± 3	38 ± 6	39 ± 5	0.65
β_γ	47 ± 3	51 ± 7	51 ± 8	0.70
β_ε	9 ± 1	11 ± 3	10 ± 4	0.15
No. experiments	5	5	3	1

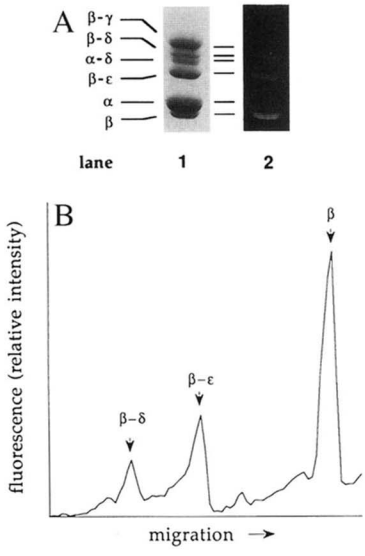

FIG. 7. **NbfCl modification of cross-linked mutant ECF$_1$ (βE381C/εS108C) followed by incorporation of [^{14}C]DCCD.** *A*, cross-links were induced by passage of mutant ECF$_1$ (7.5–12.5 μM) through two consecutive $CuCl_2$-containing centrifuge columns and the reaction stopped by the addition of 1 mM EDTA. Cross-linked mutant ECF$_1$ was transferred to a Tris pH 7.5 buffer, and incubated with 500 μM NbfCl for 1 h at 30 °C. Excess NbfCl was removed by column centrifugation and an 80-μg aliquot subjected to SDS-PAGE in the absence of DTT. *Lane 1*, Coomassie Brilliant Blue-stained gel; *lane 2*, fluorogram of the same gel. *B*, relative intensities of NbfCl incorporation into the different β subunits as observed upon UV illumination (302 nm) (*A*, *lane 2*).

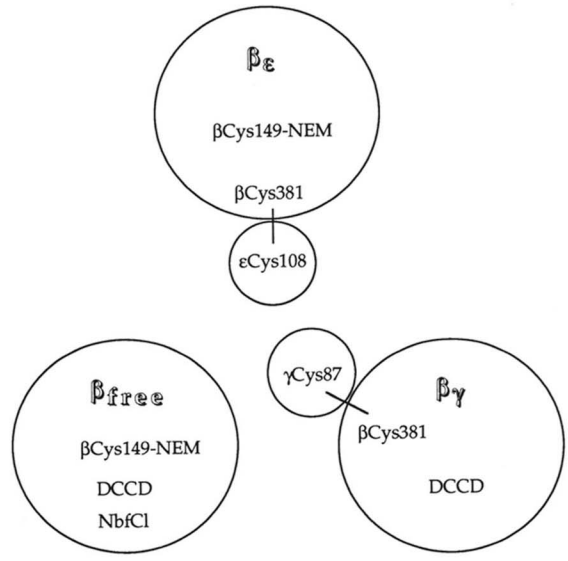

FIG. 8. **Schematic representation of the different conformations of the three β subunits of ECF$_1$, designated β_γ, β_ε, and β_{free}, relative to interactions of the α-β subunit pairs with the single-copy subunits γ and ε.** β_γ forms a cross-link between βCys^{381} and γCys^{87}, reacts with DCCD but not with NbfCl, and residue Gly^{149} → Cys (P-loop) of this β subunit is shielded from reaction with NEM; β_ε forms a cross-link between βCys^{381} and εCys^{108}, incorporates NEM into Cys^{149}, but reacts only very poorly with DCCD and NbfCl; β_{free} is the primary site of reaction with NbfCl, reacts with DCCD, and Cys^{149} of this β subunit is modified by NEM.

subunits and γ and ε subunits, respectively. Up to 2 mol of reagent were incorporated under both conditions with the same distribution of reagent between the three different β subunits. This result establishes that the asymmetrical distribution of DCCD is not induced by the incorporation of the reagent, but reflects an intrinsic asymmetry of the enzyme. Furthermore, the data with DCCD are reassurance that cross-linked enzyme fairly reflects the structure of ECF$_1$, which is not significantly altered by disulfide bond formation between β with γ, and β with ε subunits.

The key finding of the work presented here is that the three different β subunits have different conformations (shown schematically in Fig. 8), in the absence of nucleotides in catalytic sites and even when both catalytic and non-catalytic nucleotide-binding sites are empty. Therefore, there must be an intrinsic asymmetry of F$_1$ induced by interactions of the α-β pairs with the small subunits γ and ε. One β subunit, that which interacts directly with the short central α helix of the γ subunit (residues 82–99 in *E. coli*) (β_γ), is reactive to the hydrophobic carbodiimide DCCD but does not react with NbfCl. A Cys introduced at residue 149 in place of Gly in this copy of the β subunit is shielded from reaction with NEM. The β subunit linked to the ε subunit (β_ε) does not appear to bind DCCD at Glu^{192} and has very poor reactivity to NbfCl. Cys^{149} in this β subunit is the most reactive of the three to NEM. The third β subunit, the free β subunit (β_{free}), reacts readily with DCCD, Cys^{149} is modified by NEM, and this copy of the β subunit is the primary site of reaction of NbfCl.

Asymmetry of the enzyme, as reflected by the different reactivities of the three β subunits, is retained after binding of nucleotides into catalytic (and non-catalytic) sites under conditions where all sites would be occupied (*i.e.* 5 mM nucleotide). With either ATP + EDTA, AMP-PNP + Mg^{2+}, or ADP + Mg^{2+} + P_i bound, DCCD reacted with β_{free} and β_γ but not β_ε, while NEM reacted with β_ε, β_{free} but not β_γ. The recent structure determination of Abrahams *et al.* (1994) establishes an asymmetry of F$_1$ under conditions different from those used here, in this case for enzyme with ADP in one catalytic site, AMP-PNP in a second catalytic site (that which is in the β subunit linked

to the short α helix of γ and, therefore β_γ in our terminology), and a third catalytic site empty of nucleotide. It is interesting to note that Weber *et al.* (1994) have shown that the three catalytic sites have the same affinity for ATP or ADP in the absence of Mg^{2+} (*i.e.* in EDTA). Therefore, the catalytic sites can become equivalent without loss of the asymmetry induced by binding of the small subunits.

The chemical modification studies presented here provide interesting data on nucleotide-dependent conformational changes occurring in ECF_1. Binding of ATP to the enzyme under conditions that prevent hydrolysis of the substrate (ATP + EDTA or AMP-PNP + Mg^{2+}) does not greatly alter the reactivity of Cys^{149} compared with when catalytic sites are empty of nucleotide. However, binding of ADP + Mg^{2+} + P_i has a profound effect. The reactivity of the enzyme to DCCD, in contrast, is not sensitive to nucleotide conditions. It is modulated by Mg^{2+}, probably indirectly, by interaction of the cation with carboxyl groups, resulting in some shielding from the carbodiimide. Importantly, the nucleotide-dependent conformational change reflected in the altered reactivity of Cys^{149} occurs specifically in that β subunit linked to the ϵ subunit. Conformational changes have been detected in the ϵ subunit during ATP hydrolysis (Mendel-Hartvig and Capaldi, 1991; Aggeler *et al.*, 1992; Turina and Capaldi, 1994a) and ATP synthesis (Richter and McCarty, 1987), in concert with translocation of this subunit between an α and a β subunit (Wilkens and Capaldi, 1994). The results presented here, then, add evidence to the proposal (Capaldi *et al.*, 1994) that the ϵ subunit plays an important role in the functioning of ECF_1F_0.

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REFERENCES

Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994) *Nature* **370,** 621–628
Aggeler, R., and Capaldi, R. A. (1992) *J. Biol. Chem.* **267,** 21355–21359
Aggeler, R., Zhang, Y.-Z., and Capaldi, R. A. (1987) *Biochemistry* **26,** 7107–7113
Aggeler, R., Mendel-Hartvig, J., and Capaldi, R. A. (1990) *Biochemistry* **29,** 10387–10393
Aggeler, R., Chicas-Cruz, K., Cai, S.-X., Keana, J. F. W., and Capaldi, R. A. (1992) *Biochemistry* **31,** 2956–2961
Aggeler, R., Haughton, M. A., and Capaldi, R. A. (1995) *J. Biol. Chem.* **270,** 9185–9191
Andrews, W. W., Hill, F. C., and Allison, W. S. (1984) *J. Biol. Chem.* **259,** 8219–8225
Bianchet, M., Ysern, X., Hullihen, J., Pedersen, P. L., and Amzel, L. M. (1991) *J. Biol. Chem.* **266,** 21197–21201
Boekema, E. J., and Böttcher, B. (1992) *Biochim. Biophys. Acta* **1098,** 131–143
Boyer, P. D. (1993) *Biochim. Biophys. Acta* **1140,** 215–250
Capaldi, R. A., Aggeler, R., Turina, P., and Wilkens, S. (1994) *Trends Biochem. Sci.* **12,** 186–189
Cross, R. L. (1988) *J. Bioenerg. Biomembr.* **20,** 395–405
Davis, L. G., Dibner, M. D., and Battey, J. F. (1986) *Basic Methods in Molecular Biology*, pp. 89–118, 220–232 Elsevier, New York
Downer, N. W., Robinson, N. C., and Capaldi, R. A. (1976) *Biochemistry* **15,** 2930–2936
Esch, F. S., Böhlen, P., Otsuka, A. S., Yoshida, M., and Allison, W. S. (1981) *J. Biol. Chem.* **256,** 9084–9089
Foster, D. L., and Fillingame, R. H. (1979) *J. Biol. Chem.* **254,** 8230–8236
Fromme, P., and Gräber, P. (1990) *Biochim. Biophys. Acta* **1016,** 29–42
Futai, M., and Kanazawa, H. (1983) *Microbiol. Rev.* **47,** 285–312
Gogol, E. P., Aggeler, R., Sagermann, M., and Capaldi, R. A. (1989a) *Biochemistry* **28,** 4717–4724
Gogol, E. P., Lücken, U., Bork, T., and Capaldi, R. A. (1989b) *Biochemistry* **28,** 4709–4716
Iwamoto, A., Park, M.-Y., Maeda, M., and Futai, M. (1993) *J. Biol. Chem.* **268,** 3156–3160
Laemmli, U. K. (1970) *Nature* **227,** 680–685
Lötscher, H.-R., and Capaldi, R. A. (1984) *Biochem. Biophys. Res. Commun.* **121,** 331–339
Lötscher, H.-R., deJong, C., and Capaldi, R. A. (1984a) *Biochemistry* **23,** 4134–4140
Lötscher, H.-R., deJong, C., and Capaldi, R. A. (1984b) *Biochemistry* **23,** 4140–4143
Mendel-Hartvig, J., and Capaldi, R. A. (1991) *Biochemistry* **30,** 1278–1284
Pedersen, P. L., Hullihen, J., Bianchet, M., Amzel, L. M., and Lebowitz, M. S. (1995) *J. Biol. Chem.* **270,** 1775–1784
Penefsky, H. S. (1977) *J. Biol. Chem.* **252,** 2891–2899
Richter, M. L., and McCarty, R. E. (1987) *J. Biol. Chem.* **262,** 15037–15040
Sambrook, J., Tritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY
Satre, M., Lunardi, J., Pougeois, R., and Vignais, P. V. (1979) *Biochemistry* **18,** 3134–3140
Senior, A. E. (1990) *Annu. Rev. Biophys. Chem.* **19,** 7–14
Senior, A. E., Lee, R. S. F., Al-Shawi, M. K., and Weber, J. (1992) *Arch. Biochem. Biophys. Biophys.* **297,** 340–344
Stan-Lotter, H., and Bragg, P. D. (1986a) *Arch. Biochem. Biophys.* **248,** 116–120
Stan-Lotter, H., and Bragg, P. D. (1986b) *Eur. J. Biochem.* **154,** 321–327
Tommasino, M., and Capaldi, R. A. (1985) *Biochemistry* **24,** 3972–3976
Turina, P., and Capaldi, R. A. (1994a) *J. Biol. Chem.* **269,** 13465–13471
Turina, P., and Capaldi, R. A. (1994b) *Biochemistry* **33,** 14275–14280
Walker, J. E., Saraste, M., and Gay, N. J. (1984) *Biochim. Biophys. Acta* **768,** 164–200
Weber, J., Wilke-Mounts, S., and Senior, A. E. (1994) *J. Biol. Chem.* **269,** 20462–20467
Wilkens, S., and Capaldi, R. A. (1994) *Biol. Chem. Hoppe-Seyler* **375,** 43–51
Wise, J. G., Latchney, L. R., and Senior, A. E. (1981) *J. Biol. Chem.* **256,** 10383–10389
Yoshida, M., Poser, J. W., Allison, W. S., and Esch, F. S. (1981) *J. Biol. Chem.* **256,** 148–153