Solution Structure of the ε Subunit of the F1-ATPase from *Escherichia coli* and Interactions of This Subunit with β Subunits in the Complex*

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The solution structure of the ε subunit of the *Escherichia coli* F1-ATPase has been determined by NMR spectroscopy. This subunit has a two-domain structure with an N-terminal 10-stranded β sandwich and a C-terminal antiparallel two α-helix hairpin, as described previously (Wilkens, S., Dahlquist, F. W., McIntosh, L. P., Donaldson, L. W., and Capaldi, R. A. (1995) *Nat. Struct. Biol.* 2, 961–967). New data show that the two domains interact in solution in an interface formed by β strand 7 and the very C-terminal α-helix. This interface involves only hydrophobic interactions. The dynamics of the ε subunit in solution were examined. The two domains are relatively tightly associated with little or no flexibility relative to one another. The ε subunit can exist in two states in the ECF1F0 complex depending on whether ATP or ADP occupies catalytic sites. Proteolysis of the ε subunit in solution and when bound to the core F1 complex indicates that the conformation of the polypeptide in solution closely resembles the conformation of ε when bound to the F1 in the ADP state. Chemical and photo-cross-linking show that the ε subunit spans and interacts with two β subunits in the ADP state. These interactions are disrupted on binding of ATP + Mg2+, as is the interaction between the N- and C-terminal domains of the ε subunit.

An F1F0-type ATP synthase is found in the inner mitochondrial membrane, the inner membrane of bacteria, and the thylakoid membrane of chloroplasts where it functions to convert the free energy of the proton motive force into the chemical energy source ATP (for recent reviews see Refs. 1–3). This large enzyme complex is composed of two major parts, a water-soluble F1 made of three α, three β, and one copy of each of the γ, δ, and ε subunit; and a membrane-embedded F0 consisting of 1 α and 2 β, and 9–12 c subunits. The overall molecular weight of the complex is 520,000. There are three catalytic nucleotide binding sites located on the β subunits of the F1 and one proton channel formed by the α and c subunits in the membrane-embedded F0.

The two parts of the complex are linked by two 45-Å stalks, a central one formed by the ε and part of the γ subunit and a peripheral one, constituted by the hydrophilic portions of the two b subunits of the F0 and the δ subunit of the F1 (4, 5). The function of the central stalk is to transmit energy between the proton channel in the membrane and the catalytic nucleotide binding sites on the β subunits via conformational changes in the stalk-forming proteins. The peripherally located second stalk functions as a “stator” or “scaffold” to form a rigid link between the catalytic domain and the a subunit of the F0 with respect to a mobile domain formed by the γ, ε, and c subunit ring (6, 7).

The 2.8-Å crystal structure of the mitochondrial F1 provides a molecular picture of the α and β subunits and part of the γ subunit (8). The α and β subunits are arranged alternatingly in a hexagon around a central cavity in which the N and C termini of the γ subunit are located. Unfortunately, part of the γ subunit and the entire ε subunit (*Escherichia coli* nomenclature), although present, were not resolved in the crystal structure, probably because they were disordered.

Structural features of the N- and C-terminal domains of the ε subunit have been obtained in this laboratory by NMR spectroscopy (9). These data show that the N-terminal domain of the subunit is folded in a 10-stranded β sandwich, and the C-terminal third of the protein is arranged in an antiparallel two α-helix hairpin. A possible arrangement of the two domains with respect to one another was suggested based on spin-labeling experiments and on a small number of long range NOEs between the two domains. However, because all structure calculations had been performed for the two domains independently, the proposed interaction site between the two domains remained speculative. While the work described here was in progress, a crystal structure of the *E. coli* ε subunit at 2.8 Å was obtained (10), which confirmed the domain structure seen in NMR and provided new information on the interaction of the two domains. To establish that the arrangement of the two domains relative to one another was not an artifact of the crystallization, we have completed the structure determination of the ε subunit in solution by NMR. The dynamics of the polypeptide have been investigated. In the intact ECF1 and ECF1F0, the ε subunit exists in at least two arrangements (11, 12). Proteolysis studies show that in solution the ε is in the same conformation as when ADP is present on the enzyme.

**MATERIALS AND METHODS**

**Protein Purification**—Wild type ECF1, ECF1, from mutant strain eM138C, and an overexpressed ε subunit were isolated as described (13, 14).

**NMR Spectroscopy and Data Analysis**—NMR spectroscopy and data analysis were performed essentially as described previously (9). A total of 1321 experimental restraints have been collected for the full-length polypeptide, including 1212 NOE distance restraints, 30 hydrogen bond distance restraints, and 79 dihedral angle restraints. NOE intensities were sorted visually into the three classes: strong (1.8–3 Å), medium

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**Footnotes**

1 The abbreviations used are: NOE, nuclear Overhauser effect; TFPAM-3, N-(4-azide-2,3,5,6-tetrafluorobenzyl)-3-maleimidopropionamide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; MOPS, 4-morpholinepropanesulfonic acid; AMP-PNP, adenosine 5’-(β,γ-imino)triphosphate; NhC1, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole.

**Article**

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Hydrogen bonds were only added when the accepting oxygens were unambiguously determined from structure calculations without or with only a subset of hydrogen bonds. Structure calculations were performed with the program X-PLOR (15) according to standard protocols. A template with ideal local geometry was created based on standard bond length and angles. This template was then used to calculate a starting set of 30 embedded structures using distance geometry. The 30 structures were regularized and repeatedly refined by using simulated annealing and molecular dynamics protocols until stable. All 30 structures converged to give the same fold with 3 structures having NOE violations greater than 0.5 Å. All 30 structures were averaged, and the average was energy minimized to satisfy standard bond lengths and angles. 15N relaxation parameters were measured based on the inverse detection scheme from a series of two-dimensional spectra (16–18) as described by Zhou et al. (19). Overall correlation times were calculated from $T_1/T_2$ ratios on a residue-by-residue basis as described by Kay et al. (16).

Proteolysis of Isolated and $F_1$-bound $\epsilon$ Subunit—ECF$_1$ and an isolated $\epsilon$ subunit were cleaved with trypsin at a ratio of 1:2000 for ECF$_1$ and 1:80 for the $\epsilon$ subunit protease to protein (w/w) in 50 mM MOPS/Cl, pH 7.0, 6% D$_2$O, and 3 mM Na$_3$ in a 8-mm triple-resonance gradient probe at 20 °C. Labels of residues that form the C-terminal $\alpha$ domain are drawn in red. The second primary amide proton of the side chain of Gln$^{127}$ is located at a proton chemical shift of 5.6 ppm. All other amides and primary side chain amides have been assigned in the spectrum; however, the intensity of some backbone amide resonances is weak because of solvent exchange (see text). The amide proton of His$^{169}$ is exchange-broadened beyond detectability at pH 7.4, and it can be detected at pH values smaller than 7 (approximately 9.1/122 ppm). Protons of primary amides are connected by horizontal lines.

(1.8–4 Å), and weak (1.8–5.5 Å). Hydrogen bonds were only added when the accepting oxygens were unambiguously determined from structure calculations without or with only a subset of hydrogen bonds. Structure calculations were performed with the program X-PLOR (15) according to standard protocols. A template with ideal local geometry was created based on standard bond length and angles. This template was then used to calculate a starting set of 30 embedded structures using distance geometry. The 30 structures were regularized and repeatedly refined by using simulated annealing and molecular dynamics protocols until stable. All 30 structures converged to give the same fold with 3 structures having NOE violations greater than 0.5 Å. All 30 structures were averaged, and the average was energy minimized to satisfy standard bond lengths and angles. 15N relaxation parameters were measured based on the inverse detection scheme from a series of two-dimensional spectra (16–18) as described by Zhou et al. (19). Overall correlation times were calculated from $T_1/T_2$ ratios on a residue-by-residue basis as described by Kay et al. (16).

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was identical under both nucleotide conditions. Cleavage products were analyzed by SDS-polyacrylamide gel electrophoresis in 10–18% gradient gels.

**Photo- and Chemical Cross-linking Using eM138C—** 

ECF1 containing the mutation eM138C (20) was prepared using ammonium sulfate precipitation followed by two subsequent spin columns in 50 mM MOPS/Cl, pH 7, 10% glycerol, 0.5 mM EDTA. The enzyme at a protein concentration of 10 mg/ml was reacted with either TFPAM-3 (21, 22) (200 mM for 1 h in the dark) or EDC (0.5 mM for 30 min) at room temperature. Excess reagent was removed by one spin column in 50 mM Tris/Cl. The enzyme was converted to the ADP state before cross-linking by the addition of 2 mM ATP and 2.5 mM MgCl2. Half of the photolyzed sample was treated with 0.5 mM EDC as described above. Finally, all three samples (EDC-treated, photolyzed, and photolyzed and EDC-treated) were reacted with 0.8 mM NbfCl followed by one spin column to remove excess NbfCl. Cross-linked and labeled products were analyzed by denaturing polyacrylamide gel electrophoresis as described above. The composition of cross-linked bands was analyzed by monoclonal antibody blots as described (23).

**RESULTS**

**Solution Structure of the e Subunit**—Previously, we determined the global fold of the e subunit from NMR spectroscopy (9). This showed that the N-terminal two-thirds of the protein is folded in a 10-stranded \( \beta \) sandwich and the very C-terminal \( \alpha \)-helix. Based on the NOE data, the interface is formed by 5 residues within the N-terminal domain (shown in green) and 7 residues from the second \( \alpha \)-helix (red). The side chain of Ile131 is not shown, and the long range NOE involving this residue is between the amide proton and the e protons of Phe81. a was created with Molscript, and b was created in RasMol2.6.

**Fig. 2. Solution structure of the E. coli F\(_{1}\)-ATPase e subunit.** a, the minimized average structure for the entire family of 30 refined structures. b, the interface between the N-terminal \( \beta \) domain and the very C-terminal \( \alpha \)-helix hairpin. Based on the NOE data, the interface is formed by 5 residues within the N-terminal domain (shown in green) and 7 residues from the second \( \alpha \)-helix (red). The side chain of Ile131 is not shown, and the long range NOE involving this residue is between the amide proton and the e protons of Phe81. a was created with Molscript, and b was created in RasMol2.6.
C-terminal domain by 30 long range NOEs involving the side chains of 12 residues, 5 from the N-terminal domain (Gly\textsuperscript{48}, Phe\textsuperscript{61}, Tyr\textsuperscript{63}, Gly\textsuperscript{86}, and Gln\textsuperscript{87}) and 7 from the C-terminal α-helix (Ala\textsuperscript{126}, Gln\textsuperscript{127}, Val\textsuperscript{130}, Ile\textsuperscript{131}, Leu\textsuperscript{133}, Thr\textsuperscript{134}, and Ala\textsuperscript{137}). This interface is shown in Fig. 2b. The two domains are held together entirely by hydrophobic contacts, as there is no indication of any hydrogen bonds, salt bridges, or other polar interactions.

Less well defined is the interaction between the two C-terminal α-helices themselves. Only a few (17) long range NOEs could be identified because of the overlap of chemical shift values for the interacting protons. These are between the side chains of residues 91, 92, 94, 95, 98, 102, and 105 of the first helix and residues 131, 128, 125, 121, and 114 of the second helix. As reported previously, the backbone amide protons in the two C-terminal α-helices are undetectable in D\textsubscript{2}O buffer because of rapid exchange with solvent D\textsubscript{2}O (9). However, amide protons of most of the residues in the α-helices can be detected in D\textsubscript{2}O at pH values lower than 7.4 (data not shown).

Backbone Dynamics of the ε Subunit—NMR allows examination of the dynamics of a protein and, in the case of ε, the flexibility of the two domains with respect to one another. A first indication that the N- and C-terminal domains of ε are in tight contact in the solution structure is the observation that there are at least 30 resolved long range NOEs between residues of the N- and C-terminal domains (see above). On average, these have the same intensity (not decreased) compared with other long range NOEs within the N-terminal domain (not shown). To examine the flexibility of the two domains with respect to one another in more detail, a backbone\textsuperscript{15}N{1H} NOE spectrum was recorded, and the backbone relaxation parameters were measured. A \textsuperscript{15}N{1H} NOE spectrum of the ε subunit is shown in Fig. 3. Despite the fact that some of the peaks are weakened because of the solvent exchange (the same ones that are weak in amide correlation spectra recorded at pH 7.4, see above), all of the peaks, including the ones of residues in the linker region and the interface, have a positive intensity, consistent with the ε subunit tumbling as one entity in solution. Fig. 4 shows a summary of the measured relaxation parameters for a total of 100 of the 138 residues. The average \( T_1 \) and \( T_2 \) times for the ε subunit backbone amides are 571 ± 27 ms (residues 3–136, excluding residues 14, 17, 39, 74, and 107 because of their large errors) and 65 ± 3 ms, respectively.

The measured \( T_1 \) and \( T_2 \) relaxation parameters shown in Fig. 4 provide no evidence of a flexible linker connecting the

![Fig. 3. \textsuperscript{15}N\textsuperscript{1}{H} NOE spectrum of the ε subunit. The heteronuclear \textsuperscript{15}N\textsuperscript{1}{H} NOE spectrum of the ε subunit was recorded with a solution of uniformly \textsuperscript{15}N-labeled protein at a concentration of 1 mM in 5 mM potassium phosphate buffer, pH 7.4, 3 mM NaN\textsubscript{3}, 5% D\textsubscript{2}O, and 5% D\textsubscript{6}-Me\textsubscript{2}SO at 18 °C. Some of the amide peaks are folded. Negative intensities are drawn in red.](image-url)
two domains in ε. Only Leu\textsuperscript{89} shows a somewhat larger than average $T_1$, which is not uncommon for a turn position. Moreover, residues involved in the interface between the two domains (Gly\textsuperscript{48}, Phe\textsuperscript{61}, Tyr\textsuperscript{63}, Gly\textsuperscript{86}, Gln\textsuperscript{87}, Ala\textsuperscript{126}, Gln\textsuperscript{127}, Val\textsuperscript{130}, Ile\textsuperscript{131}, Leu\textsuperscript{133}, Thr\textsuperscript{134}, and Ala\textsuperscript{137}) show none of the unusual relaxation behavior expected for residues undergoing conformational exchange. Thus, any such conformational change must be faster than $10^{-3}$–$10^{-4}$ s or slower than the $T_2$ relaxation time above.

The Conformation of the Isolated ε Subunit Corresponds to the Conformation of the Subunit When Bound to ECF\textsubscript{1} in the ADP State—Previous experiments from this laboratory showed that the ε subunit can adopt at least two conformations, when bound to ECF\textsubscript{1} (11) or in ECF\textsubscript{1}F\textsubscript{0} (12), depending on whether the enzyme is in the ATP form (e.g., ATP/EDTA or AMP-PNP•Mg\textsuperscript{2+}) or in the ADP state (ADP/Mg/P\textsubscript{i} present). For example, the rate with which trypsin cleaves the ε subunit is different whether ADP or ATP is in catalytic sites, with the ADP form of ε being more resistant to protease attack (11). This difference in rates of cleavage of ε under the two conditions is clearly evident in the experiment shown in Fig. 5. With ECF\textsubscript{1} in ATP (lanes 2–5), the first cleavage product observed runs above and subsequent products run below the unmodified ε subunit. In the presence of ADP (lanes 7–10), the rate of cleavage of ε is
much slower under otherwise identical proteolysis conditions (see also Ref. 11). Lanes 11–15 of Fig. 5 show the cleavage pattern of the isolated ε subunit in the presence of much higher levels of trypsin than used for ECF1. Similar to the ε subunit bound to ECF1 with ADP in catalytic sites, there is only very slow cleavage of the polypeptide. The various cleavage sites obtained after prolonged trypsin treatment of the isolated ε subunit were determined from mass spectrometric analysis. Peptides with molecular weights for residues 1–123, 1–100, 1–99, 1–98, and 1–93 were generated sequentially in time course experiments. These are the same cleavage products found by trypsin treatment of ε in ECF1 in the presence of either ATP or ADP. It is the rate of the cleavage, not the site of cleavage, which varies under different nucleotide conditions in ECF1. These sites are in both α-helices of the C-terminal domain. The different nucleotide conditions had no effect on cleavage rates in isolated ε subunit (results not shown).

Arrangement of the ε Subunit from Cross-linking Studies—The orientation of the N-terminal β sandwich domain in the ATP synthase complex is well defined based on a large number of chemical and photo-cross-linking results (20, 22, 24, 25). From these data, interfaces to the γ subunit of the F0 and to the ε subunit oligomer of the F0 could be identified, involving one face and the bottom of the β sandwich, respectively.

Several cross-linking studies have shown that there is binding of the ε subunit to α and β subunits and that this is through the C-terminal α-helix-loop-α-helix domain. For example, cross-linking can be induced between a β and ε subunit by the water-soluble carbodiimide EDC (26), a cross-link subsequently shown to involve Glu381 of β and one of three Ser residues (106–108) in ε (27). Cross-linking from α or β to ε can also be obtained by replacing Glu381 of β (24) or Ser411 of α (28) along with Ser108 of ε with Cys residues. Finally, a Cys replacing the C-terminal residue Met138 of ε can be cross-linked to a β subunit when modified with the photoactivatable cross-linker TFPAM-3 (20). As Ser108 and Met138 are at the opposite ends of the helix-loop-helix region of ε, it seems likely that this subunit spans two β subunits.

To test this possibility, cross-linking from the two sites was performed sequentially using ECF1 from the mutant εM138C. As shown in Fig. 6, EDC reaction, or photoactivation after TFPAM-3 modification of Cys138 alone, gave a major cross-linked product of M(r)app 70,000. In contrast, when both reactions were performed sequentially, a significant amount of cross-linked product of M(r)app 130,000 was produced. This large product must contain ε cross-linked to two β subunits, i.e. it is a β-ε-β adduct. The presence of ε and β in the 130,000 cross-link product was confirmed by monoclonal antibodies (results not shown).

In one set of experiments, ECF1 from the mutant εM138C was modified with NbfCl after the cross-linking reaction (29). No fluorescence because of bound NbfCl was observed in the β-ε cross-link generated with either EDC or via photo-cross-linking from the Cys at position 138. However,bound reagent was readily apparent in a noncross-linked β subunit (not shown).

**DISCUSSION**

The 2.8-Å crystal structure of a major part of the F1 from beef heart mitochondria has provided important insight into cooperative catalytic by ATP synthases (8). However, to fully understand energy coupling within this enzyme, it will almost certainly be necessary to obtain a high resolution structure of the entire complex. The lack of any high resolution structural data for the stalk region and membrane-bound F0 makes it particularly difficult to establish the molecular mechanism for the coupling of ATP hydrolysis to proton translocation.

We have been examining individual purified subunits of the stalk region to study their structure and interaction. Previously, we obtained structural models of the N- and C-terminal domains of the ε subunit from NMR spectroscopy (9). Here, we describe the solution structure of the entire polypeptide, which adds important information on the interaction sites between the two domains, and the dynamics of the molecule as a whole. The interaction between the two domains involves side chains from β strand 7 of the N-terminal domain and one face of the very C-terminal α-helix. No interactions were observed between the first C-terminal α-helix (residues 91–106) and the N-terminal domain. Recently, the crystal structure of the E. coli ε subunit has been determined at 2.3-Å resolution (10). This crystal structure confirmed our previously determined structures of the two domains and also showed a tight interaction of the N- and C-terminal domains mainly via hydrophobic residues. The tight interaction between the two domains of ε seen in the crystal structure determination could have been enforced by the crystal packing. The fact that the same interaction is now seen by NMR adds confidence to it being a preferred conformation of the polypeptide.

In conjunction with the NMR structure determination, we have been examining the interactions of the ε subunit with other subunits of ECF1 and ε subunits by cross-linking experiments. Here, we extend these studies and show that the C-terminal domain of the ε subunit binds in such a way as to span two β subunits, with the region around Ser108 interacting with one and Met138 being close to the other. The distance between Ser108 and Met138 from the structure shown in Fig. 2 is approximately 40 Å and, therefore, sufficient to interact with two β subunits at the same time.

It is clear from the x-ray structure of MF1 (8), as well as from biochemical studies, that the three β subunits are in different conformations that appear to be determined by nucleotide bind-
cysteine at position 108 in the \( \epsilon \) subunit cross-links preferentially to the \( \alpha \) subunit when going from the ADP to the ATP state, and a gold particle bound to the \( \epsilon \) subunit at position 38 can be seen superimposed on either an \( \alpha \) (ADP form) or a \( \beta \) subunit (ATP form). Taken together, our data suggest that the rotation of the \( \gamma \) domain in the intact F\(_{1}\)F\(_{0}\) ATP synthase requires conformational rearrangements of the \( \epsilon \) subunit in each step. The possibility that rotation is six-steped, rather than three-steped, warrants further study.

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REFERENCES

1. Capaldi, R. A., Aggeler, R., Turina, P., and Wilkens, S. (1994) Trends Biochem. Sci. 19, 284–289
2. Engelbrecht, S., and Junge, W. (1997) FEBS Lett. 41, 485–491
3. Senier, A. K. (1990) Annu. Rev. Biophys. 19, 7–41
4. Gogol, E. P., Lucken, U., and Capaldi, R. A. (1987) FEBS Lett. 219, 374–378
5. Wilkens, S., and Capaldi, R. A. (1995) Nature 373, 29
6. Ogilvie, I., Aggeler, R., and Capaldi, R. A. (1997) J. Biol. Chem. 272, 16552–16556
7. Wilkens, S., Dunn, S. D., Chandler, J., Dahlquist, F. W., and Capaldi, R. A. (1997) Nat. Struct. Biol. 4, 198–201
8. Abrahams, J. P., Leslie, A. G., Lutter, R., and Walker, J. E. (1994) Nature 370, 621–628
9. Wilkens, S., Dahlquist, F. W., McIntosh, L. P., Donaldson, L. W., and Capaldi, R. A. (1995) Nat. Struct. Biol. 2, 961–967
10. Uhl, C., Cox, G. B., and Guss, J. M. (1997) Structure 5, 1219–1230
11. Mendel-Hartvig, J., and Capaldi, R. A. (1991) Biochemistry 30, 1278–1284
12. Wilkens, S., and Capaldi, R. A. (1991) Biochemistry 30, 1998–2011
13. Gogol, E. P., Aggeler, R., Sagermann, M., and Capaldi, R. A. (1990) Biochemistry 29, 4717–4724
14. Patel, A. M., Dallmann, H. G., Skakono, E. N., Kapala, T. D., and Dunn, S. D. (1992) Mol. Microbiol. 6, 1941–1946
15. Brunger, A. (1993) X-FLOR, Version 3.1, Yale University Press, New Haven, CT
16. Kay, L. E., Torchia, D. A., and Bax, A. (1989) Biochemistry 28, 8972–8979
17. Clere, G. M., Driscoll, P. C., Wingfield, P. T., and Gorenstein, A. M. (1990) Biochemistry 29, 7387–7401
18. Barbato, G., Icura, M., Kay, L. E., Pastor, R. W., and Bax, A. (1992) Biochemistry 31, 5269–5278
19. Zhou, H., Lowry, D. L., Swanson, R. V., Simon, M. I., and Dahlquist, F. W. (1995) Biochemistry 34, 13858–13870
20. Tang, C., and Capaldi, R. A. (1996) J. Biol. Chem. 271, 3018–3024
21. Aggeler, R., Chicas-Cruz, K., Cai, X. S., Keana, J. F., and Capaldi, R. A. (1992) Biochemistry 31, 2956–2961
22. Aggeler, R., Weinreich, F., and Capaldi, R. A. (1995b) Biochim. Biophys. Acta 1230, 62–68
23. Aggeler, R., Mendel-Hartvig, J., and Capaldi, R. A. (1999) Biochemistry 38, 10387–10395
24. Aggeler, R., Haughton, M. A., and Capaldi, R. A. (1985) J. Biol. Chem. 260, 9185–9191
25. Zhang, Y., Oldenburg, M., and Fillingame, R. H. (1994) J. Biol. Chem. 269, 10221–10224
26. Lässcher, H. R., deJong, C., and Capaldi, R. A. (1984) Biochemistry 23, 4134–4140
27. Dallmann, H. G., Flynn, T. G., and Dunn, S. D. (1992) J. Biol. Chem. 267, 10953–10960
28. Aggeler, R., and Capaldi, R. A. (1996) J. Biol. Chem. 271, 13888–13891
29. Haughton, M. A., and Capaldi, R. A. (1996) Biochim. Biophys. Acta 1276, 164–169
30. Capaldi, R. A., Aggeler, R., Wilkens, S., and Gruber, G. (1996) J. Bioenerg. Biomembr. 28, 397–401
31. Gogol, E. P., Johnson, E., Aggeler, R., and Capaldi, R. A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5053–5059
32. Duncan, T. M., Bulygin, V. V., Zhou, Y., Hutcheon, M. L., and Cross, R. L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10964–10968
33. Sabbert, D., Engelbrecht, S., and Junge, W. (1996) Nature 381, 623–626
34. Noji, H., Yasuda, R., Yoshida, M., and Kinosita, K., Jr. (1997) Nature 386, 299–302
35. Aggeler, R., Ogilvie, I., and Capaldi, R. A. (1997) J. Biol. Chem. 272, 19621–19624
36. Watts, S. D., Tang, C., and Capaldi, R. A. (1996) J. Biol. Chem. 271, 28341–28347
37. Junge, W., Lill, H., and Engelbrecht, S. (1997) Trends Biochem. Sci. 22, 420–423
38. Wilkens, S., and Capaldi, R. A. (1994) Biol. Chem. Hoppe-Seyler 375, 43–51

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\(^2\) J. E. Walker, personal communication.