Fluidity of Structure and Swiveling of Helices in the Subunit c Ring of Escherichia coli ATP Synthase as Revealed by Cysteine–Cysteine Cross-Linking

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Subunit c in the membrane-traversing F0 sector of Escherichia coli ATP synthase is known to fold with two transmembrane helices and form an oligomeric ring of 10 or more subunits in the membrane. Models for the E. coli ring structure have been proposed based upon NMR solution structures and intersubunit cross-linking of Cys residues in the membrane. The E. coli models differ from the recent x-ray diffraction structure of the isolated Ilyobacter tartaricus c-ring. Furthermore, key cross-linking results supporting the E. coli model prove to be incompatible with the I. tartaricus structure. To test the applicability of the I. tartaricus model to the E. coli c-ring, we compared the cross-linking of a pair of doubly Cys substituted c-subunits, each of which was compatible with one model but not the other. The key finding of this study is that both A21C/M65C and A21C/166C doubly substituted c-subunits form high yield oligomeric structures, ε2, ε3 . . . ε10, via intersubunit disulfide bond formation. The results indicate that helical swiveling, with resultant interconversion of the two conformers predicted by the E. coli and I. tartaricus models, must be occurring over the time course of the cross-linking experiment. In the additional experiments reported here, we tried to ascertain the preferred conformation in the membrane to help define the most likely structural model. We conclude that both structures must be able to form in the membrane, but that the helical swiveling that promotes their interconversion may not be necessary during rotary function.

The F1F0-ATP synthases of oxidative phosphorylation utilize the energy of a transmembrane electrochemical gradient of H+ or Na+ to mechanically drive the synthesis of ATP via two coupled rotary motors in the F1 and F0 sectors of the enzyme (1–3). In the intact enzyme, ATP synthesis or hydrolysis takes place in the F1 sector at the surface of the membrane, with synthesis being coupled to H+ or Na+ transport through the transmembrane F0 sector. Homologous enzymes are found in mitochondria, chloroplasts, and many bacteria (4). In Escherichia coli and other eubacteria, F1 consists of five subunits in an αβγδε1 stoichiometry (4). F0 is composed of three subunits in a likely ratio of a,b,c10 in E. coli and Bacillus PS3 or a,b,c11 in the Na+-translocating Ilyobacter tartaricus ATP synthase (3, 5–7). A 3.9-Å resolution crystal structure of a yeast mitochondrial F1-c10 depicts 10 c-subunits arranged in a ring-like structure (8). Other bacterial c-rings may have as many as 15 c-subunits (9). Subunit c spans the membrane as a hairpin of two transmembrane helices (TMHs),2 with the first TMH on the inside and the second TMH on the periphery of the c-ring (7, 10, 11).

Helical hairpin structures resembling those predicted for the membrane have been solved by NMR using chloroform/methanol solvent mixtures (12–14). A high resolution x-ray structure of the I. tartaricus c11-ring, which differs significantly from the NMR structures, has revealed the Na+-binding site at the periphery of the ring, with the chelating groups to the Na+ ion extending from two interacting subunits (7). In the complete membranous enzyme, H+ or Na+ transport through F0 is proposed to drive c-ring rotation and the coupled rotation of the y-subunit within the αβγδε1-hexamer of F1, leading to synthesis and release of ATP (1–3). In the H+-transporting E. coli enzyme, Asp64 at the center of cTMH2 is thought to undergo protonation and deprotonation as each subunit of the c-ring moves past a stationary a subunit. Subunit a is thought to provide access channels to the H+-binding Asp64 residue, and candidate residues lining possible aqueous access pathways were tentatively identified (15–18).

The model proposed by Dmitriev et al. (11) for the E. coli c-ring was based upon the initial NMR structure at pH 5 (12) and the cross-linking of Cys residues introduced into the protein in native membranes (10). In the E. coli model (11), cTMH1 on the inside of the ring is positioned as it is in the I. tartaricus x-ray structure (7). High yield disulfide cross-links are formed between Cys residues introduced at position 30 (10). Cys30/33 formation does not significantly reduce H+-pumping activity, indicating that this region of the protein could remain relatively immobile during function (5). The E. coli model differs most significantly from the I. tartaricus x-ray structure in the positioning of cTMH2 relative to cTMH1. In the E. coli model, Asp64 packs between the helices of interacting subunits and projects toward cTMH1 at the interior of the ring (Figs. 1A and 2A). In the I. tartaricus structure, Glu65

2 The abbreviations used are: TMHs, transmembrane helices; CuP, Cu2+ (phenanthroline)2; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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The prediction that the 21/66 Cys/Cys pair should form multimeric ladders if the *I. tartaricus* structure is applicable to the *E. coli* c-ring led us to the experiments described in this study. After constructing the 21/66 Cys mutant, we compared Cu\(^{2+}\)-catalyzed cross-linking with that in the 21/65 Cys mutant and were surprised that multimeric ladders were formed in both double mutants. These findings immediately suggested that helical swiveling with resultant interconversion of the two conformers could be occurring over the time course of the experiment. Previously, the possibility of rare helical swiveling was suggested as an explanation for formation of low yield dimers when single Cys residues were introduced in the region of residues 62–66 near the essential Asp\(^{61}\) residue (10). In the additional experiments reported here, we tried to ascertain the preferred conformation in the membrane to help define the most likely structural model. We conclude that both structures must be able to form in the membrane, but also that the helical swiveling promoting the interconversion of structures may not be a necessary feature of rotary function.

EXPERIMENTAL PROCEDURES

Mutant Plasmid Construction—The Cys substitutions were generated in plasmid pCMA113 (16). Plasmid pCMA113 plasmid encodes the *unc (atp)* operon and additionally encodes a C-terminal His\(_6\) tag on subunit *a*, a C21S substitution in subunit *b*, and Ala substitutions for the remaining endogenous Cys residues in *F*\(_1\) (20). Cys substitutions were created by a two-step PCR method of site-directed mutagenesis using a synthetic oligonucleotide with the codon change and two wild-type primers encoding known restriction sites (21). Mutant PCR products were ligated directly into plasmids pCMA113 and plasmid pPRS081 (pCMA113 with a deletion of the *uncB* gene encoding subunit *a*). Plasmid pPRS081 was generated by cloning the HindIII/BsrGI DNA fragment containing the *uncB* deletion from plasmid pVF208 (22) into the equivalent restriction sites in plasmid pCMA113. All experiments were carried out with plasmid transformants of strain JWP292, which carries a chromosomal deletion of the *unc* operon (5).

Copper-catalyzed Cross-linking—Cells were grown and membrane vesicles were prepared as described (23). Membranes were centrifuged and resuspended in TMG buffer (50 mM Tris–HCl, 5 mM MgCl\(_2\), and 10% (v/v) glycerol) at pH 6.5, 7.0, 7.5, 8.0, and 8.5 at a concentration of 5 mg/ml, and 45-μl aliquots were mixed with 5 μl of 15 mM CuSO\(_4\) and 45

Cys/Cys pairs in the two models are shown in Fig. 1 (A and B). In the *E. coli* model (Fig. 1A), these Cys/Cys pairs are positioned such that intersubunit cross-links could form. After rotation of cTMH2 by 160° to a position equivalent to that seen in the *I. tartaricus* model (Fig. 1B), both the 20/66 and 21/65 Cys/Cys pairs are positioned such that intrasubunit (but not intersubunit) cross-links could form. The 14/72 intermolecular cross-link could also form from the positions shown in Fig. 1A, but not in the model shown in Fig. 1B. On the other hand, after a 160° clockwise rotation of TMH2, one might expect to see intersubunit cross-links with the 21/66 Cys/Cys pair (Fig. 1B).

FIGURE 1. Positions of residues subject to intersubunit cross-linking for A21C/M65C, A20C/I66C, and A21C/I66C substituted subunit *c* in the *E. coli* versus *I. tartaricus* models. The helical wheels are based upon the pH 5 NMR structure of *E. coli* subunit *c* and depicted by Jones et al. (10). A, shown is the juxtaposition of helices as seen in the pH 5 NMR structure. In this conformation, A21C/M65C and A20C/I66C are positioned such that intermolecular cross-links could form between subunits (blue arrows). The essential Asp\(^{61}\) residue points toward TMH1 and away from the membrane bilayer and site of interaction with subunit *a*, *b*, in the conformation shown, TMH2 in the pH 5 structure was rotated clockwise by 160° as viewed here from the cytoplasm. In this position, Asp\(^{61}\) points toward the periphery of *I. tartaricus* would look like if equivalent residues were overlaid on the structure, Meier et al. (7) have modeled what the *E. coli* versus *I. tartaricus* models. The helical wheels are based upon the pH 5 NMR structure of *E. coli* subunit *c* (10). B, a C21S substitution in subunit *c* (11), cTMH2 would have to be rotated by ~160° to place Asp\(^{61}\) in the equivalent position (Figs. 1B and 2C). Upon comparison of the two models shown in Fig. 1 (A and B), we immediately realized that the *I. tartaricus*-based model with TMH2 rotated by 160° was incompatible with key cross-linking constraints that had been used to derive the original *E. coli* model (11). Four sets of double Cys substitutions could be cross-linked into multimeric *c*-subunit “ladders” on an SDS-polyacrylamide gel that extended up to *c*\(_{10}\)-oligomers (10). The multimeric ladders formed with the 14/72, 20/66, and 21/65 Cys/Cys pairs could not be explained by models mimicking the *I. tartaricus* structure, whereas the multimeric ladder seen with the 70/72 Cys/Cys pair could be explained by either model. The positions of the 20/66 and 21/65 Cys/Cys pairs in the two models are shown in Fig. 1 (A and B). In the *E. coli* model (Fig. 1A), these Cys/Cys pairs are positioned such that intersubunit cross-links could form. After rotation of cTMH2 by 160° to a position equivalent to that seen in the *I. tartaricus* model (Fig. 1B), both the 20/66 and 21/65 Cys/Cys pairs are positioned such that intrasubunit (but not intersubunit) cross-links could form. The 14/72 intermolecular cross-link could also form from the positions shown in Fig. 1A, but not in the model shown in Fig. 1B. On the other hand, after a 160° clockwise rotation of TMH2, one might expect to see intersubunit cross-links with the 21/66 Cys/Cys pair (Fig. 1B).
mM o-phenanthroline (Cu$^{2+}$ (phenanthroline)$_2$ (CuP)) stock solution in TMG buffer (at the appropriate pH) to initiate the reaction. Cross-linking reactions were incubated for variable times at variable temperatures. All reactions were stopped by the addition of 0.1 volume of 0.5M EDTA (pH 8.0) and 0.1 volume 0.5M N-ethylmaleimide in ethanol and incubated at room temperature for 30 min. The sample was then mixed with an equal volume of 2× SDS sample buffer (100 mM Tris-HCl (pH 8.0), 8% SDS, 24% glycerol, and 0.04% bromphenol blue) at room temperature for 1 h prior to SDS-PAGE.

**SDS Electrophoresis and Immunoblotting**—Each sample was run via SDS-PAGE using the Tricine-buffered gel system described by Schägger and von Jagow (24) with 12% acrylamide and 0.375% bisacrylamide. Following SDS-PAGE, proteins were transferred to a polyvinylidene difluoride membrane. The polyvinylidene difluoride membrane and transferred proteins were then blocked with 5% (w/v) blocking agent (GE Healthcare) in PBS-T (0.8% (w/v) NaCl, 6.5 mM Na$_2$HPO$_4$, 1.5 mM NaH$_2$PO$_4$, and 0.1% Tween 20). Immunoblotting was carried out in a 1:40,000 dilution of rabbit anti-subunit c serum (25) in PBS-T containing 2% (w/v) bovine serum albumin with a 1-h incubation at room temperature. Following washing with PBS-T, the membrane was incubated in a 1:40,000 dilution of anti-rabbit IgG coupled to horseradish peroxidase (GE Healthcare) in PBS-T for 1 h at room temperature. Following washing, each immunoblot was covered with West Pico chemiluminescence solution (Pierce) for 5 min and then exposed to HyBlot autoradiography film (Denville Scientific Inc., Metuchen, NJ) for protein visualization.

**RESULTS**

**Comparison of Cross-link Formation in 21/65 Versus 21/66 Double Cys Mutants**—Membranes from the 21/65 and 21/66 double Cys mutants were treated with CuP to catalyze oxidative formation of Cys–Cys disulfide bonds. Subunit c and its cross-linked products were detected by Western blotting (Fig. 3). Prominent oligomeric subunit c products were observed in both the 21/65 and the 21/66 double Cys mutants. Oligomers of the two mutants were found to migrate at slightly different rates on SDS-polyacrylamide gel. Oligomers were absent in membranes that were not treated with CuP and also in wild-type membranes lacking Cys in subunit c. A prominent immunoreactive band of unknown identity had been reported previously (10). Distinct multimeric products ($c_2$, $c_3$, ..., $c_{10}$) maximizing at $c_{10}$ were clearly observed with the Cys$^{21}$/Cys$^{66}$ membranes, as had been reported previously (5). The bands seen in the Cys$^{21}$/Cys$^{66}$ membranes were always more diffuse, suggesting cross-linking heterogeneity. This heterogeneity was most apparent upon examination of the $c_2$ and $c_4$ products. We think that the heterogeneity is most likely due to dimer formation between Cys$^{66}$ in two different subunits in addition to Cys$^{21}$/Cys$^{66}$ dimer and oligomer formation. As we have reported previously (10), mutant proteins with single Cys...
residues substituted into residues 62–66 of cTMH2 do form low yield dimers, with the yield of dimer being much more prominent for the Cys66 mutant versus the Cys65 mutant. The lower yield of the Cys65 dimer may explain the apparent absence of band heterogeneity in the 21/65 double Cys mutant. Finally, the oligomers formed with the 21/66 membranes do not maximize as cleanly at c10 and may suggest minor amounts of c11 and perhaps c12 products.

Effects of Time, Temperature, and pH on Cross-link Formation—The cross-link formation seen in Fig. 3 occurred in reactions conducted over 60 min at room temperature at pH 8.0. We attempted to distinguish whether one type of cross-link occurred more rapidly than the other by varying the length of the reaction from 1 to 60 min (Fig. 4). The 21/66 mutant formed greater amounts of c4 product at the 5- and 10-min intervals, and the intensity of oligomeric products >c5 was also greater in this mutant at the 30- and 60-min intervals. However, the most obvious conclusion from the experiment is that cross-linking is generally slow in both membrane types. Cross-linking with both membrane types showed a strong temperature dependence, with virtually no cross-linking occurring at 0 °C over a period of 60 min (Fig. 5). Nearly equivalent amounts of oligomeric products ≈c5 were observed in the two membrane types at 16 °C. As the temperature was raised further, the 21/66 membranes showed greater formation of oligomeric products >c5. Cross-linking in both mutant membranes increased at alkaline pH, as would be expected if the Cys thiolate were the reactive species (Fig. 6). Virtually no cross-linking was observed at pH ≈7.0. The increase in extent of cross-linking seen between pH 8.0 and 8.5 was considerably greater for 21/65 than for 21/66 membranes and may indicate a difference in pKaq for Cys65 versus Cys66.

Cross-linking Is Enhanced by Removal of Subunit a—The structure of c-subunits packed next to subunit a in the membrane may differ from those elsewhere in the ring, and conceivably, the interaction with subunit a could affect the structures
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**DISCUSSION**

The key finding in this study is that Cys$^{21}$/Cys$^{65}$ and Cys$^{21}$/Cys$^{66}$ substituted $c$-subunits both form oligomeric ladders via CuP-catalyzed disulfide bond formation. Multimeric subunit $c$ ladder formation with the Cys$^{21}$/Cys$^{65}$ mutant is incompatible with a model of the c-ring based upon the *I. tartaricus* x-ray structure (7), and ladder formation with the Cys$^{21}$/Cys$^{66}$ mutant is incompatible with the c-ring model based upon the NMR structure of subunit $c$ at pH 5.5 and other cross-linking data (11). Other models that would accommodate cross-linking of both Cys$^{21}$/Cys$^{65}$ and Cys$^{21}$/Cys$^{66}$ from a single structure, e.g. if TMH2 was rotated counterclockwise by 60° in Fig. 1A, would be incompatible with other cross-links observed by Jones et al. (10). We believe that the most likely explanation for the apparently conflicting observations in the literature (7, 10, 11, 13) is that the c-ring structure is inherently flexible, at least in *E. coli*, and that thermally induced helical movement and swiveling can take place in the membrane. Such movements may be relatively slow and normally may be rapidly reversed to the preferred structure. The observed cross-links may have trapped the swiveled helices in conformations that are inherently improbable. The distances between Cys $\beta$-carbons in the two modeled structures are 5.8 Å for Cys$^{21}$/Cys$^{65}$ (Fig. 1A) and 6.0 Å for Cys$^{21}$/Cys$^{66}$ (Fig. 1B). The average distance between $\beta$-carbons of disulfide-linked Cys--Cys residues in proteins is ~4 Å (26), and some motion is therefore likely necessary to facilitate cross-link formation. The cross-linking observed here did require relatively long periods of incubation and was markedly inhibited by reductions in temperature. The removal of subunit $a$ led to increased rates of cross-linking, indicating that the c-ring might be stabilized while complexed with subunit $a$, with a resultant decrease in helical swiveling. None of these factors clearly distinguished the Cys$^{21}$--Cys$^{65}$ versus Cys$^{21}$--Cys$^{66}$ cross-linking reactions. The inherent flexibility of c-ring structure and slowness of the cross-linking reactions preclude use of this type of approach in distinguishing mechanistic models.

The molecular dynamics calculations of Aksimentiev et al. (27) with a modeled *E. coli* c-ring indicate that TMH2 can freely swivel at the TMH1 interface to move the Asp$^{61}$ carboxyl group from the position shown in Fig. 1A to that shown in Fig. 1B. The NMR studies of Rastogi and Girvin (13) indicated that subunit $c$ adopts two stable conformations in solution depending upon the pH. At pH 5, the structure is as shown in Figs. 1A and 2A. At pH 8, TMH2 rotates 140° clockwise to a position similar to that shown in Figs. 1B and 2B and C. In the experiments in which we measured the effects of pH on cross-linking, we hypothesized that the more alkaline pH values might favor the Cys$^{21}$--Cys$^{66}$ cross-linking reaction because TMH2 is predicted to be turned as shown in Fig. 1B when Asp$^{61}$ is ionized. However, in contrast to this prediction, the more alkaline incubation conditions at pH 8.5 enhanced Cys$^{21}$--Cys$^{65}$ cross-link formation.

Upon comparison of the pH 5 and 8 NMR structures, major differences are seen in the short polar loop connecting TMH1 and TMH2 (13). Similarly, the major differences of all c-subunits in the ring in a cooperative manner. These packing interactions might also reduce helical swiveling within subunit c. To test the effects of subunit a on the cross-linking reactions, we generated the 21/65 and 21/66 substitutions in a plasmid in which the uncB gene encoding subunit a was deleted. Comparisons of cross-linking in membranes containing or lacking subunit a are shown in Fig. 7. Deletion of subunit a led to significant increases in the extent of cross-linking during a 30-min incubation for both the 21/65 and 21/66 membranes.
between the pH 5 structure (Fig. 2A) and the modeled E. coli WHATIF structure (Fig. 2B) are in the loop region. When the dihedral angles for loop residues 37–49 are replaced with the equivalent dihedral angles from the I. tartaricus structure (Fig. 2D), a swiveling of helices and repositioning of residues occur to very closely mimic the structures shown in Fig. 2 (B and C). This exercise suggests that helical swiveling in the membrane may be mediated by structural changes in the loop residues.

Does helical swiveling between the two conformations displayed in Fig. 1 play a functional role in the mechanical rotation of the c-ring, as suggested previously by our models (27–29) and those of Rastogi and Girvin (13)? These models now seem less likely, particularly if the swiveling is caused by major structural changes in loop residues. Mutations in loop residues are known to weaken the binding and coupling of F1 to F0 (30–34), and the sites of binding of γ- and ε-subunits to loop residues of the c-ring have been mapped by Cys–Cys cross-linking (35–38). The γ-subunit can be permanently cross-linked to subunit c and the enzyme retain nearly normal ATP-driven H+ translocation function (2, 39, 40), so the γε-complex need not be displaced from the c-ring surface during multiple 360° c-ring rotations. Major changes in the loop region such as those observed in the pH 5 and 8 structures by Rastogi and Girvin (13) would be expected to alter γ binding and possibly displace F1 from F0. We think that the helical swiveling observed here could be taking place predominantly at subunits of the c-ring that are not binding γε or possibly occur during the periodic dissociation of F1 from F0 under these dilute solution conditions.

The I. tartaricus c-ring structure is proposed to be very stable because of the liganding of Na+ between residues of two interacting subunits (3, 7, 41). For example, the c11-ring is stable in Tris-buffered octyl glucoside solution at 95 °C in the presence of Na+, but dissociates into monomeric subunits in the absence of Na+ (41). It will be of interest to know whether the I. tartaricus ring is more resistant to helical swiveling than its E. coli counterpart and whether swiveling is inhibited by the binding of Na+. This can be tested by cross-linking experiments similar to those reported here.

At present, the I. tartaricus c-ring structure, with the helices of all subunits positioned as shown in Fig. 1B, does provide structural explanations for several experimental observations made with E. coli F0. Using Cys–Cys cross-linking, Jiang and Fillingame (42) identified seven residues on one helical face of cTMH2 that can be cross-linked to Cys substituted into TMH4 of subunit a. The side chains of the seven membrane-spanning residues are positioned on the exposed, outer helical face of cTMH2 in the WHATIF model of the E. coli subunit c (Fig. 2B) (7). The WHATIF model also provides a facile structural explanation for the function of the Asp interchange mutants, in which the essential aspartate was moved from position 61 in cTMH2 to position 24 in cTMH1 with retention of function (43, 44). As shown in Fig. 2 (B–D), the wild-type Ala24 side chain packs at a position where an Asp substitution would place the essential carboxyl in essentially the same position between the helices as when anchored at Asp61.

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**REFERENCES**

1. Yoshida, M., Muneyuki, E., and Hisabori, T. (2001) Nat. Rev. Mol. Cell Biol. 2, 669–677
2. Capaldi, R. A., and Aaggeler, R. (2002) Trends Biochem. Sci. 27, 154–160
3. Dimroth, P., von Ballmoos, C., and Meier, T. (2006) EMBO Rep. 7, 276–282
4. Senior, A. E. (1988) Physiol. Rev. 68, 177–231
5. Jiang, W., Hermolin, J., and Fillingame, R. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 4966–4971
6. Mitome, N., Suzuki, T., Hayashi, S., and Yoshida, M. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 12159–12164
7. Meier, T., Polzer, P., Diedrichs, K., Welte, W., and Dimroth, P. (2005) Science 308, 659–662
8. Stock, D., Leslie, A. G., and Walker, J. E. (1999) Science 286, 1700–1705
9. Pogoryelov, D., Yu, J., Meier, T., Vonck, J., Dimroth, P., and Muller, D. J. (2005) EMBO Rep. 6, 1040–1044
10. Jones, P. C., Jiang, W., and Fillingame, R. H. (1998) J. Biol. Chem. 273, 17178–17185
11. Dmitriev, O. Y., Jones, P. C., and Fillingame, R. H. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 7785–7790
12. Girvin, M. E., Rastogi, V. K., Abildgaard, F., Markley, J. L., and Fillingame, R. H. (1998) Biochemistry 37, 8817–8824
13. Rastogi, V. K., and Girvin, M. E. (1999) Nature 402, 263–268
14. Nakano, T., Ikegami, T., Suzuki, T., Yoshida, M., and Akutsu, H. (2006) J. Mol. Biol. 358, 132–144
15. Cain, B. D. (2000) J. Bioenerg. Biomembr. 32, 365–371
16. Angervle, C. M., and Fillingame, R. H. (2003) J. Biol. Chem. 278, 6068–6074
17. Angervle, C. M., Herold, K. A., and Fillingame, R. H. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 13179–13183
18. Angervle, C. M., Herold, K. A., Vincent, O. D., and Fillingame, R. H. (2007) J. Biol. Chem. 282, 9001–9007
19. Friend, G. (1990) J. Mol. Graph. 8, 52–56
20. Kuo, P. H., Ketchum, C. J., and Nakamoto, R. K. (1998) FERS Lett. 426, 217–220
21. Barik, S. (1996) Methods Mol. Biol. 68, 203–215
22. Valiavettil, F. I., and Fillingame, R. H. (1997) J. Biol. Chem. 272, 32635–32641
23. Schwem, B. E., and Fillingame, R. H. (2006) J. Biol. Chem. 281, 37861–37867
24. Schägger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368–379
25. Hermolin, J., and Fillingame, R. H. (1995) J. Biol. Chem. 270, 2813–2817
26. Richardson, J. S., and Richardson, D. C. (1989) in Prediction of Protein Structure and the Principles of Protein Conformation (Fasman, G., ed) pp. 1–98. Plenum Publishing Corp., New York
27. Aksimentiev, A., Balabin, I. A., Fillingame, R. H., and Schulten, K. (2004) Biochim. Biophys. Acta 13179–13183
28. Fillingame, R. H., Jiang, W., and Dmitriev, O. Y. (2000) J. Biol. Chem. 275, 9–17
29. Fillingame, R. H., Angervle, C. M., and Dmitriev, O. Y. (2002) Biochim. Biophys. Acta 1555, 29–36
30. Mosher, M. E., White, L. K., Hermolin, J., and Fillingame, R. H. (1985) J. Biol. Chem. 260, 4807–4814
31. Miller, M. J., Fraga, D., Paule, C. R., and Fillingame, R. H. (1989) J. Biol. Chem. 264, 305–311
32. Fraga, D., and Fillingame, R. H. (1989) J. Biol. Chem. 264, 6797–6803
33. Fraga, D., Hermolin, J., Oldenburg, M., Miller, M., and Fillingame, R. H. (1994) J. Biol. Chem. 269, 7532–7537
34. Zhang, Y., Oldenburg, M., and Fillingame, R. H. (1994) J. Biol. Chem. 269, 10221–10224
35. Zhang, Y., and Fillingame, R. H. (1995) J. Biol. Chem. 270, 24609–24614
36. Watts, S. D., Zhang, Y., Fillingame, R. H., and Capaldi, R. A. (1995) FEBS Lett. 368, 235–238
37. Hermolin, J., Dmitriev, O. Y., Zhang, Y., and Fillingame, R. H. (1999) J. Biol. Chem. 274, 17011–17016
38. Watts, S. D., Tang, C., and Capaldi, R. A. (1996) J. Biol. Chem. 271, 28341–28347
39. Schulenberg, B., Aggeler, R., Murray, J., and Capaldi, R. A. (1999) J. Biol. Chem. 274, 34233–34237
40. Tsunoda, S. P., Aggeler, R., Yoshida, M., and Capaldi, R. A. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 898–902
41. Meier, T., and Dimroth, P. (2002) EMBO Rep. 3, 1094–1098
42. Jiang, W., and Fillingame, R. H. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6607–6612
43. Miller, M. J., Oldenburg, M., and Fillingame, R. H. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4900–4904
44. Zhang, Y., and Fillingame, R. H. (1994) J. Biol. Chem. 269, 5473–5479