The 20 C-terminal Amino Acid Residues of the Chloroplast ATP Synthase γ Subunit Are Not Essential for Activity*  

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It has been suggested that the last seven to nine amino acid residues at the C terminus of the γ subunit of the ATP synthase act as a spindle for rotation of the γ subunit with respect to the αβ subunits during catalysis (Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994) Nature 370, 621–628). To test this hypothesis we selectively deleted C-terminal residues from the chloroplast γ subunit, two at a time starting at the sixth residue from the end and finishing at the 20th residue from the end. The mutant γ genes were overexpressed in Escherichia coli and assembled with a native αβγ complex. All the mutant forms of γ assembled as effectively as the wild-type γ. Deletion of the terminal 6 residues of γ resulted in a significant increase (>50%) in the Ca-dependent ATPase activity when compared with the wild-type assembly. The increased activity persisted even after deletion of the C-terminal 14 residues, well beyond the seven residues proposed to form the spindle. Further deletions resulted in a decreased activity to ~19% of that of the wild-type enzyme after deleting all 20 C-terminal residues. The results indicate that the tip of the γ C terminus is not essential for catalysis and raise questions about the role of the C terminus as a spindle for rotation.

The ATP synthase enzymes of the inner membranes of mitochondria, chloroplasts and of the bacterial cytoplasmic membrane, couple the energy of a transmembrane electrochemical proton gradient to the synthesis of ATP from ADP and inorganic phosphate. The general structural features of the enzyme are highly conserved from one organism to another. It is comprised of an integral membrane-spanning H₁-translocating segment (F₀ or factor O) and a peripheral membrane segment (F₁ or factor 1) which contains the catalytic sites for ATP synthesis and hydrolysis. The F₁ subunit is comprised of five different polypeptide subunits designated α to ε in order of decreasing molecular weight. The subunit stoichiometry is α₃β₂γδε, and ε. Nucleotide binding is associated with the α and β subunits, whereas the γ and ε subunits play regulatory and/or structural roles. The δ subunit is likely to be involved in binding the F₁ segment to the F₀ segment (reviewed in Ref. 1).

A high resolution crystal structure of the core catalytic portion of the mitochondrial F₁ enzyme was reported recently (2). The α and β subunits alternate with each other to form a hexameric ring with one nucleotide binding site located at each of the six αβ subunit interfaces. Part of the structure of the γ subunit was also solved, including well conserved regions of the N and C termini. The C terminus, from residues 209 to 272 forms a single α helix that stretches from below the base to the top of the αβ hexamer (see Fig. 5). The last nine residues of this remarkably long helix are predominantly hydrophobic in nature and pass through a greasy sleeve formed by a ring of hydrophobic residues provided by interacting N-terminal β barrel domains of all six of the α and β subunits. On the basis of this unusual asymmetric structure, it was suggested that the C-terminal helix of the γ subunit forms a spindle around which the αβ hexamer rotates, rotation being facilitated by the hydrophobic (greasy) nature of the amino acids involved. That is, the αβ subunits provide a bearing through which the tip of the γ subunit passes and within which the γ subunit rotates. Although the amino acid sequences of γ subunits from different organisms show little overall homology, segments near the N and C termini are quite well conserved suggesting that they may be involved in forming important contacts with other F₁ subunits (3, 4).

The crystal structure of F₁ indicated that the three αβ pairs of the αβ hexamer also make direct contact with other regions of the γ subunit to induce different conformational states of the nucleotide binding sites at the αβ subunit interfaces. During rotation, each nucleotide binding site would sequentially alternate between three different conformational states, each state dictated by a different type of interaction with the γ subunit. Such rotation has been predicted from kinetic studies (5, 6), has been supported by several recent experiments (7–9), and is now widely considered to be a general mechanistic feature of all of the F₁ enzymes (reviewed in Ref. 10).

In this study we have tested the “bearing” hypothesis specifically suggested by the crystal structure, by selectively deleting amino acid residues from the extreme C-terminal end of the γ subunit, which, in the mitochondrial enzyme, extends through the greasy sleeve of the αβ hexamer. To do this we utilized an efficient reconstitution system reported earlier (11) in which the native γ subunit isolated from CF₁,1 was reconstituted with an isolated αβ subunit complex. The cloned γ subunit could effectively replace the native γ subunit in reconstitution of the core enzyme complex.2 Eight genetically engineered γ subunits, lacking between 6 and 20 of the C-terminal amino acids, were tested for assembly with the αβ subunits, and the catalytic activities of the assembled complexes were examined. The results

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1 The abbreviations used are: CF₁, chloroplast coupling factor 1; CF₁(–δε), CF₁ deficient in the δ and ε subunits; PCR, polymerase chain reaction; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; WT, wild type.
2 M. Sokolov, L. Lu, W. Tucker, F. Gao, P. A. Gegenheimer, and M. L. Richter, manuscript in preparation.
sults demonstrate that the tip of the C terminus of the γ subunit, from residues 304 to 323 (chloroplast numbering), is not essential for rapid turnover by CF1 subunit. The isolated γ subunit was stored in the isolation buffer at 4 °C. An ab subunit, from residues 304 to 323 (chloroplast numbering), is not essential for rapid turnover by CF1 complex. Results demonstrated that the tip of the C terminus of the γ subunit, from residues 304 to 323 (chloroplast numbering), is not essential for rapid turnover by CF1.

**EXPERIMENTAL PROCEDURES**

**Materials—**CF1 and CF2, lacking the δ and ε subunits, CF1(−δε) were prepared from fresh market spinach as described previously (12) and stored as ammonium sulfate precipitates. Prior to use the proteins were desalted on Sephadex G-50 centrifuge columns (13). The isolated γ subunit (14) was stored in the isolation buffer at 4 °C. An ab complex and the γ subunit were isolated from CF1(−δε) as described previously (11). The ab γ subunit complex was recycled through the isolation procedure to ensure that trace amounts of contaminating γ subunit were removed.

ATP (grade I and II) and antibiotics (ampicillin, chloramphenicol, and tetracyclin) were purchased from Sigma. Stock solutions of tetracytin were prepared by dissolving the inhibitor in ethanol to a final concentration of 5 mM and stored at −70 °C. Pfu DNA polymerase and its reaction buffer were purchased from Strategene. T4 DNA ligase and its reaction buffer were obtained from Promega. DNAse I was from Roche Molecular Biochemicals. Trypsin and yeast extract were obtained from Difco. Urea (ultra pure) was purchased from Fluka and hydroxylapatite from Bio-Rad. All other chemicals were of the highest quality reagent grade available.

**Plasmid Construction—**Most of the recombinant DNA methods used in this study have been described elsewhere (15, 16). Escherichia coli transformation protocols were as described by Hanahan (17). Plasmid pSG101 (4), generously supplied by Dr. M. Futai, contains the full-length cDNA for the spinach (Spinacia oleracea) chloroplast atpC gene encoding the ATP synthase γ subunit. A 1.1-kilobase pair Bsal-BamHI fragment of pSG101 was cloned into the Ncol and BamHI cleaved expression vector pET16b (18) via an Ncol-Bsal adaptor. The resulting plasmid pET8cgam bb1 was transformed into the expression host E. coli BL21(DE3)pLysS (19). Plasmid DNA for sequencing was prepared by alkaline-SDS lysis and polyethylene glycol precipitation (20).

**Generation of atpC Gene Mutants—**Eight deletion mutants of γ were generated by “inverse” PCR with a forward primer that was complementary to the terminus of the native γ subunit and the downstream sequence of the pET8gam bb1 plasmid. The reverse primer was complementary to the required C-terminal amino acid and its adjacent upstream sequence. PCR primers were 24–31 base pairs long and were 5′-phosphorylated. Oligonucleotides were synthesized by Macromolecular Resources, Colorado State University. Plasmid DNA for PCR was prepared by ethanol precipitation after phenol-chloroform extraction (17). PCR was carried out in 50 μl of cloned Pfu DNA polymerase reaction buffer, which also contained 60 ng of the pET8gam bb1 plasmid, 4 mM total MgSO4, 22 pmol of each primer, 0.4 mM dNTPs, and 2.5 units of cloned Pfu DNA polymerase. The components were mixed on ice and placed in a GenAmp PCR System 2400 (Perkin-Elmer) prewarmed to 94 °C. Cycling parameters were: 94 °C for 1 min, 56 °C for 1 min, 72 °C for 12 min, for 20 cycles. The PCR product was purified by agarose gel electrophoresis followed by electroelution into an ISCO micro-trap. The eluted DNA was precipitated with ethanol and circularized (14). For this 100–200 ng of the DNA was incubated with 3 units of T4 DNA ligase in the T4 DNA ligase buffer overnight at room temperature (−22 °C). The resulting plasmid was transformed into E. coli XL1-Blue cells for amplification. The amplified plasmid was isolated using the protocol after sonication with a Branson 250 sonifier for 2 × 15 s at an output of 4 and a duty cycle of 10. After the sonication cells were kept on ice for additional 20 min. Inclusion bodies, together with some cell debris, were sedimented at 4000 × g for 10 min. The pellet, containing mostly insoluble γ polypeptide, was washed three times with 25 ml of TE50/2 before solubilization. The insoluble γ polypeptide was dissolved in a solution containing 4 mM urea, 50 mM NaHCO3-NaOH, pH 9.5, 1 mM EDTA, 5 mM dithiothreitol, 20% (v/v) glycerol, and slowly dialyzed for 24 h against a solution containing 0.2 M LiCl, 50 mM NaHCO3-NaOH, pH 9.5, 1 mM EDTA, 5 mM dithiothreitol, 20% (v/v) glycerol. The final concentration of protein was approximately 1 mg/ml. The protein was stored at −70 °C.

**Assembly of γ Mutants—**The purified ab γ mixture was diluted to about 100 μg/ml with a solution containing 20% glycerol, 50 mM Heps-NaOH, pH 7.0, 2 mM MgCl2, 2 mM ATP, and 2 mM dithiothreitol and kept on ice. The γ subunit preparation was added dropwise to the ab γ mixture to give a final molar ratio of 3γ/1βγ. The mixture was gently mixed and left to sit at room temperature (−22 °C) for 2 h. Unreconstituted subunits were separated from the reconstituted abγ by anion exchange chromatography as described previously (11).

**RESULTS**

**Overexpression of the Spinach atpC Gene in E. coli—**The atpC gene encoding the full-length γ subunit of the spinach chloroplast ATP synthase was inserted into the pET8c expression vector and overexpressed at high levels (>100 mg/liter of cells at the end of log-phase growth). The overexpressed protein was solubilized from insoluble inclusion bodies into 4 mM urea and recovered by slow dialysis. The cloned protein was identical to the native protein (11) in its ability to reconstitute with native ab γ subunits to form a fully active core enzyme complex. Two C-terminal fragments of the C-terminal fragment of the full-length γ subunit and the eight deletion mutants are shown in Fig. 1. Also shown in Fig. 1 is the corresponding sequence at the C terminus of the bovine mitochondrial F1 γ subunit. The sequence underlined corresponds to that part of the γ subunit that is in the immediate vicinity of the hydrophobic sleeve, the last seven residues (267–273) actually passing through the sleeve (2). Deletion of all ten C-terminal residues would arguably be sufficient to test the bearing hypothesis. The C-terminal segment of γ shown in Fig. 1 is one of the most highly conserved regions among γ subunits from different species. This is evident from the more than 50% direct sequence identity between the bovine mitochondrial and chloroplast subunits (Fig. 1).

**Assembly of the γ Mutants—**Each of the γ constructs was tested for its ability to organize the ab γ subunits into a stable abγ core enzyme complex. For this, folded γ polypeptide was incubated with the isolated ab complex and the resulting abγ assembly was purified by DEAE-cellulose column chromatography as described earlier for purifying abγ assembly using the native F1 subunits (11). Incubation of each of the γ constructs with the ab γ subunits resulted in formation of an abγ complex, which is eluted from DEAE-cellulose at the same salt concentration as the native complex and which is significantly higher than that required to elute unassembled subunits (11).
**C-terminal Deletion of the Chloroplast ATP Synthase γ Subunit**

The polypeptide profiles of all of the assemblies were very similar to each other as indicated for the αβγ12 and αβγ20 assemblies, which are compared with the αβγ WT assembly in Fig. 2. This suggests that all of the γ mutants were capable of assembling with the αβ subunits.

The results shown in Table I compare the ATPase activities of protein assemblies reconstituted with the first two mutants, γD6 and γD8 with the wild-type γ. Remarkably, both mutant assemblies were significantly more active than the wild-type assembly in calcium-dependent ATP hydrolysis. The magnesium-dependent activities of the two mutants, however, were significantly reduced. The apparent Kₘ and Kcat for Ca-ATP hydrolysis of the γD6 mutant were measured and compared with the wild-type assembly (Table I). Only the Kcat exhibited a measurable change in the mutant.

Fig. 3 compares the relative rates of ATP hydrolysis of the remaining mutants, γD10 through γD20, in the presence of either Ca²⁺, Mg²⁺, or Mn²⁺ as the divalent cation substrate. The γD10, γD12, and γD14 mutant assemblies all showed similar responses to those of the γD6 and γD8 mutant assemblies in that their Ca-ATPase activities were significantly higher than that of the wild-type enzyme. The maximum activity was obtained with the γD14 mutant, which had a specific activity of 55 μmol min⁻¹ mg⁻¹, which is the highest rate of Ca-ATP hydrolysis that we have ever observed with the chloroplast enzyme. However, deletion of 16 residues from the γC terminus resulted in a sharp decrease in Ca-ATPase activity, which continued upon deletion of additional residues ending with an activity that was ~19% of the wild-type control at γD20. In contrast to the Ca-ATPase activity, the Mg-ATPase and Mn-ATPase activities declined continuously with each additional pair of residues deleted. Nevertheless, even after deleting 20 residues from the C terminus, the enzyme exhibited significant rates of catalysis: 17% of the wild-type Mg-ATPase activity and 20% of the wild-type Mn-ATPase activity.

Activation of the latent Mg-ATPase and Mn-ATPase activities of CF₄ normally requires, in addition to removing the inhibitory ε subunit (14), the presence of oxyanions such as ethanol, carbonate, or sulfite, which overcome a strong inhibition caused by free metal ions binding to and stabilizing bound ADP at the catalytic site(s) (24). The degree of stimulation by oxyanions usually varies between 10- and 100-fold depending on the divalent cation and the oxyanion concentrations. The Ca-ATPase activity, however, is already high once ε is removed and is slightly inhibited by oxyanions (25). So the magnesium- and manganese-dependent ATPase activities listed in Table I and shown in Fig. 3 were measured in the presence of high concentrations (25 mM) of sulfite ions. It was of interest to examine the effects of the γ deletions on the Mg-ATPase activities in the absence of the stimulatory oxyanions. The results of this study are shown in Table II. The Mg-ATPase activity in the absence of sulfite was, like the Ca-ATPase activity, stimulated by deletion of residues from the γC terminus and was highest in the γD14 mutant. The activity of this mutant was almost 4-fold that of the wild-type enzyme, and in parallel to the Ca-ATPase activity, it decreased markedly upon deletion of 16 or more residues. The γD20 mutant still retained a readily measurable activity, which was ~45% of that of the wild-type enzyme (Table II).

**Sensitivity of the Mutant Assemblies to Inhibitors**—The responses of the different assemblies to the inhibitory ε subunit and to the fungal inhibitor tentoxin were examined, in part to evaluate the effect of the deletions on the ability of the two inhibitors to block activity and in part to verify that the observed activities are representative of the normal activity of CF₄, which responds to these inhibitors with absolute specificity. The inhibitory responses of the Ca-ATPase activities of the different constructs to a fixed concentration (10-fold molar excess) of added ε subunit are summarized in Table III. All of the enzyme assemblies, including the enzyme assembled with the γD20 mutant, were strongly inhibited by ε, although there was a significant variation (between 64 and 83%) in the extent of inhibition observed, and all were less inhibited than the wild-type assembly (91%). The γD14 mutant, which exhibited the highest activity, was the least inhibited in the presence of a 10-fold molar excess of ε. However, in the presence of a 30-fold molar excess of the ε subunit, the γD14 mutant was inhibited by the same extent as the wild-type enzyme (results not shown), indicating that the deletion had reduced the apparent affinity of the enzyme for ε but not the maximal extent of inhibition.

The results of titrating the αβγ assemblies with tentoxin are shown in Fig. 4. All of the assemblies, with the exception of the γD20 mutant, were sensitive to inhibition by tentoxin. There were, however, significant differences among the mutant en-
**C-terminal Deletion of the Chloroplast ATP Synthase γ Subunit**

**TABLE I**

| Protein assembly | Ca-ATPase | Mg-ATPase |
|------------------|-----------|-----------|
|                  | Units mg⁻¹ | % Kcat   | Kₘ (app) | Units mg⁻¹ | % |
| αβγWT            | 24.1 ± 2.0 | 100      | 149      | 1.2        | 42.8 ± 6.9 | 100 |
| αβγD10           | 35.3 ± 7.6 | 147      | 218      | 1.2        | 29.6 ± 8.3 | 70  |
| αβγD12           | 42.7 ± 8.4 | 177      | ND       | ND         | 27.3 ± 5.0 | 64  |

*αβγ* assemblies were purified by anion exchange chromatography (11) prior to assay. ATPase values listed are the averages and standard deviations for three separate determinations.

**TABLE II**

| Protein preparation | Mg-ATPase activity |
|---------------------|--------------------|
|                     | No additions       | +25 mM Na₂SO₃ |
| αβγWT               | 2.2                | 47.2         |
| αβγD10              | 5.8                | 34.6         |
| αβγD12              | 5.6                | 21.2         |
| αβγD14              | 8.5                | 14.8         |
| αβγD16              | 4.7                | 9.4          |
| αβγD18              | 1.7                | 6.2          |
| αβγD20              | 1.0                | 8.6          |

**TABLE III**

| Protein preparation | Ca-ATPase activity |
|---------------------|--------------------|
|                     | No additions       | +ε subunit    |
| αβγWT               | 20.6               | 1.9 (9%)      |
| αβγD10              | 34.3               | 10.1 (29%)    |
| αβγD12              | 39.2               | 14.0 (36%)    |
| αβγD14              | 31.0               | 9.6 (31%)     |
| αβγD16              | 29.6               | 4.6 (22%)     |
| αβγD18              | 4.8                | 0.8 (17%)     |

**DISCUSSION**

A cross-sectional view through the structure of the beef heart mitochondrial F₁, shown in Fig. 5, the tip of the C terminus of the γ subunit, more specifically the last 7-10 residues, is surrounded by a sleeve of residues formed by part of the tightly packed β barrel domains of the six α and β subunits. The sleeve residues, located in the region marked A on the β subunit in Fig. 5, have an overall hydrophobic character as do the nearby residues on the γ subunit. A hydrophobic contact between γ and the surrounding sleeve could allow the γ subunit to act as a spindle around which the αβ hexamer could rotate with minimum frictional resistance (2). The base of the C-terminal helix...
of γ is offset from the central axis of the hexamer by ~7 Å, so that, provided it remained rigid, it would sequentially and reversibly come into contact with regions of the α and β subunits during rotation to create the required asymmetry among the nucleotide binding sites.

The remarkably high amino acid sequence conservation among the α and β subunits of F₁ enzymes from different species, together with the fact that the structures of the α and β subunits of a thermophilic bacterium can be essentially superimposed upon those of the mitochondrial F₁ subunits (26), are cogent reasons for assuming that all of the F₁ enzymes have a very similar overall structure and utilize the same basic mechanism for ATP synthesis. There is, however, some evidence suggesting that there may be some minor structural differences among the F₁ enzymes. For example, site mapping studies of the chloroplast F₁ using fluorescence resonance energy transfer (27) as well as chemical cross-linking experiments (28) have indicated that cysteine 322, which is the second last amino acid residue at the C terminus of the CF₁ γ subunit, is located near the base of the αβ hexamer, more than 60 Å away from its position in the mitochondrial F₁. The reason for this difference is not understood at this time but is particularly intriguing given the significant amino acid sequence homology which is apparent in the C-terminal domains of γ subunits from different organisms (Ref. 4; also see Fig. 1). Moreover, a different location for the γC terminus implies that the idea that the C-terminal helix of γ acts as a spindle for rotation is probably not correct, at least not as originally envisioned based on the mitochondrial F₁ structure (2).

We have selectively deleted part of γC terminus reasoning that if this region of γ was indeed acting as the tip of a spindle for rotation, or if it was in any way critical for catalysis by CF₁, the deletion should result in a complete loss of catalytic activity. However, the enzyme containing mutant γ subunits missing up to 20 amino acids from the C terminus was still capable of significant catalytic activity, which, with the exception of the D20 mutant, was sensitive to specific allosteric inhibitors of CF₁; a strong indication that the mutant enzymes followed the usual cooperative catalytic pathway and that their activities were not artifactual. It is noteworthy that a similar result was obtained for E. coli F₁ (29). In that case, membranes containing the mutant enzyme retained a limited catalytic activity (~10% of both ATP hydrolysis and synthesis) following deletion of 10 residues from the C terminus. Deletion more than 10 residues from the C terminus resulted in a complete loss of activity in that case, although the enzyme still apparently correctly assembled on the membrane. The greater sensitivity of the E. coli enzyme to deletion of the C terminus may reflect a different structural requirement for catalysis by the F₉-F₃ complex than for the isolated F₁. The activity of the E. coli F₁ mutants following isolation from the membrane was not investigated in that study.

The initial activation of enzyme turnover upon deletion of up to 14 residues from the C terminus of γ occurred for both the calcium- and the magnesium-dependent ATPase activities. One likely explanation for this effect is that the deletions resulted in a partial loosening of the structure of the enzyme to a point where it weakened binding of the cation-ADP reaction product at the catalytic site(s) in the interfacial region between α and β subunits. Since the off-rate of the cation-ADP limits the overall reaction rate, the end result is to increase the Kₐₑₚ of the enzyme. This would also explain why the sulfite-stimulated activity is inhibited rather than stimulated by the deletions. Assuming that the presence of high concentrations of sulfite maximally reduce the off-rate of cation-ADP so that the on-rate of the cation-ATP now becomes rate-limiting, a further reduction in nucleotide affinity at the catalytic site caused by the γ deletions would result in a reduction in the on-rate for the cation-ATP substrate and thus a reduction in the K₌ₑₚ. This could also explain the reduced apparent affinities for the ε subunit and for tentionox, which resulted from the C-terminal deletions. Both inhibitors are known to block cooperative release of bound nucleotides, probably by stabilizing a rigid inhibited conformation of CF₁ (1, 12). Thus a loosening of the F₁ structure might favor the activated conformation over the inhibited conformation. The structural change resulting in altered inhibitor affinity does not necessarily have to be large, since small perturbations of γ structure, such as reduction of the γ disulfide bond or single-site cleavage of γ by trypsin, are known to markedly decrease the affinity of CF₁ for the ε subunit (30).

The chloroplast γ subunit has a glycine residue at position 310, 14 residues in from the C-terminal end (Fig. 1). Most secondary structural prediction algorithms predict a break in secondary structure near this position (29). In that case, the γ subunit might also be preserved in CF₁. Deleting the 14 C-terminal residues from CF₁ γ would remove the third helix from the central bundle, possibly decreasing the number of contacts between γ and the αβ subunits. This could feasibly have the effect of loosening the structure, thereby weakening the nucleotide affinity. The sharp decrease in catalytic activity, which occurred upon deleting residues beyond the first 14, may have resulted from an interference with the important γ-αβ interactions. For example, the arginines at positions 254 and 256 in MF₁ are surrounded by a ring of 9 charged residues located on six loop segments of the α and β subunits marked “B” on one of the β subunits in Fig. 5. In the MF₁ structure,
Arg-254 and Gln-255 form hydrogen bonds with adjacent residues in the \( \beta \) subunit loop to form one of the few sites of direct contact between \( \gamma \) and the \( \alpha \) and \( \beta \) subunits (2). Assuming that \( CF_1 \) has the same arrangement in this region of \( \gamma \), deleting residues in the near vicinity of the site of contact would be expected to significantly compromise catalytic function as was observed.

Interestingly, an earlier study of the \( E. \ coli \) enzyme (31) showed that mutations near the C terminus of the \( \gamma \) subunit were able to restore catalytic function to functionally impaired enzymes which contained mutations near the N terminus. The original interpretation of these results was that the two mutations are in close proximity to each other. If this is true it would place the C terminus of the \( E. \ coli \ \gamma \) in a location very close to that of the chloroplast \( \gamma \) as determined by the fluorescence mapping experiments, assuming of course that the position of the N terminus of the \( E. \ coli \ \gamma \) is similar to that of the mitochondrial enzyme.

In conclusion, the results of this study have demonstrated that the extreme C-terminal 20 residues of the \( \gamma \) subunit of \( CF_1 \) are not essential for normal cooperative catalytic turnover by the isolated enzyme. The results eliminate the possibility of a catalytic mechanism that is universal to all \( F_1 \) enzymes in which the tip of the C terminus of the \( \gamma \) subunit must act as a spindle for rotational catalysis. The lack of functional importance of this part of the \( \gamma \) subunit for rapid turnover by \( CF_1 \) is consistent with earlier work indicating that the conformation of the C-terminal portion of the \( \gamma \) subunit of the chloroplast ATP synthase may differ from that of the mitochondrial enzyme. The results further suggest that the contacts between the \( \alpha \), \( \beta \), and \( \gamma \) subunits of the enzyme, which are essential for rotational catalysis must be provided by regions of the \( \gamma \) subunit other than the extreme C terminus.

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