

Observation of Calcium-dependent Unidirectional Rotational Motion in Recombinant Photosynthetic F_1 -ATPase Molecules*

Received for publication, June 9, 2004,
and in revised form, September 14, 2004
Published, JBC Papers in Press, September 17, 2004,
DOI 10.1074/jbc.C400269200

Ward C. Tucker^{‡§¶}, Alon Schwarz^{||},
Tiferet Levine^{||}, Ziyun Du^{§**},
Zippora Gromet-Elhanan[§], Mark L. Richter[‡],
and Gilad Haran^{||‡‡}

From the [‡]Department of Molecular Biosciences,
University of Kansas, Lawrence, Kansas 66045 and the
Departments of ^{||}Chemical Physics and [§]Biological
Chemistry, Weizmann Institute of Science,
Rehovot 71600, Israel

ATP hydrolysis and synthesis by the F_0F_1 -ATP synthase are coupled to proton translocation across the membrane in the presence of magnesium. Calcium is known, however, to disrupt this coupling in the photosynthetic enzyme in a unique way: it does not support ATP synthesis, and CaATP hydrolysis is decoupled from any proton translocation, but the membrane does not become leaky to protons. Understanding the molecular basis of these calcium-dependent effects can shed light on the as yet unclear mechanism of coupling between proton transport and rotational catalysis. We show here, using an actin filament γ -rotation assay, that CaATP is capable of sustaining rotational motion in a highly active hybrid photosynthetic F_1 -ATPase consisting of α and β subunits from *Rhodospirillum rubrum* and γ subunit from spinach chloroplasts ($\alpha^R\beta^R\gamma^C$). The rotation was found to be similar to that induced by MgATP in *Escherichia coli* F_1 -ATPase molecules. Our results suggest a possible long range pathway that enables the bound CaATP to induce full rotational motion of γ but might block transmission of this rotational motion into proton translocation by the F_0 part of the ATP synthase.

Recent experiments have provided strong evidence for the rotational catalysis mechanism proposed for the ubiquitous enzyme F_0F_1 -ATPase/ATP synthase (1, 2). Particularly striking is the effort led by several laboratories to measure the

* This work was supported in part by a grant from the Avron-Wilstätter Minerva Center for Research in Photosynthesis (to G. H.), National Science Foundation Grant MCB0212908 (to M. L. R.), and National Institutes of Health Grant GM08545 (to W. C. T.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[¶] Present address: Dept. of Physiology, University of Wisconsin, Madison, WI 53706.

** Present address: Dept. of Pathology, University of Tennessee Health Science Center, Rm. 411, Molecular Science Bldg., Memphis, TN 38163.

^{‡‡} To whom correspondence should be addressed. E-mail: gilad.haran@weizmann.ac.il.

rotational motion of the F_1 part of the enzyme on the single-molecule level. F_1 is constructed of five subunits, with a stoichiometry of $\alpha_3\beta_3\gamma\delta\epsilon$. Fluorescent actin filaments attached to the γ subunit of engineered surface-immobilized $\alpha_3\beta_3\gamma$ subcomplexes of the enzyme, either from a thermophilic bacterium, TF₁¹ (3), or from *Escherichia coli*, EF₁ (4, 5), were shown to rotate unidirectionally while hydrolyzing MgATP. This rotation was not only dependent on ATP concentration but was shown to have almost 100% efficiency of chemical to mechanical energy conversion. At very low ATP concentration the stepwise motion of the enzyme was exposed (6). Small beads attached to γ in place of the actin filament allowed Yasuda *et al.* (7) to follow the motion at the limit of small load and watch rotation at a rate as high as 130 revolutions per second. Most spectacularly, forced clockwise rotation was shown very recently to lead to ATP synthesis, thus confirming the essential role of γ subunit rotation in both directions of the reaction (8). Some single-molecule work also addressed the operation of the F_0 part of the F_0F_1 -ATP synthase. It was shown that the F_0 -c subunit oligomer, which consists of 10–14 identical c subunits, is capable of rotating together with γ (9, 10).

While much new information has been collected regarding rotational catalysis in the ATP synthase, there are still many open questions, mainly in relation to the coupling between enzymatic activity and rotational motion. The photosynthetic version of the ATP synthase is an attractive system to probe some of these questions, since it presents several distinct functional properties. These include the tight regulation of ATP hydrolysis, which is especially important in photosynthetic cells, where it prevents the depletion of essential ATP pools in the dark (11–13). Indeed, plant chloroplasts have a unique regulatory system, termed thiol modulation, which leads to reduction of a disulfide bond formed between Cys-199 and Cys-205, located in the so-called switch region of the CF₁- γ subunit that does not appear in any other type of CF₁- γ (14). The oxidation of the disulfide bond inhibits the ATPase activity of the enzyme, while its reduction stimulates it. Thiol modulation was studied indirectly on the single-molecule level by Bald *et al.* (15) who transferred the switch region from the CF₁- γ subunit to a TF₁- γ subunit. A second unique feature of ATP synthases of both chloroplasts (16) and chromatophores (17) is their high sensitivity to inhibition by excess free Mg²⁺ ions, which results in a drastic reduction of their MgATPase activities at a Mg/ATP ratio exceeding 0.5. Another very interesting phenomenon, found in both chloroplasts (18) and chromatophores (17), is the decoupling of Ca²⁺-induced ATP hydrolysis from proton translocation, although their membranes do not become leaky to protons. Both systems also show no Ca²⁺-dependent ATP synthesis.

Understanding the mechanism by which calcium decouples ATP hydrolysis and proton translocation can enhance our knowledge of the interaction between various parts of the F_0F_1 molecule in relation to its functionality. We provide here the first step toward this goal by demonstrating that the assembled hybrid photosynthetic F_1 - $\alpha^R\beta^R\gamma^C$ complex, recently described

¹ The abbreviations used are: CF₁, EF₁, RF₁, and TF₁, F_1 -ATPases from chloroplasts, *E. coli*, *R. rubrum*, and thermophilic *Bacillus* PS3, respectively; α^R , β^R - α , and β subunits from *Rhodospirillum rubrum*; γ^C , γ subunit from spinach chloroplast; HRP-Ni-NTA, horseradish peroxidase nickel-nitrilotriacetic acid; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; N, newton.

in our laboratory (19), shows rotational motion in the presence of CaATP. This result indicates that the decoupling effect of calcium, which does not interrupt the connection between ATP hydrolysis and γ^C rotation, might be mediated by a long range effect on the membrane bound F_0 subunits.

EXPERIMENTAL PROCEDURES

Materials—Biotinylated G-actin was obtained from Cytoskeleton. Biotin-PEAC₅-maleimide was from Dojindo. The phalloidin-tetramethylrhodamine B isothiocyanate conjugate was from Fluka, and the HRP-Ni-NTA conjugate was from Qiagen. ATP (grade II), glucose oxidase, catalase, and streptavidin were purchased from Sigma. All other chemicals were of the highest quality reagent grade available.

Reconstitution and Biotinylation of the Hybrid F_1 Enzyme—Recombinant α^R containing an N-terminal His₆ tag was obtained by amplification of the α^R gene from pTZ α (20) and ligation into the pET14b expression vector. This His-tagged α^R , as well as the recombinant β^R (21), and γ^C (22) were expressed as inclusion bodies in *E. coli* BL21(DE3)/pLysS cells grown to steady state at 37 °C. These inclusion bodies were individually solubilized and the expressed proteins were diluted with the refolding buffer (20), assembled into the hybrid $\alpha^R_3\beta^R_3\gamma^C$ complex, and purified by high performance liquid chromatography as described by Du *et al.* (19). The purity of the isolated hybrid complex was evaluated by SDS-PAGE (Fig. 1A).

Specific biotinylation of the $\alpha^R_3\beta^R_3\gamma^C$ complex was achieved by using the reversible disulfide bond formed between cysteine residues 199 and 205 of the γ^C subunit, as described by Hisabori *et al.* (23). Purified $\alpha^R_3\beta^R_3\gamma^C$ was oxidized with 200 μ M CuCl₂ for 1 h at 22 °C, to promote disulfide bond formation before the addition of 1 mM *N*-ethylmaleimide to block all other exposed cysteines. The γ^C disulfide of the *N*-ethylmaleimide-treated complex was then reduced by incubation with 20 mM dithiothreitol for 30 min at 22 °C. After passage through two successive Sephadex G-50 centrifuge columns to remove the excess reductant, the enzyme was labeled with a 3-fold excess of biotin-PEAC₅-maleimide for 30 min at 22 °C. The labeling was stopped by the addition of 200 μ M cysteine followed by passage through a centrifuge column. The specificity of labeling was confirmed by Western blots (Fig. 1B).

$EF_1\text{-}\alpha_3\beta_3\gamma$ Complexes—These complexes were prepared according to Noji *et al.* (5) and Panke *et al.* (10) and kindly supplied by Drs. W. Junge and S. Engelbrecht.

Polymerization of the Biotinylated G-actin—Stocks of biotinylated G-actin were maintained at a concentration of 1 mg/ml in 2 mM Hepes-NaOH (pH 7.2), 0.2 mM CaCl₂, and 0.2 mM ATP at -80 °C. To obtain fluorescently labeled F-actin, the protein was diluted to 18 μ M into the polymerization buffer at a final concentration of 20 mM Hepes-NaOH (pH 7.2), 100 mM KCl, 10 mM MgCl₂, 2 mM ATP, and 36 μ M phalloidin-tetramethylrhodamine B isothiocyanate conjugate and polymerized overnight at 4 °C.

Rotational Assays—Construction of the flow cells, adherence of the His₆-tagged and biotinylated $\alpha^R_3\beta^R_3\gamma^C$, and infusion of the actin filaments were carried out essentially as described previously for TF₁- and EF₁- $\alpha_3\beta_3\gamma$ complexes (3, 4, 10). Briefly, flow cells with a chamber volume of 20–30 μ l were infused with the following series of buffers (2 \times 25 μ l per step, 5-min incubation): 1) Buffer A (10 mM Hepes-NaOH (pH 7.2), 25 mM KCl, 5 mM MgCl₂) with 2.4 μ M HRP-Ni-NTA; 2) Buffer B (Buffer A plus 10 mg/ml bovine serum albumin) with a 3 nM concentration of the biotinylated $\alpha^R_3\beta^R_3\gamma^C$ complex; 3) Buffer B with 50 nM streptavidin; and 4) Buffer B with 50 nM fluorescently labeled actin filaments. Each step was followed with a wash of 100 μ l of Buffer B, except for step 4, after which we performed a wash with 100 μ l of Buffer B and 100 μ l of Buffer A, both lacking MgCl₂. CaATP-dependent rotation was initiated by the infusion of 50 mM Tricine-NaOH (pH 8.0), 4 mM CaCl₂, 4 mM ATP, 216 μ g/ml glucose oxidase, 36 μ g/ml catalase, 25 mM glucose, and 1% β -mercaptoethanol. The EF₁- $\alpha_3\beta_3\gamma$ ATPase was adsorbed into the flow cell in a similar fashion, but since its MgATP-dependent rotation was tested, the CaCl₂ in the reaction mixture was replaced with 2 mM MgCl₂.

Immediately following infusion of the reaction buffer, the flow cell was mounted on an inverted microscope (Olympus) and illuminated with a mercury lamp. Fluorescent images were taken using a digital intensified CCD camera (Roper Scientific). Typically, 500 frames were collected from each microscope field of view at a rate of 15 frames per second. Images were processed using custom-written Matlab routines.

Other Methods—Ca²⁺-dependent ATP hydrolysis activities were measured for 5 min at 37 °C in the presence of 50 mM Tricine-NaOH (pH 8.0), 4 mM ATP, 4 mM CaCl₂, 50 mM NaCl, and 5 μ g of enzyme. For measuring MgATPase activity the 4 mM CaCl₂ was replaced by 2 mM

TABLE I
The ATP hydrolysis turnover rates of the biotinylated $\alpha^R_3\beta^R_3\gamma^C$ complex
The $\alpha^R_3\beta^R_3\gamma^C$ complexes were prepared, biotinylated (biot), and assayed as described under "Experimental Procedures."

Complex	Temperature °C	MgATPase s^{-1}	CaATPase s^{-1}
$\alpha^R_3\beta^R_3\gamma^C$	37	95	226
	22	36	173
$\alpha^R_3\beta^R_3\gamma^C$ -biot	37	100	218
	22	42	167

MgCl₂, and 10 μ g of enzyme was used. P_i release was determined as described by Taussky and Shorr (24). Protein concentration was determined by the method of Lowry (25) or according to Bradford (26). SDS-PAGE was carried out on Novex Pre-Cast 10–20% Tris-glycine gradient gels.

RESULTS AND DISCUSSION

We have recently assembled in our laboratory a hybrid photosynthetic F_1 -ATPase consisting of RF₁- α^R and β^R subunits and a CF₁- γ^C subunit, all expressed in *E. coli* (19). The isolated hybrid $\alpha^R_3\beta^R_3\gamma^C$ complex showed high ATPase activity, particularly in the presence of CaATP as a substrate, reaching 167 s⁻¹ even when assayed at 22 °C in its biotinylated state (Table I). The complex fully retained the unique properties of its parent ATPases, which include sensitivity to inhibition by free magnesium (16, 17), and a higher CaATPase than MgATPase activity (Table I), both being modulated by γ^C oxidation and reduction (19, 27). Single-molecule studies of extracted native CF₁ (lacking a δ subunit) have already provided some evidence for its γ subunit rotation (23). But this native CF₁ (- δ) showed a very low MgATPase activity of 1.3 s⁻¹ at 25 °C and could be fixed to the glass surface only spontaneously. The availability of our highly active recombinant $\alpha^R_3\beta^R_3\gamma^C$ enabled us to apply protein engineering tools and other experimental methods similar to those employed with the bacterial TF₁ (3, 6) and EF₁-ATPases (4, 5, 9, 10).

The single-molecule study of the $\alpha^R_3\beta^R_3\gamma^C$ complex reported here was motivated by the intriguing effects of CaATP on the photosynthetic enzyme. The much higher activity of the enzyme in the presence of CaATP, as compared with MgATP (Table I), suggests that it must be caused by multiple site catalysis (28, 29) and should therefore involve γ^C subunit rotation within our hybrid complex. We therefore started monitoring rotational motion of individual $\alpha^R_3\beta^R_3\gamma^C$ hybrid F_1 -ATPase molecules in the presence of calcium, using the relatively simple and broadly applicable actin filament assay. A limitation of this assay is the load exerted by the large actin filament on the rotating enzyme, but this limitation is also an asset, as it allows determining the torque developed by the protein through the relation between filament length and rotation speed (6).

To facilitate the attachment of our hybrid complex to the glass surface, via absorption to an HRP-Ni-NTA conjugate, a His₆-tag was introduced at the N terminus of the α^R subunit, as described by Omote *et al.* (4). Attachment of the fluorescently labeled actin filament at the opposite end of the molecule was achieved by forming a biotin-streptavidin-biotin linkage between the filament and the specific cysteine pair in γ^C . Using a streptavidin-conjugated peroxidase we demonstrated that the biotinylation appears specifically in the γ^C subunit and is dependent on the prereduction of its disulfide (Fig. 1B, lanes 1 and 2). The binding of biotin had no significant effect on either Mg²⁺-dependent or Ca²⁺-dependent ATPase activity. However, at 22 °C, the temperature of the rotation assays, MgATPase activity was reduced much more than CaATPase activity (Table I).

Molecules of our $\alpha^R_3\beta^R_3\gamma^C$ complex readily adsorbed to a

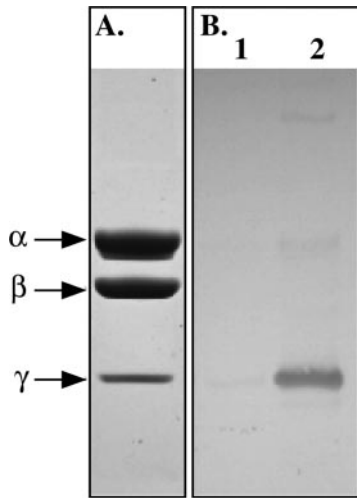


FIG. 1. Specific biotinylation of the γ^C subunit disulfide cysteines in the $\alpha_3\beta_3\gamma^C$ complex. The assembled hybrid complex was purified and biotinylated as described under "Experimental Procedures." The enzyme was subjected to SDS-PAGE and stained with Coomassie Brilliant Blue R (A) or transferred to nitrocellulose and probed with streptavidin-conjugated peroxidase (B). To demonstrate specific biotinylation of the γ^C subunit, labeling was carried out following incubation without (lane 1) or with (lane 2) reduction by dithiothreitol.

glass surface covered with HRP-Ni-NTA, but no enzyme attachment occurred in the absence of the HRP-Ni-NTA conjugate. The fluorescent actin filaments attached to the immobilized proteins only after addition of streptavidin and were easily washed out if the biotinylated $\alpha_3\beta_3\gamma^C$ complex was not present in the flow cell (data not shown). About 100 fluorescent actin filaments could be observed in each field of view of the microscope, and on the average one molecule in a field showed rotational motion in presence of the CaATP buffer, a percentage that is comparable with previous reports (6, 9). An example of a rotating filament is shown in Fig. 2A, and the rotational trajectories of several molecules are plotted in Fig. 2B.

Since a CaATPase induced rotation has never been studied before, we performed identical studies using the $EF_1\text{-}\alpha_3\beta_3\gamma$ MgATPase, whose rotational motion was already thoroughly investigated (4, 5, 9, 10). A comparison of the EF_1 -MgATPase and $\alpha_3\beta_3\gamma^C$ -CaATPase-induced rotation is presented in Fig. 3, in which the rotational velocity (in inverse seconds) is plotted as a function of filament length for the two groups. Only molecules that showed at least 5 full rotations (>70% of the rotating molecules) were included in this figure. The solid lines (see legend to Fig. 3) were calculated based on the following relation between the rotation velocity in Hz, ν , and the applied torque N : $\nu = 1/3\tau_{\text{cat}} + 2\pi\zeta/N$, in which τ_{cat} is the turnover time of the enzyme, and ζ is the rotational friction coefficient that depends on the filament length L . For a rigid filament ζ is given by $4\pi\eta L^3/3[\ln(L/2r) - 0.447]$, in which η is the viscosity of the solution and r is the filament radius, taken as 5 nm (6, 30). The torque developed by each molecule was calculated by inverting the above equation. The average torque was 14.6 ± 1.5 pN·nm for the hybrid $\alpha_3\beta_3\gamma^C$ and 16.8 ± 1.4 pN·nm for the $EF_1\text{-}\alpha_3\beta_3\gamma$ molecules. While these values are smaller than previously reported (4–6), the high similarity between the two groups of molecules is quite striking and suggests that they rotate in a very similar fashion.

We also found that the $\alpha_3\beta_3\gamma^C$ complexes could rotate in the presence of MgATP, very similarly to the CaATP-induced rotation, but only when 100 mM of bicarbonate were added (data not shown). This finding is on par with earlier reports that the MgATPase activity of both CF_1 (16) and RF_1 (17) is

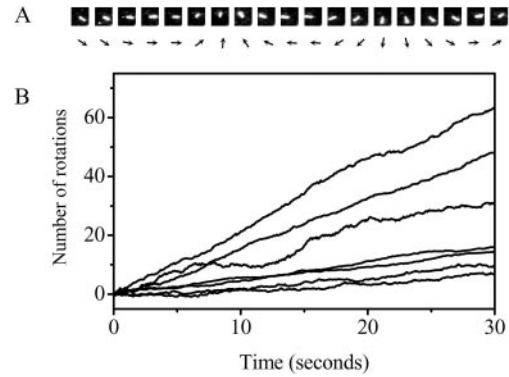


FIG. 2. A, 30-ms snapshots of a rotating $\alpha_3\beta_3\gamma^C$ -attached actin filament ($\sim 2\text{-}\mu\text{m}$ length) in the presence of CaATP. The arrows mimic the position of the filament and indicate the anti-clockwise direction of the observed rotation. **B**, rotational trajectories of actin filaments attached to $\alpha_3\beta_3\gamma^C$ complexes in the presence of CaATP.

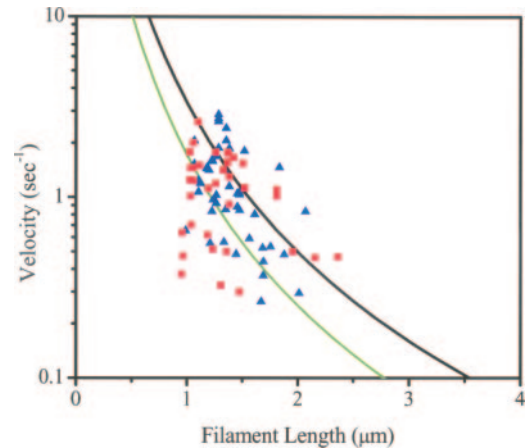


FIG. 3. Rotational rate as a function of actin filament length for $\alpha_3\beta_3\gamma^C$ complexes (red squares) in the presence of CaATP and for EF_1 molecules (blue triangles) in the presence of MgATP. The solid lines were calculated as described in the text. The green line is for $N = 10$ pN·nm, and the black line for $N = 20$ pN·nm.

very sensitive to inhibition by free Mg^{2+} , and in both cases high rates of catalysis require addition of oxyanions such as bicarbonate or sulfite (19, 31). In addition, at 22 °C under the conditions of the rotation assay, the MgATPase activity of the $\alpha_3\beta_3\gamma^C$ complex was 4-fold lower than its CaATPase activity (Table I). This difference could account for the lack of any MgATP-induced γ^C rotation in the absence of bicarbonate. Indeed, a strong, nonlinear relation between ATPase activity and the number of rotating molecules was already noted by Müller *et al.* (32).

How can CaATP-induced rotational motion of the photosynthetic $\alpha_3\beta_3\gamma^C$ complex be explained, in view of the decoupling activity of calcium? Current models of the function of F_0F_1 -ATP synthase assert that the protonmotive force created by proton translocation through the F_0 part drives rotation of the c subunit oligomer, which in turn causes rotation of the γ subunit, leading to ATP synthesis by the F_1 part (33, 34). A reverse sequence of events is expected to occur following ATP hydrolysis, which should drive rotation of the γ subunit, leading to rotation of the c subunit oligomer that is coupled to proton translocation. Indeed, it has been shown that γ rotation is contemporaneous with rotation of the c subunit oligomer in both TF_1 and EF_1 (9, 35). Boyer's binding change mechanism recognizes three asymmetric catalytic sites on the three β subunits (1). These three sites interconvert as the γ subunit rotates. Efficient ATP synthesis without waste of energy requires coupling between events occurring at the three sites and proton

translocation. Thus proton translocation must occur only after ADP and P_i bind at a loose catalytic site, while at the same time an ATP molecule is bound at the tight site (36). This scheme was borne out by experiments carried out by Cross and co-workers (37) who showed that a protonmotive force cannot sustain rotation in the absence of ADP and P_i in the medium.

Consequently, there has to be a switch mechanism that couples or decouples the F_1 and F_0 parts and transmits information between them in both directions (38, 39). It could involve some form of a conformational change induced by substrate binding at the β catalytic sites and further transmitted through the γ subunit to the F_0 -c subunits. The combined effects of calcium, which include both a modulation of F_1 activity and decoupling of F_1 and F_0 , point to its involvement with the same switch mechanism suggested by Cross (36). Since the current study shows that the $\alpha^R\beta_3^R\gamma^C$ decoupled CaATPase activity does *not* hinder γ^C rotation, it is likely that the switch works in parallel to, but outside, the rotational pathway. It might involve, for example, the F_1 - ϵ subunit, which is a well known inhibitor of both CaATPase and MgATPase activity in the photosynthetic F_1 (27, 40).

We cannot currently rule out a direct effect of calcium at a more "downstream" position than suggested here, *e.g.* at the F_0 -c subunit. However, we believe that our results, combined with previous indications in the literature (36, 37), do suggest that the calcium effect is related to a *long range pathway for allosteric communication* between the F_0 and F_1 parts of the ATP synthase. The experimental system described in this work provides an opportunity to directly check this hypothesis. In particular, the difference between CaATP and MgATP binding at the active sites on the interface between the α and β subunits should be probed. This can now be studied in our photosynthetic complex, since we have already assembled a hybrid $\alpha^R\beta_3^R\gamma^C$ containing the catalytic site mutant β^R -T159S (41), which shows exceptionally high MgATPase but very low CaATPase activities (19). Carmeli and co-workers (42) suggested recently, based on a simulation, that the average bond length of a Ca^{2+} ion at the active site of F_1 is longer by 0.3 Å than that of Mg^{2+} . It is as yet unknown how such local differences in binding can propagate through the structure of the β subunit into the CF_1 γ and the F_0 c and a subunits. It will therefore be important to attempt to assemble the c subunit oligomer into the hybrid protein and test rotation of this part of the enzyme in the presence of calcium. These and other future studies will be facilitated by the current availability of a fully active recombinant photosynthetic protein that shows rotational motion.

Acknowledgments—We thank Drs. Sigfried Engelbrecht and Wolfgang Junge for kindly supplying us with *E. Coli* F_1 -ATPase and for carefully reading the manuscript.

REFERENCES

- Boyer, P. D. (1993) *Biochim. Biophys. Acta* **1140**, 215–250
- Yoshida, M., Muneyuki, E., and Hisabori, T. (2001) *Nat. Rev. Mol. Cell. Biol.* **2**, 669–677
- Noji, H., Yasuda, R., Yoshida, M., and Kinoshita, K. (1997) *Nature* **386**, 299–302
- Omote, H., Sambonmatsu, N., Saito, K., Sambongi, Y., Iwamoto-Kihara, A., Yanagida, T., Wada, Y., and Futai, M. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 7780–7784
- Noji, H., Hasler, K., Junge, W., Kinoshita, K., Jr., Yoshida, M., and Engelbrecht, S. (1999) *Biochem. Biophys. Res. Commun.* **260**, 597–599
- Yasuda, R., Noji, H., Kinoshita, K., and Yoshida, M. (1998) *Cell* **93**, 1117–1124
- Yasuda, R., Noji, H., Yoshida, M., Kinoshita, K., Jr., and Itoh, H. (2001) *Nature* **410**, 898–904
- Itoh, H., Takahashi, A., Adachi, K., Noji, H., Yasuda, R., Yoshida, M., and Kinoshita, K. (2004) *Nature* **427**, 465–468
- Sambongi, Y., Iko, Y., Tanabe, M., Omote, H., Iwamoto-Kihara, A., Ueda, I., Yanagida, T., Wada, Y., and Futai, M. (1999) *Science* **286**, 1722–1724
- Pänke, O., Gumbiowski, K., Junge, W., and Engelbrecht, S. (2000) *FEBS Lett.* **472**, 34–38
- Gromet-Elhanan, Z. (1995) in *Anoxygenic Photosynthetic Bacteria* (Blankenship, R. E., Madigan, M. T., and Bauer, C. E., eds) pp. 807–830, Kluwer Academic Publishers Group, Dordrecht, The Netherlands
- Richter, M. L., Hein, R., and Huchzermeyer, B. (2000) *Biochim. Biophys. Acta* **1458**, 326–342
- McCarty, R. E., Evron, Y., and Johnson, E. A. (2000) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **51**, 83–109
- Miki, J., Maeda, M., Mukohata, Y., and Futai, M. (1988) *FEBS Lett.* **232**, 221–226
- Bald, D., Noji, H., Yoshida, M., Hirono-Hara, Y., and Hisabori, T. (2001) *J. Biol. Chem.* **276**, 39505–39507
- Hochman, Y., Lanir, A., and Carmeli, C. (1976) *FEBS Lett.* **61**, 255–259
- Gromet-Elhanan, Z., and Weiss, S. (1989) *Biochemistry* **28**, 3645–3650
- Pick, U., and Weiss, M. (1988) *Eur. J. Biochem.* **173**, 623–628
- Du, Z., Tucker, W. C., Richter, M. L., and Gromet-Elhanan, Z. (2001) *J. Biol. Chem.* **276**, 11517–11523
- Du, Z., and Gromet-Elhanan, Z. (1999) *Eur. J. Biochem.* **263**, 430–437
- Nathanson, L., and Gromet-Elhanan, Z. (1998) *J. Biol. Chem.* **273**, 10933–10938
- Sokolov, M., Lu, L., Tucker, W., Gao, F., Gegenheimer, P. A., and Richter, M. L. (1999) *J. Biol. Chem.* **274**, 13524–13529
- Hisabori, T., Kondoh, A., and Yoshida, M. (1999) *FEBS Lett.* **463**, 35–38
- Taussky, H. H., and Shorr, E. (1953) *J. Biol. Chem.* **202**, 675–685
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Tucker, W. C., Du, Z., Gromet-Elhanan, Z., and Richter, M. L. (2001) *Eur. J. Biochem.* **268**, 2179–2186
- Boyer, P. D. (2000) *Biochim. Biophys. Acta* **1458**, 252–262
- Weber, J., and Senior, A. E. (2000) *Biochim. Biophys. Acta* **1458**, 300–309
- Hunt, A. J., Gittes, F., and Howard, J. (1994) *Biophys. J.* **67**, 766–781
- Nelson, N., Nelson, H., and Racker, E. (1972) *J. Biol. Chem.* **247**, 6506–6510
- Müller, M., Pänke, O., Junge, W., and Engelbrecht, S. (2002) *J. Biol. Chem.* **277**, 23308–23313
- Junge, W., Lill, H., and Engelbrecht, S. (1997) *Trends Biochem. Sci.* **22**, 420–423
- Oster, G., and Wang, H. (2000) *Biochim. Biophys. Acta* **1458**, 482–510
- Tsunoda, S. P., Aggeler, R., Yoshida, M., and Capaldi, R. A. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98**, 898–902
- Cross, R. L. (2000) *Biochim. Biophys. Acta* **1458**, 270–275
- Zhou, Y., Duncan, T. M., and Cross, R. L. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 10583–10587
- Penefsky, H. S. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82**, 1589–1593
- Matsuno-Yagi, A., and Hatefi, Y. (1993) *J. Biol. Chem.* **268**, 1539–1545
- Nowak, K. F., and McCarty, R. E. (2004) *Biochemistry* **43**, 3273–3279
- Nathanson, L., and Gromet-Elhanan, Z. (2000) *J. Biol. Chem.* **275**, 901–905
- Hochman, Y., Gong, X. M., Lifshitz, Y., and Carmeli, C. (2000) *Indian J. Biochem. Biophys.* **37**, 459–469