Biochemical and Biophysical Research Communications 391 (2010) 182–186

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

F_oF_1-ATPase activity regulated by external links on β subunits

Jie Cheng [a,c], Xiao-ai Zhang [a,c], Yao-Gen Shu [b,*], Jia-Chang Yue [a,*]

[a] National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
[b] Key Laboratory of Frontiers in Theoretical Physics, Institute of Theoretical Physics, Chinese Academy of Sciences, Beijing 100190, China
[c] Graduate School of the Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 21 October 2009
Available online 10 November 2009

Keywords:
F_oF_1-ATPase
Activity regulation
Western blotting
ESR

ABSTRACT

F_oF_1-ATPase activity is regulated by external links on β subunits with different molecular weight. It is inhibited when anti-β subunit antibody, streptavidin and H9 antibody link on the β subunits successively, but is activated when virus was binded. Western blotting indicated that the employed anti-β antibody target was on the non-catalytic site of the β subunit. Furthermore, an ESR study of spin-labeled ATP (SL-ATP) showed that the affinity of ATP to the holoenzyme increases with increasing external links on the β subunits. This simple regulation method may have great potential in the design of rapid, free labeled, sensitive and selective biosensors.

Introduction

F_oF_1-ATPase is the ubiquitous rotary motor that uses the transmembrane electrochemical potential to synthesize ATP in bacterial cell membranes, chloroplasts, mitochondria and on the plasma membrane of endothelial and tumor cells. The holoenzyme is a complex of two rotary motors, F_o and F_1, which are mechanically coupled by a common central stalk ("rotor"), $c_n\varepsilon\gamma$. The membrane-embedded F_o unit converts the proton motive force (p.m.f) into mechanical rotation of the "rotor", thereby causing the cyclic conformational change of the $\alpha_3\beta_3$ crown ("stator") in F_1, and driving ATP synthesis. A striking characteristic of this motor is its reversibility. It may rotate in the reverse direction for ATP hydrolysis and utilize the excess energy to pump protons across the membrane [1–6]. Furthermore, single molecule experiments have revealed that the enzyme activity can be regulated by manipulating its physical rotation because of tight mechanochemical coupling [7–9].

The regulation of the holoenzyme activity is still an interesting topic [10]. Traditional methods of describing enzyme regulation are chemical in nature, and utilize either the concentration of the substrate or the transmembrane electrochemical potential [11–13]. In fact, the F_oF_1 motor actively converts a chemical reaction (ATP hydrolysis in F_1 or proton transfer in F_o) into the unidirectional physical rotation of the "rotor" that is accompanied with the cyclic conformational change of the "stator". Single molecule assays have indicated that the activity of F_1 or F_o [7–9] can be regulated by varying the load on the "rotor," and the eccentric rotation of the γ subunit is mechanically coupled with the cyclic conformational change of the $\alpha_3\beta_3$ crown at a high efficiency. However, the physical regulation of the activity of the holoenzyme (including F_o and F_1), instead of chemical regulation, is still difficult to understand. One of the reasons is that the "rotor" of F_oF_1 motor is mostly enwrapped by the "stator" $\alpha_3\beta_3$ crown. The other reason is that the exposed fraction of the "rotor" is also partly shaded by the b_2 subunit. Thus, it becomes very difficult to directly regulate the holoenzyme activity by a load on the "rotor".

In view of the fact that the unidirectional rotation of the "rotor" is tightly coupled with the cyclic conformational change of the "stator", what will happen to the activity of the rotary motor if external complexes bind to the "stator"? Our previous investigations [14–18] have demonstrated that the holoenzyme activity can be regulated by external links on β subunits. In this article, we studied the holoenzyme activity regulation in detail with different external links on β subunits, including Western blotting to check the epitope of anti-β antibody, and ESR to analyze the affinity of ATP to the modified holoenzyme.

Materials and methods

Materials. 3-Carboxy-1-oxyl-2,2,5,5-tetramethyl pyrrolidine was purchased from Fisher Scientific Co. (USA). HAT (hypoxanthine aminopterin thymidine), HT (hypoxanthine thymidine), PEG (MW 1500), luciferin, luciferase, adenosine 5′-triphosphate (ATP) (disodium salt) and adenosine-5′-diphosphate (ADP) (disodium salt), were purchased from Sigma–Aldrich Co. (USA). DMEM high glucose medium was purchased from Gibco (USA). Fetal bovine serum was purchased from PAA Co. (Location?). Ni–NTA His Bind Resins was purchased from Qiagen (USA). pET-30a was purchased from Nova-

* Corresponding authors. Fax: +8610 64871293.
E-mail addresses: shuyg@itp.ac.cn (Y.-G. Shu), yuejc@sun5.ibp.ac.cn (J.-C. Yue).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.11.028

gen (USA). pMD19-T Simple vector was purchased from TaKaRa Co (Japan). All other analytical purified reagents were purchased domestically.

Preparation of the anti-β subunit monoclonal antibody. The β subunit of F_oF_1-ATPase from the thermophilic bacterium *Bacillus. PS3* ($F_1\beta$) was expressed in *Escherichia coli* JM103 and purified according to Ref. [9]. Six-to-eight-week-old female mice were first injected with Freund's complete adjuvant, then by incomplete adjuvant, and finally by splenocyte intravenous booster. The antigen without adjuvant was injected to splenocytes. After three days, splenocytes were isolated and fused with Sp2/0 mouse myeloma cells using polyethylene glycol. Hybridomas were obtained in 96-well culture plates in RPMI-1640 supplemented with 20% FBS and HAT medium. Cell supernatants were screened for antibodies binding to the β subunit of F_1-ATPase. The positive clones were screened by ELISA, using the recombinant ATPase β subunit as the target antigen [19]. Subcloned cells in 96-well plates were recovered, and injected intraperitoneally into BALB/c mice (10^6 per mouse). Antibodies in the ascites were purified by gel filtration on Sephadex G-200 (Pharmacia?, USA). The monoclonal anti-β subunit antibody (2D5) was prepared according to Ref. [20] and stored at −20 °C.

Cloning of the fragments. The fragments of the β subunit, including domains 1–2, domain 2, and domains 2–3, were PCR-amplified using specific primers. Primers were designed to introduce an NdeI restriction site at the 5′ end, and an HindIII site at the 3′COOH-terminal end (Table 1). Domain boundaries were determined based on structural motifs (Fig. 2A–C). PCR products were purified from 1.5% Tris–acetate/EDTA agarose gels using a gel band purification kit (Amersham), and subcloned into pMD™ 19-T Simple Vector (TAKARA). Competent *E. coli* DH5α (Life Technologies) were transformed with 1 μl ligation mixture/100 μl cells, plated on LB-ampicillin (50 μg/ml) and X-Gal agarose plates, and grown overnight at 37 °C. Colonies were screened for inserts with NdeI and HindIII (TAKARA) digestion, followed by gel purification. The subcloned inserts were ligated into the NdeI- and HindIII-digested pET30a vector (Novagen), using the Clonables kit (Novagen). Competent *E. coli* Novablue cells (Novagen) were transformed with the ligation mixture and grown on LB-kanamycin agarose plates. Colonies were screened for insertion by restriction enzyme digestion and DNA sequencing.

Purification of the fragments and β subunit. BL21(DE3) cells (Solarbio, China) were transformed with pET30a vector containing β subunit or its domains, plated on LB-kanamycin agarose plates, and grown overnight at 37 °C. Then, 2.5 ml LB, containing 30 μg/ml kanamycin was inoculated with one colony and grown at 37 °C, with shaking at 200 rpm, to an $A_{600\ nm}$ of 0.6, and stored overnight at 4 °C. A 50 ml culture (LB, 30 μg/ml kanamycin) was inoculated with 2 ml of the noninduced overnight culture and grown at 37 °C, 250 rpm, to an $A_{600\ nm}$ of 0.60. Isopropyl thio-β-D-galactosidase was added to a final concentration of 1 mM/l and cultures were grown an additional 3 h at 37 °C, 250 rpm. Cells were harvested by centrifugation at 5000*g* for 5 min and stored at −20 °C. Lysates were prepared by denaturing with 8 M/l urea for all proteins. Lysates were purified using Ni–NTA His Bind Resin columns (Novagen) and the resulting proteins were dialyzed against PBS (pH 7.0). The β subunit was isolated and purified from *Rhodopseudomonas palustris* according to Ref. [21].

Table 1
Primer sequences used for construction of fragments. Sequences are based on the DNA of *Rhodopseudomonas palustris* ATP synthase (gene accession RPA0176 KEGG GENES Database). Restriction enzyme sites, in bold, represent for 5′ end primers, an NdeI cleavage site, and for 3′ primers, an HindIII site. Primers read from 5′ to 3′ according with gene sequence.

Domains	5′ Primer sequence	3′ Primer sequence
1 and 2	TT**CATATG**GCTACAC CCGCCAATCAGACCG	AT**AAGCTT**GACGATC GAGGCGGACAGCATG
2	TT**CATATG**CAGTCGA CCGAGGCTGAAATTC	AT**AAGCTT**GACGATC GAGGCGGACAGCATG
2 and 3	TT**CATATG**CAGTCGA CCGAGGCTGAAATTC	TAT**AAGCTT**GGCGGC GAGCTTCTTGCCCTTC
Full length	TT**CATATG**GCTACAC CCGCCAATCAGACCG	TAT**AAGCTT**GGCCGCC TCGGCGGCGAGCTTCT

Western blotting. Purified, recombinant β domain proteins under reducing conditions were separated by SDS–PAGE and transferred to polyvinylidene difluoride. Membranes were blocked by 5% BSA overnight at 4 °C, then incubated with the anti-β subunit monoclonal antibody at 2.5 mg/ml for 2 h at room temperature. After washing three times, the polyvinylidene difluoride membrane was incubated with goat anti-mouse, HRP-conjugated secondary antibody. The blot was developed by using a super-enhanced chemiluminescence detection kit (Applygen Technologies Inc., Beijing, China).

Preparation of chromatophores. Chromatophores were prepared from the cells of *R. palustris*, according to Ref. [22] in TSM buffer (0.1 mM Tricine–NaOH, 0.25 M sucrose, 5 mM $MgCl_2$, pH 8.0). *R. palustris* cells were grown in the medium for 72 h at 33 °C and illuminated with white light. Harvested cells were washed twice in TSM buffer and then disrupted by ultrasonication. The suspension was centrifuged for 30 min at 25,000*g*. The chromatophore in the supernatant was collected by centrifugation at 25,000*g* for 90 min, and then resuspended in TSM buffer. Bacteriochlorophll (BChl) was determined by using the spectrophotometric extinction coefficient (860 nm) according to Clayton [23]. Under the electron microscope, the diameter of the obtained chromatophore was estimated to be about 100 nm.

Preparation of samples with different external links on the β subunit. To compare the holoenzyme activity with different external links, we have prepared 5 samples: (1) sample 1 is the native F_oF_1-ATPase within the chromatophore; (2) sample 2: 100 μl (50 mg/ml) chromatophore (sample 1) was incubated with 1 μl (10 mg/ml) anti-β antibody at 37 °C for 60 min, and then the free anti-β antibody was washed away by centrifugation at 4 °C for 30 min. (The anti-β antibody was labeled with biotin in the 1:10 and free biotin was dialyzed); (3) sample 3: sample 2 was incubated with equivalent streptavidin in 37 °C for 40 min; (4) sample 4: sample 3 was incubated with biotinylated influenza virus anti-H9 antibody at 37 °C for 30 min, and the free antibody was washed; (5) sample 5: sample 4 was incubated with 10 μl influenza H9 virus for 60 min at 37 °C and then the sample was washed three times. All the samples with different links were treated as Fig. 1.

Measurement of F_oF_1-ATPase activity. The ATP synthesis activity was measured by the luciferin–luciferase method with a computerized ultra-weak luminescence analyzer (type BPCL manufactured at the Institute of Biophysics, Academia Sinica, Beijing, China). Three microliters (1 μg) of chromatophores were reacted in 50 μl ATP synthesis buffer (50 mM Tris–HCl, 5 mM $MgCl_2$, 5 mM K_2HPO_4, 2 mM ADP, 10% glycerol, pH 8.0) at 37 °C for 5 min. The ATP synthesis reaction was stopped by adding 1/10 volume of 4% TCA, and then 3 μl liquor was diluted with 100 μl assay mix dilution buffer. The luminescence was measured for 10 s after the injection of luciferin–luciferase reagent. The ATP hydrolysis activity was measured with the same method. Similarly, 1 μg chromatophores were incubated at 37 °C for 5 min in 50 μl ATP hydrolysis buffer (50 mM Tris–HCl, 2 mM ATP, 2 mM $MgCl_2$, 10 mM KCl, pH 8.0). The ATP synthesis/hydrolysis reaction was stopped by TCA, and then 3 μl liquor was tested in the same way.

Preparation of spin-labeled ATP (SL-ATP). The spin-labeled ATP, 3′-*O*-(1-oxyl-2,2,5,5-tetramethyl-3-carbonyl pyrrolidine) adenosine 5′-triphosphate, was synthesized by the method described in Refs. [24,25]. ATP was esterified by 3-carboxy-1-oxyl-2,2,5,5-tetramethyl

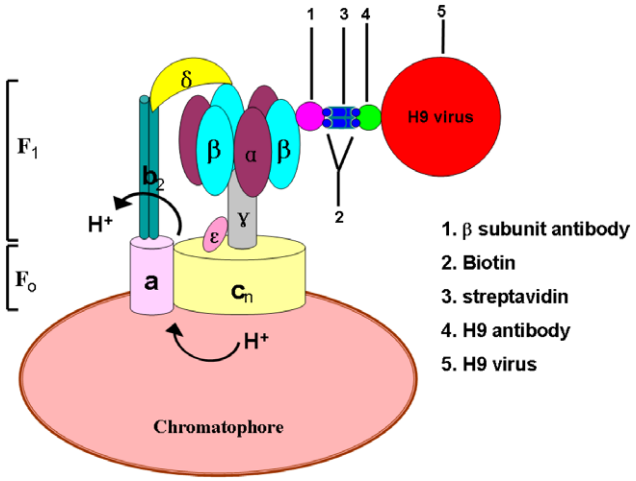

Fig. 1. The schematic illustration of the activity regulation assay, showing the experimental geometry (not to scale). The other two same links are not painted. The holoenzyme is a complex of two rotary motors F_o and F_1. The F_o motor is constituted by the a subunit (a, stator) and n channels (c_n, rotor). The $\alpha_3\beta_3$ crown (stator) and the γ subunit (rotor) form the F_1 motor. The complex of b_2 and δ subunits fix the two stators, whereas the two rotors are mechanically coupled by the ε subunit. External complexes bind to the β subunits to regulate the holoenzyme activity.

pyrrolidine. Separation of SL-ATP from the complex product was achieved by reversed-phase HPLC, with a linear gradient including buffer A (10% aqueous 2-propanol) and buffer B (water) on a self-packed C8 column (Beijing Jinouya Technology and Development Co. Ltd.). SL-ATP was eluted at least 3 times, and the volume was monitored at 214 and 257 nm. Fractions exhibiting both a 257 nm absorption and an ESR signal were collected according to Ref. [24].

ESR signal measurement. For each sample of F_oF_1-ATPase with different external links, 0.1 μM SL-ATP in 10 mM Hepes buffer (pH 8) and 1 mM $MgCl_2$ were added to 200 μl chromatophores ([Bchl] = 0.5 μg/μl), and incubated for 25 min. A 60-fold excess of SL-ATP over the enzyme, corresponding to a 10-fold excess per potential binding site was required. The free SL-ATP was separated by gel centrifugation chromatography on G-50 fine [26]. ESR spectra were recorded with an ER 200D-SRC electron spin resonance spectrometer, in the X-band mode at 15–20 mW microwave power, and modulation amplitude of 1 G. Each spectrum was recorded four independence test.

Results

Model of activity regulation

A simple method to study the regulation of F_oF_1-ATPase activity is shown in Fig. 1. The program was operated step by step, as specified in Section 2.7, which describes the generation of 5 different samples. First, the chromatophore was prepared as described in Section 2.6, in which F_o was embedded [where?], whereas F_1 protruded outside (sample 1), Next, the anti-β antibody was bound to each β subunit (sample 2). Then, streptavidin was added to the anti-β antibody (sample 3), and the H9 antibody was bound to the streptavidin in series via biotin (sample 4). Finally, H9 virus was captured specifically by the H9 antibody (sample 5).

The holoenzyme activities of the 5 samples were measured and compared as depicted in Section 2.8. All samples with different external links were treated as Fig. 1.

Mapping of the anti-β antibody recognition site

The $F_1\alpha$ and β subunits are structurally similar, and each comprises three domains. The apical N-terminal domains (domain 1, red) interact to form a β barrel (Fig. 2A–b,c). The central domains (domain 2, green) contain the nucleotide-binding sites, which are

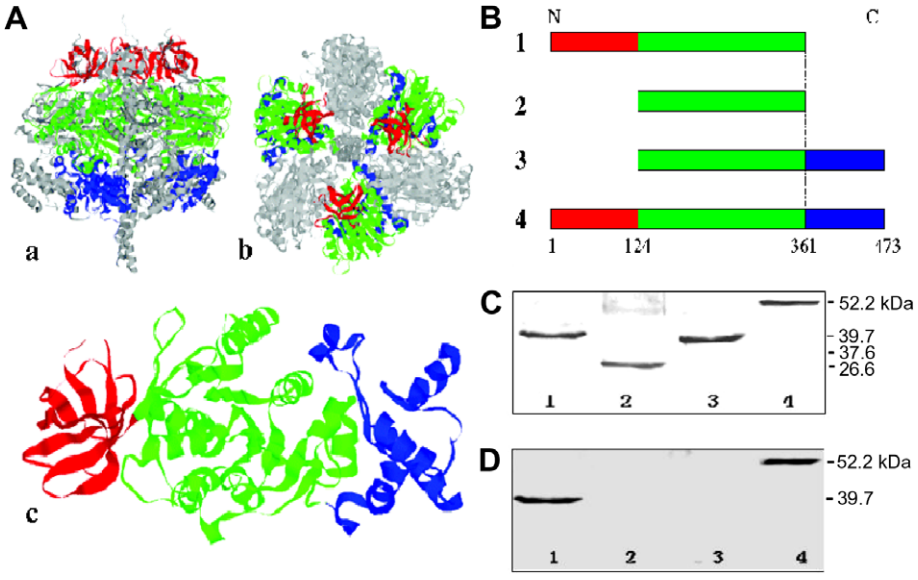

Fig. 2. (A) Relative position of three domains (indicated in different colors) of the $F_1\beta$ subunit (a, b). Three domains of the β subunit (c): domain 1 (red), domain 2 (green) and domain 3 (blue). ATP synthesis and hydrolysis occur in domain 2. Three domains undergo conformational changes during enzymatic catalysis, with domain 3 interacting with the rotating "rotor". Domain 1 remains relatively fixed in interactions with the β barrel domains of adjacent α subunits (gray), forming a hexameric crown as seen in (b). Reprinted with permission from Ref [6]. (B) Expression and purification of fragments of the β subunit. Fragments 1, 2, 3, and 4 contain domains 1 and 2, domain 2, domains 2 and 3, and the full length β subunit, respectively. (C) Purification of 4 recombinant fragments. Purified proteins were analyzed by SDS–PAGE. Lane 1, 2, 3 and 4 correspond to fragment 1, 2, 3 and 4, respectively. Molecular weights of the constructs were identical to the PVDF used in (D) to determine the antigen recognition site of β antibody. (D) Immunoreactivity of β antibody to β subunit. Purified, recombinant fragments submitted to SDS–PAGE and Western blotting were detected by anti-β antibody. Immunoblotting with anti-β antibody did not detect the constructs of individual domain 2 (lane 2) or domains 2 and 3 (lane 3), but clearly detected domains 1 and 2 (lane 1) and the entire β subunit (lane 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

catalytic in the β subunit, but not in the α subunit (Fig. 2A–c). The C-terminal domains (domain 3, blue) fold in α-helices and interact with the eccentrically rotating γ.

To determine the epitope recognized by the anti-β antibody, we selected an antibody (2D5) and generated recombinant domains of the $F_1\beta$ subunit. The four fragments were designated as domains 1 and 2, domain 2, domains 2 and 3, and the full length β subunit, as shown in Fig. 2B and C. In Western blot assays, the anti-β antibody strongly detected the fragments 1 and 4, as shown in Fig. 2D. The anti-β antibody did not recognize the individual domain 2, or domains 2 and 3. Although the expression construct of the individual domain 1 was not generated, these results revealed that the employed anti-β antibody targets the non-catalytic domain 1 of the β subunit. This implies that the external linked complexes do not directly affect the function of the catalytic site of the β subunit.

The activity of the holoenzyme with different external links on the β subunits

ATP synthesis and hydrolysis activities were measured for different samples. To show the regulation effect, the activity of native F_oF_1-ATPase was used as a control and the sample's activity was compared with the control. When the anti-β antibody was bound, the F_oF_1-ATPase (sample 2) activities in both synthesis and hydrolysis decreased, and its relative value was about 85% of the native form (sample 1). If streptavidin was joined to the anti-β antibody (sample 3), the enzyme synthesis/hydrolysis activities decreased to 87% of the activity of sample. After the H9 antibody was connected to streptavidin (sample 4), enzyme activity was inhibited further, with a relative value of 75% of sample 3. Once the H9 virus was captured by its antibody (sample 5), however, the motor was activated, and its relative synthesis and hydrolysis activities were about 210% of that of sample 4. It is surprising that the activity of F_oF_1-ATPase in which a series of external complexes are linked on the β subunits, can exceed the native level. These results are indicated in columns 2, 3, 4 and 5 of Fig. 3.

To identify whether H9 virus affects F_oF_1-ATPase activity directly, the activity of the native buffer (sample 1) incubated with the H9 virus (sample 6) was measured. Fig. 3 shows that the H9 virus does not have any direct effect on the holoenzyme activity, whether the enzyme functions as a synthase or hydrolase (note that the value of column 6 is almost the same as that of column 1).

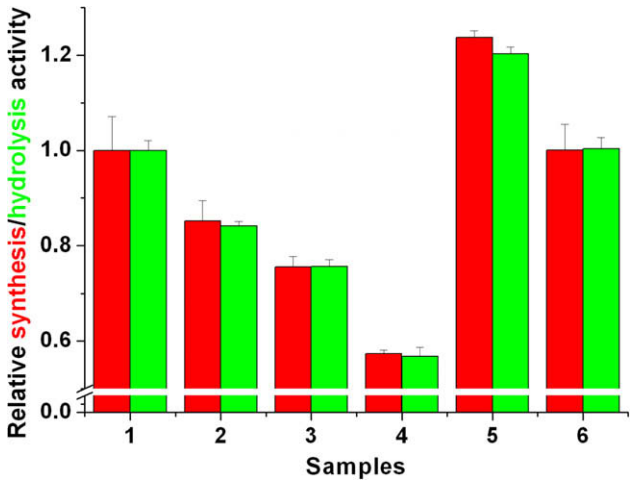

Fig. 3. Relative synthesis (red) and hydrolysis (green) activities of the holoenzyme with different links on β subunits shown in Fig. 1. The statistical value (mean ± SEM) of each sample was computed by 50~60 samples. The experiment has been repeated independently more than five times (10 samples for each time). The native F_oF_1-ATPase (sample 1) activity was taken as a control, and the others were expressed in proportion to the control. Sample 2, 3, 4 and 5 represent F_oF_1-ATPase binding to anti-β antibody, streptavidin, H9 antibody and H9 virus, respectively. Sample 6 represents the holoenzyme activity when buffer was mixed directly with H9 virus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The affinity of ATP studied with ESR

To further investigate the mechanism of the regulation of enzyme activity, Electron Spin Resonance (ESR) was employed to study the affinity of ATP to F_oF_1-ATPase with different external links. As an ATP analog, spin-labeled ATP is rarely hydrolyzed, and thus has been widely employed in various studies of structure–function relationship of F_oF_1-ATPase [27]. The holoenzyme exhibits 3 nucleotide-binding sites in the cleft between the β and α subunits. Fig. 4 shows the ESR signal intensity of the covalent complex of SL-ATP. The binding rate of ATP to F_oF_1-ATPase correlated positively with the molecular weight of the adducts on the β subunit. Curve A shows the ESR signal intensity of SL-ATP with the native F_oF_1-ATPase (sample 1). The intensity of the ESR signal from the β subunit linked with the anti-β antibody–biotin–streptavidin–biotin–H9 antibody complex (sample 4; curve B) increased by 65% above that of the control. Furthermore, when the H9 virus was captured (sample 5), the ESR intensity increased (curve C) to 213% of that of the native enzyme (curve A). These results suggest that the external complex bound to the non-catalytic sites of the β subunits might induce conformational changes of the catalytic sites, and also facilitate ATP binding. This conformational change of the catalytic site may arise from the interaction between the external complex and the non-catalytic domain 1 over a long distance [28].

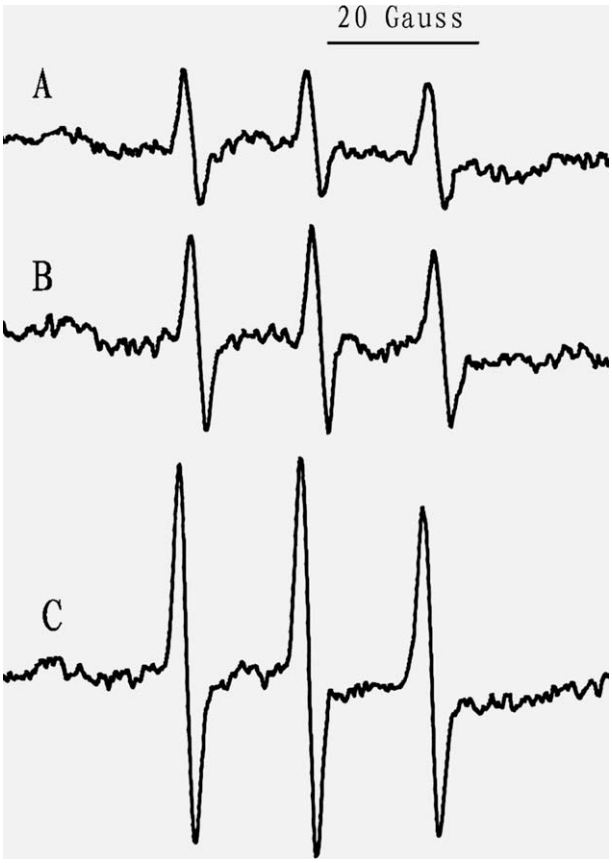

Fig. 4. ESR spectrum of covalently incorporated SL-ATP in the F_oF_1-ATPase chromatophore. (A) Native F_oF_1-ATPase (sample 1); (B) β subunit bound to anti-β antibody–biotin–streptavidin–biotin–H9-antibody (sample 4); (C) the β subunit was bound to anti-β antibody–biotin–streptavidin–biotin–H9 antibody-H9 virus (sample 5).

Discussion

We have presented a simple method to regulate the F_oF_1-ATPase activity through external complexes linking to the exposed "stator" (β subunits). The holoenzyme activity was inhibited with more external links, but activated if the virus binds its antibody. The change in synthesis activity was almost the same as hydrolysis activity. That the holoenzyme was activated significantly by the binding of a large structure, such as a virus, was unexpected. Any potential direct effect of the virus on holoenzyme activity was excluded (column 6 in Fig. 3).

The ESR study shows that the affinity of ATP for the holoenzyme increases monotonously with the increase in the molecular weight of the external links. This is different from the trend in the regulation of enzyme activity. The conformational change of the β subunit induced by external links may be of great benefit to substrate binding, and the rate-limiting step may be the release of the product, not the binding of the substrate [10].

In addition, Western blotting has proved that the employed anti-β antibody targets the non-catalytic site. Considering that the conformational changes of the β subunit are coupled with that of the neighboring α subunit [28–30], it can be concluded that external links on α subunits may also regulate holoenzyme activity.

The large difference in the activity between sample 4 and 5 indicates that sample 4 has great potential for the design of rapid, free labeled, sensitive and selective biosensors [32]. Nevertheless, the underlying mechanism of regulation is still unclear.

Acknowledgments

We thank Dr. Zhi-Qian Ni (Harbin Veterinaty Research Institute, CAAS) for the generous gift of the influenza H9 virus and its antibody. This work was supported by the National Basic Research Program of China (973 Program) under the Grant Nos. 2007CB935901 and 2007CB935903, and National Nature Science Foundation of China (20873176, 90923009).

References

[1] P.D. Boyer, The ATP synthase—a splendid molecular machine, Annu. Rev. Biochem. 66 (1997) 717–749.
[2] J.P. Abrahams, A.G. Leslie, R. Lutter, et al., Structure at 2.8 Å, resolution of F_1-ATPase from bovine heart mitochondria, Nature 370 (1994) 621–628.
[3] H. Noji, R. Yasuda, M. Yoshida, et al., Direct observation of the rotation of F_1-ATPase, Nature 386 (1997) 299–302.
[4] W. Junge, Protons, proteins and ATP, Photosynth. Res. 80 (2004) 197–221.
[5] J. Weber, A.E. Senior, ATP synthesis driven by proton transport in F_oF_1-ATP synthase, FEBS Lett. 545 (2003) 61–70.
[6] S.L. Chi, M.L. Wahl, Y.M. Mowery, et al., Angiostatin-like activity of a monoclonal antibody to the catalytic subunit of F_oF_1 ATP synthase, Cancer Res. 67 (2007) 4716–4724.
[7] R. Yasuda, H. Noji, K. Kinosita Jr, et al., F1-ATPase is a highly efficient molecular motor that rotates with discrete 120° step, Cell 93 (1998) 1117–1124.
[8] H. Itoh, A. Takahashi, K. Adachi, et al., Mechanically driven ATP synthesis by F_1-ATPase, Nature 427 (2004) 465–468.
[9] Y.H. Zhang, J. Wang, Y.B. Cui, et al., Rotary torque produced by proton motive force in F_oF_1 motor, Biochem. Biophys. Res. Commun. 331 (2005) 370–374.
[10] Y.G. Shu, P.Y. Lai, Systematic kinetics study of F_oF_1-ATPase, J. Phys. Chem. B. 112 (2008) 13453–13459.
[11] O. Pänke, B. Rumberg, Kinetic modeling of the proton translocating CF_oCF_1-ATP synthase from spinach, FEBS Lett. 383 (1996) 196–200.
[12] M. Diez, B. Zimmermann, M. Börsch, et al., Proton-powered subunit rotation in single membrane-bound FoF1-ATP synthase, Nat. Struct. Mol. Biol. 11 (2004) 135–141.
[13] S. Steigmiller, P. Turina, P. Gräber, The thermodynamic H^+/ATP ratios of the H^+-ATPsynthases from chloroplasts and *Escherichia coli*, Proc. Natl. Acad. Sci. USA 105 (2008) 3745–3750.
[14] Y.B. Cui, Z. Fan, J.C. Yue, Detecting proton flux across chromatophores driven by F_oF_1-ATPase using *N*-(fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt, Anal. Biochem. 344 (2005) 102–107.
[15] X.L. Liu, Y. Zhang, J.C. Yue, et al., F_oF_1-ATPase as biosensor to detect single virus, Biochem. Biophys. Res. Commun. 342 (2006) 1319–1322.
[16] Y. Zhang, Z.T. Deng, J.C. Yue, et al., Using cadmium telluride quantum dots as a proton flux sensor and applying to detect H9 avian influenza virus, Anal. Biochem. 364 (2007) 122–127.
[17] Z.T. Deng, Y. Zhang, J.C. Yue, et al., Green and orange CdTe quantum dots as effective pH-sensitive fluorescent probes for dual simultaneous and independent detection of viruses, J. Phys. Chem. B 111 (2007) 12024–12031.
[18] X.L. Liu, J.C. Yue, G. Yu, et al., Fabrication of F_oF_1-ATPase nanostructure on gold surface through dip-pen nanolithography, J. Nanosci. Nanotechnol. 8 (2008) 5753–5756.
[19] G. Köhler, C. Milstein, Continuous cultures of fused cells secreting antibody of predefined specificity, Nature 256 (1975) 495–497.
[20] R.A. Machado de Avila, L.M. Alvarenga, C.A. Tavares, et al., Molecular characterization of protective antibodies raised in mice by *Tityus serrulatus* scorpion venom toxins conjugated to bovine serum albumin, Tixicon 44 (2004) 233–241.
[21] D. Khananshvili, Z. Gromet-Elhanan, Isolation and purification of an active gamma-subunit of the FoF1-ATP synthase from chromatophore membranes of *Rhodospirillum rubrum*. The role of gamma in ATP synthesis and hydrolysis as compared to proton translocation, J. Biol. Chem. 257 (1982) 11377–11383.
[22] S. Philosoph, A. Binder, Z. Gromet-Elhanan, Coupling factor ATPase complex of *Rhodospirillum rubrum*. Purification and properties of a reconstitutively active single subunit, J. Biol. Chem. 252 (1977) 8747–8752.
[23] R.K. Clayton, Two light reactions of bacteriochlorophyll *in vivo*, Proc. Natl. Acad. Sci. USA 50 (1963) 583–587.
[24] B. Streckenbach, D. Schwarz, K.H.R. Repke, Analysis of phosphoryl transfer mechanism and catalytic centre geometries of transport ATPase by means of spin-labelled ATP, Biochim. Biophys. Acta 601 (1980) 34–36.
[25] P. Vogel-Claude, G. Schäfer, W.E. Trommer, Synthesis of a photoaffinity-spin-labeled derivative of ATP and its first application to F_1-ATPase, FEBS Lett. 227 (1988) 107–109.
[26] H.S. Penefsky, A centrifuged-column procedure for the measurement of ligand binding by beef heart F_1, Methods Enzymol. 56 (1979) 527–530.
[27] P.D. Vogel, Insight into ATP synthase structure and function using affinity and site-specific spin labeling, J. Bioenerg. Biomembr. 32 (2000) 413–421.
[28] D. Bray, T. Duke, Conformational spread: the propagation of allosteric states in large multiprotein complexes, Annu. Rev. Biophys. Biomol. Struct. 33 (2004) 53–73.
[29] G. Oster, H. Wang, How protein motors convert chemical energy into mechanical work, in: Manfred Schliwa (Ed.), Molecular Motors, Wiley-VCH Verlag, GmbH, Weinheim, Germany, 2003, pp. 207–227.
[30] M. Strajbl, A. Shurki, A. Warshel, Converting conformational changes to electrostatic energy in molecular motors: The energetics of ATP synthase, Proc. Natl. Acad. Sci. USA 100 (2003) 14834–14839.
[32] J. York, D. Spetzler, F.S. Xiong, et al., Single-molecule detection of DNA via sequence-specific links between F_1-ATPase motors and gold nanorod sensors, Lab Chip 8 (2008) 415–419.