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Rapid detection of several foodborne pathogens by F_0F_1-ATPase molecular motor biosensor

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ABSTRACT

F_0F_1-ATPase within chromatophore was constructed as a molecular motor biosensor through ε-subunit antibody–biotin–streptavidin–biotin–AC5-Sulfo-Osu system. Based on probe-DNA specific binding, DNA of several foodborne pathogens *Listeria monocytogenes*, *Salmonella typhimurium*, *Vibrio parahaemolyticus* and *Vibrio cholerae* was specifically captured by F_0F_1-ATPase molecular motor biosensors. Loads of DNA decreased the rotation rate of F_0F_1-ATPase, and led to the decrease of ATP synthesis. The detection of pathogens based on proton flux change driven by ATP-synthesis of F_0F_1-ATPase, which was indicated by F-DHPE, was monitored by a fluorescence spectrometer. The results demonstrate that the F_0F_1-ATPase molecular motor biosensor can specifically detect bacterial DNA at low concentration level, and will be a convenient, quick, and promising tool for detecting pathogens.

1. Introduction

F_0F_1-ATP synthase is a key enzyme in the biological world and is one of the most ubiquitous abundant proteins on the earth. F_0F_1-ATP synthase in bacterial plasma membranes, mitochondrial inner membranes, and chloroplast thylakoid membranes catalyzes the endergonic synthesis of ATP from ADP and phosphate using a transmembrane proton-motive force (PMF) generated by oxidative phosphorylation or photosynthesis (Boyer, 1997; Weber and Senior, 2003; Fillingame et al., 2000). The holoenzyme is a complex of two rotary motors, F_0 and F_1, mechanically coupled by a common central stalk. The membrane-embedded F_0 efficiently converts the PMF into mechanical rotation of the central stalk inside the F_1, and the rotation causes cyclic conformational changes in F_1, driving ATP synthesis. The enzyme has a striking characteristic of its reversibility. It may rotate in the reverse direction for ATP hydrolysis and utilize the excess energy to pump protons across the membrane (Fillingame, 1997; Yoshida et al., 2001; Cross, 2000; Weber and Senior, 2000).

The development of nanotechnology has enabled the design and production of a variety of nanodevices and molecular machines. The F_0F_1-ATP synthase is a nanoscale rotary biological motor. Because of its transduction of energy, nanoscale size, and possible practicability in micro/nanotechnology, many scientists have attempted to develop the rotation of F_0F_1-ATP synthase motors as microdevices for applying in nanotechnology (Montemagno and Bachand, 1999; Haiqing et al., 2002). But, it is difficult to use the power generated by mechano-chemical coupling of the motor outside the cell. The fluorescence technique is one of the most powerful methods for studying the structure and function of biomacromolecules (Lakowicz, 1999).

N-(fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (F-DHPE) is a lipid fluorescent probe that is sensitive to pH changes and has been used to measure pH changes adjacent to the bilayer surface (Serowy et al., 2003). The F-DHPE labeled on the surface of chromatophores can be used to detect the proton flux through F_0F_1-ATP synthase driven by ATP hydrolysis or synthesis. It was shown that the F_0F_1-ATP synthase motor would be a novel application as biosensor to detect loads of molecules at single molecular level (Cui et al., 2005a). Liu et al. (2006) reported using F-DHPE labeled on the surface of chromatophores to detect single virus.

Rapid, selective, and sensitive detection of nucleic acids and proteins is vital for the identification of pathogens of food safety importance providing inspecting evidences, and for the identification of known genotypes using hybrid biological/inorganic devices (Fauci, 2002; Lane and Fauci, 2001; Van Den Heuvel and Dekker, 2007; Patolsky et al., 2004). Many methods can be used to detect the pathogens, including biochemical assays, immunological assays and polymerase chain reaction (PCR)-based testing of bacterial nucleic acids. However, most of them are time consuming and require purifying the samples, which make them tedious and inappropriate for clinic and diagnostics. Therefore, it is an important challenge to develop methods that do not rely on target-amplification systems, such as PCR or ligase chain reaction (LCR), that increase the detection time and the potential for error (Li and Rothberg, 2004a, 2004b; Storhoff et al., 2004; Demers et al., 2000).

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The aim of this study is to develop a novel method to detect single molecule of target DNA of several foodborne pathogens *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus* and *V. cholerae* by constructing a molecular motor biosensor using F_0F_1-ATPase within chromatophore. The fluorescence probe F-DHPE labeled on the surface of chromatophores was used as a proton flux indicator. The ε-subunit antibody–biotin–streptavidin–biotin–AC5-Sulfo-Osu system was used as a capture reaction receptor. The detection of pathogens based on proton flux changes driven by ATP-synthesis of F_0F_1-ATPase was monitored by fluorescence spectrometer. It is demonstrated that the F_0F_1-ATPase molecular motor biosensor will be a quick, exact and promising tool for detecting pathogens at single molecule level with high specificity.

2. Materials and methods

2.1. Chemicals and materials

S. typhimurium (ATCC 14028), *Thermomicrobium roseum* (*T. roseum*, ATCC 27502), *V. parahaemolyticus* (ATCC 17802), *V. cholerae* (ATCC 14035), *L. monocytogenes* (ATCC 15313-1), and *Escherichia coli* (*E. coli*, ATCC 25922) were purchased from ATCC (USA). Biotin-AC5-Sulfo-Osu was purchased from Dojindo (Japan). Streptavidin and adenosine diphosphate (ADP) were purchased from Sigma (St. Louis, USA). N-(fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (F-DHPE) was purchased from Invitrogen (California, USA). *E. coli* BL21 and Nickel-nitrilotriacetic acid (Ni-NTA) Sepharose column was purchased from Novagen (Darmstadt, Germany). DNA extraction kit (DP 320) was purchased from Tiangen (Beijing, China). BPW (Buffered peptone water) and BS (Bismuth sulfite) agar for culture of *S. typhimurium*, APW (Alkaline peptone water) supplemented with 3% NaCL and TCBS (Thiosulfate citrate bile salts sucrose) agar for culture of *V. parahaemolyticus*, APW for culture of *V. cholerae*, LB_1 (Listeria enrichment broth base supplemented with 0.002% nalidixic acid and 0.001% acridine yellow) and PALCAM for *L. monocytogenes*, and LST (Lauryl sulfonate tryptone) broth, EC broth and EMB (Eosin methylene blue) agar for *E. coli* were purchased from Land Bridge (Beijing, China). All other analytically purified reagents were purchased domestically.

2.2. Culture of microbes and extraction of DNA

S. typhimurium, *V. parahaemolyticus*, *V. cholerae*, *L. monocytogenes*, and *E. coli* were incubated in liquid medium, inoculated on selective medium, and then inoculated on nutrient agar, respectively. After incubation, bacteria were harvested to extract DNA.

DNA extraction was performed according to the instruction of DNA extraction kit (DP 320). The resulting DNA suspension was stored at −20 °C, and used for the following experiments. Extracted DNA was used as the template of PCR and detecting target of F_0F_1-ATPase molecular motor.

2.3. Preparation of chromatophores and labeling with F-DHPE

Chromatophores were prepared from the cells of *T. roseum* according to Suzuki et al. (2003) and Cui et al. (2005b) with slight modification. *T. roseum* was under 150 rpm shake culture for 24 h at 60 °C, and harvested by centrifugation at 4000 *g* at 4 °C for 30 min. The bacterial pellets were resuspended in extracting buffer (20 mM Tris–Cl, 100 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT, pH 8.0), and harvested by centrifugation at 6000 *g* at 4 °C for 10 min, then resuspended in extracting buffer. The suspension was sonicated on ice for 30 min (5 s on, 5 s off) after addition of 1 mM PMSF, causing the plasma membrane to break up and invert to form vesicles with the F_0F_1-ATPases inside out. The suspension was centrifuged at 25,000 *g* at 4 °C for 30 min and the supernatant fluid was transferred to a new tube and centrifuged at 145,000 at 4 °C for 1 h. The pellets, which are called chromatophores, were resuspended in extraction buffer with 50% glycerol, frozen immediately in liquid nitrogen, and then stored at −80 °C. The concentration of chromatophores in the samples was determined spectrophotometrically (880 nm) according to the method of Clayton (1963).

The fluorescence probe F-DHPE was labeled onto the surface of chromatophores as described previously (Su et al., 2006) with slight modification. Aliquots of 10 μL F-DHPE (200 mg/mL, dissolved in ethanol) were mixed with 200 μL chromatophores, and incubated for 15 min in dark with gentle shaking at room temperature. The solution of 10 mM phosphate buffered saline (PBS, pH 7.4) was added to a volume of 1.3 mL. Labeled chromatophores were harvested by centrifugation at 30,000 *g* at 4 °C for 15 min, and free F-DHPE was washed away with 10 mM PBS by centrifugation at 10,000 *g* at 4 °C for 15 min three times. The resulting pellets were resuspended with 200 μL PBS. The labeled chromatophores are called fluorescent chromatophores in the following text.

2.4. Preparation of anti-epsilon subunit antibody and labeling with biotin

The ε-subunit of F_0F_1-ATPase from *T. roseum* was expressed in *E. coli* BL21 and purified commercially. Approximately, the DNA fragment encoding the peptide of ε-subunit was subcloned to pET22b (+) to generate the expression construct pET22b (+)/ε-subunit with an N-terminal 6× His tag. *E. coli* BL21 transformation and isopropyl β-D-thiogalactoside (IPTG) inducing procedures followed the methods specified by the manufacturer. ε-Subunit expressed in *E. coli* was purified using a Ni-NTA sepharose column according to the manufacturer's protocols, and analyzed using SDS-polyacrylamide gel electrophoresis.

The antibody was prepared as described previously (Hanley et al., 1995), purified by precipitation with 33% $(NH_4)_2SO_4$, and stored at −20 °C before use.

The ε-subunit antibody was labeled with biotin–AC5-Sulfo-Osu on the N′ end as follows: 2 μL of 2 μM biotin–AC5-Sulfo-Os was added in 20 μL ε-subunit antibody at room temperature for 30 min.

2.5. Synthesis of biotin-labeled probe

Probes from housekeeping genes of *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus* and *V. cholerae* were synthesized and labeled with biotin–AC5-Sulfo-Osu on the 5′ end commercially. Probes were described in Table 1.

2.6. Construction of F_0F_1-ATPase molecular motor biosensor

The F_0F_1-ATPase molecular motor biosensor was constructed as follows: Aliquots of 40 μg of biotin-labeled ε-subunit antibody were added to 200 μL fluorescent chromatophores and 10 mM PBS was added to a final volume of 1 mL. After incubating at 37 °C for 1 h, PBS was added to a final volume of 1.4 mL, the pellets were harvested by centrifugation at 30,000 *g* at 4 °C for 10 min and resuspended with 500 μL. Aliquots of 2 μL (2 mg/mL) of streptavidin were added and PBS was added to a final volume of 1 mL. The mixture was shaken at 50–100 rpm at room temperature for 10 min. PBS was added to a

Table 1
Specific probes of bacteria.

Bacteria	Gene	Probes
L. monocytogenes	*prfA*	5′-biotin-GATACAGAAACATCGGTTGGC-3′
S. typhimurium	*invA*	5′-biotin-GTGAAATTATCGCCACGTTCGGGCAA-3′
V. parahaemolyticus	*toxR*	5′-biotin-GTGTTCTGACGCAATCGTTG-3′
V. cholerae	*ompW*	5′-biotin-CACCAAGAAGGTGACTTTATTGTG-3′

prfA, gene encoding positive regulative factor A. *invA*, gene encoding invasion protein A. *toxR*, gene encoding toxin transcriptional activator. *ompW*, gene encoding outer membrane protein W.

final volume of 1.4 mL. The pellets were harvested by centrifugation at 30,000 *g* at 4 °C for 10 min and resuspended with 500 μL PBS. Subsequently, a volume of 50 μL (10 μM) of biotin-labeled probe was added and PBS was added to a final volume of 1 mL. The mixture was shaken at 50–100 rpm at room temperature for 10 min, PBS was added to a final volume of 1.4 mL, the pellets were harvested by centrifugation at 30,000 *g* at 4 °C for 10 min and resuspended with 150 μL glycerol (30%, V/V). The prepared F_0F_1-ATPase molecular motor biosensors were called Chro-*prfA*, Chro-*invA*, Chro-*toxR* and Chro-*ompW* in the following test and stored at −20 °C before use.

2.7. Detection of bacteria by F_0F_1-ATPase molecular motor biosensors

Detection of bacteria by F_0F_1-ATPase molecular motor biosensors was performed as follows: Aliquots of 10 μL of 10 ng/mL DNA from *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus* and *V. cholerae* were added into 1.5 mL eppendorf tubes, respectively. The tubes were transferred on ice for complete cooling after incubating in boiling bath for 3 min. Aliquots of 2 μL Chro-*prfA*, Chro-*invA*, Chro-*toxR* and Chro-*ompW* were diluted with synthetic buffer (0.1 mM Tricine, 10% glycerol, 5 mM NaH_2PO_4, 5 mM $MgCl_2$, pH 9.0) and 10 μL was added to the tubes, respectively. Aliquots of 30 μL initiating buffer (0.1 mM Tricine, 10% glycerol, 5 mM NaH_2PO_4, 5 mM $MgCl_2$, ADP 0.35 mM, NADH 2 mM, pH 9.0) were added respectively and mixed homogenously. Then the reaction tubes were initiated at 37 °C for 30 min, 450 μL of PBS was added respectively and mixed homogenously. Subsequently, aliquots of 50 μL of reaction solution above were applied to 96-well microplate. Then the 96-well microplate was monitored by a F4500 fluorescence spectrometer (Hitachi, Tokyo, Japan). Fluorescence was excited at 496 nm and registered at 519 nm. The controls were performed in the presence of 10 μL H_2O instead of Chro-*prfA*, Chro-*invA*, Chro-*toxR* and Chro-*ompW*. The true fluorescence of sample was calculated by subtracting the background value from the reading value.

To test the specificity of F_0F_1-ATPase molecular motor biosensors to the bacteria, aliquots of 10 μL of 40 ng/mL DNAs from *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus*, *V. cholerae* and *E. coli* were detected by 0.052 mg/mL Chro-*prfA*, 10 μL of 10 ng/mL DNA from *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus*, *V. cholerae* and *E. coli* were detected by 0.026 mg/mL Chro-*toxR*, 10 μL of 60 ng/mL DNA from *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus*, *V. cholerae* and *E. coli* were detected by 0.026 mg/mL Chro-*invA*, and 10 μL of 40 ng/mL DNA from *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus*, *V. cholerae* and *E. coli* were detected by 0.078 mg/mL Chro-*ompW*.

To get the optimal concentrations of F_0F_1-ATPase molecular motor biosensors for the detection experiments, ten-fold serial dilutions of the Chro-*prfA*, Chro-*invA*, Chro-*toxR* and Chro-*ompW* were used to detect 10 μL of 10 ng/mL DNA from *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus* and *V. cholerae*, respectively. Otherwise, the optimal concentrations of bacterial DNA were gotten by detecting ten-fold serial dilutions of DNA with the optimal concentrations of Chro-*prfA*, Chro-*invA*, Chro-*toxR* or Chro-*ompW* obtained above.

The detection experiments were performed in triplicate, and repeated three times. Statistical analyses were performed using the computer program SPSS 13.0. The statistical significance of the difference between mean values was determined by ANOVA, and the difference at $p<0.05$ was considered significant. All the data were expressed as mean ± SD.

3. Results

F-DHPE is known as a lipid fluorescent probe and can be easily labeled on the bilayer surface. F-DHPE is a pH indicator and has been used to measure pH changes adjacent to the bilayer surface. In the range of pH 7.0 to 9.0, F-DHPE is sensitive to pH changes and has a positive correlation with the changes of pH (Cui et al., 2005b). In our study, F-DHPE was labeled on the surface of chromatophores.

As mentioned earlier, F_0F_1-ATPase is a rotary motor. During ATP synthesis, protons are pumped out of the chromatophores, resulting in an increase of concentration of H^+ out of the chromatophores. Thus, the pH out of the chromatophores will decrease, and the fluorescence probe F-DHPE is expected to detect the pH decrease (Cui et al., 2005b). However, loads of target DNA decreased the rotation rate of F_0F_1-ATPase, resulting in the rate of fluorescence intensity decreases slower. Thus, the fluorescence intensity is higher significantly than that of without loads. However, because of Brownian motion, the fluorescence intensity is often lower than that of without loads. That is to say, the significant differences in fluorescence intensity compared with negative control suggest the successful capture of target molecule. The results above are just as this.

The specificity for the target DNA is vital to F_0F_1-ATPase molecular motor biosensors. It was shown that the wells containing *L. monocytogenes* DNA-Chro-*prfA* reaction mixture had a significant decrease in fluorescence value ($p<0.05$, Fig. 1A) compared with those of H_2O-Chro-*prfA* reaction mixture and other bacterial DNA-Chro-*prfA* reaction mixture, the wells containing *S. typhimurium* DNA-Chro-*invA* reaction mixture had a significant decrease in fluorescence value ($p<0.05$, Fig. 1B) compared with those of H_2O-Chro-*invA* reaction mixture and other bacterial DNA-Chro-*invA* reaction mixture, the wells containing *V. parahaemolyticus* DNA-Chro-*toxR* reaction mixture had a significant decrease in fluorescence value ($p<0.05$, Fig. 1C) compared with those of H_2O-Chro-*toxR* reaction mixture and other bacterial DNA-Chro-*toxR* reaction mixture, and the wells containing *V. cholerae* DNA-Chro-*ompW* reaction mixture had a significant decrease in fluorescence value ($p<0.05$, Fig. 1D) compared with those of H_2O-Chro-*ompW* reaction mixture and other bacterial DNA-Chro-*ompW* reaction mixture. However, there were no significant differences in fluorescence value among the wells containing H_2O-chromatophores reaction mixture and other non-target DNA-chromatophores reaction mixture. These demonstrated that Chro-*prfA*, Chro-*invA*, Chro-*toxR* and Chro-*ompW* could capture specifically DNA of *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus* and *V. cholerae*, respectively.

The value obtained by subtracting the fluorescence value of bacterial DNA-chromatophores reaction mixture with that of H_2O-chromatophores reaction mixture was used to demonstrate the detection performance of chromatophores, and called Δ value here. A larger Δ value suggests more DNA bound to chromatophores and better detection performance. In our study, it was shown that 0.052 mg/mL Chro-*prfA*, 0.026 mg/mL Chro-*invA*, 0.1558 mg/mL Chro-*toxR* and 0.078 mg/mL Chro-*ompW* could result in a larger Δ value (data not shown) than other dilutions, and 40 ng/mL *L. monocytogenes*, 60 ng/mL *S. typhimurium*, 40 ng/mL *V. parahaemolyticus* and 40 ng/mL *V. cholerae* could result in a larger Δ value (data not shown) than other dilutions. Therefore, the optimal concentrations of Chro-*prfA*, Chro-*invA*, Chro-*toxR* and Chro-*ompW* were 0.078 mg/mL, 0.026 mg/mL, 0.052 mg/mL and 0.1558 mg/mL, respectively. The optimal DNA concentrations of *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus* and *V. cholerae* were 40 ng/mL, 60 ng/mL, 40 ng/mL and 40 ng/mL, respectively.

4. Discussion

F_0F_1-ATPase is a reversible motor for ATP synthesis/hydrolysis, the protons are pumped out of chromatophores when the F_0F_1-ATPase rotates clockwise and the ATP is synthesized; reversely, hydrolysis of ATP results in counter clockwise rotation of F_0F_1-ATPase and drives protons pump into the chromatophores. F-DHPE is pH dependent. The fluorescence intensity of F-DHPE increases with the increasing of pH. So, the rate of fluorescence intensity change can reflect the rotation rate of F_0F_1-ATPase and further reflect the weight of loads. Based on this principle, the F_0F_1-ATPase within chromatophores can be used as biosensor. In our study, F_0F_1-ATPase within chromatophore

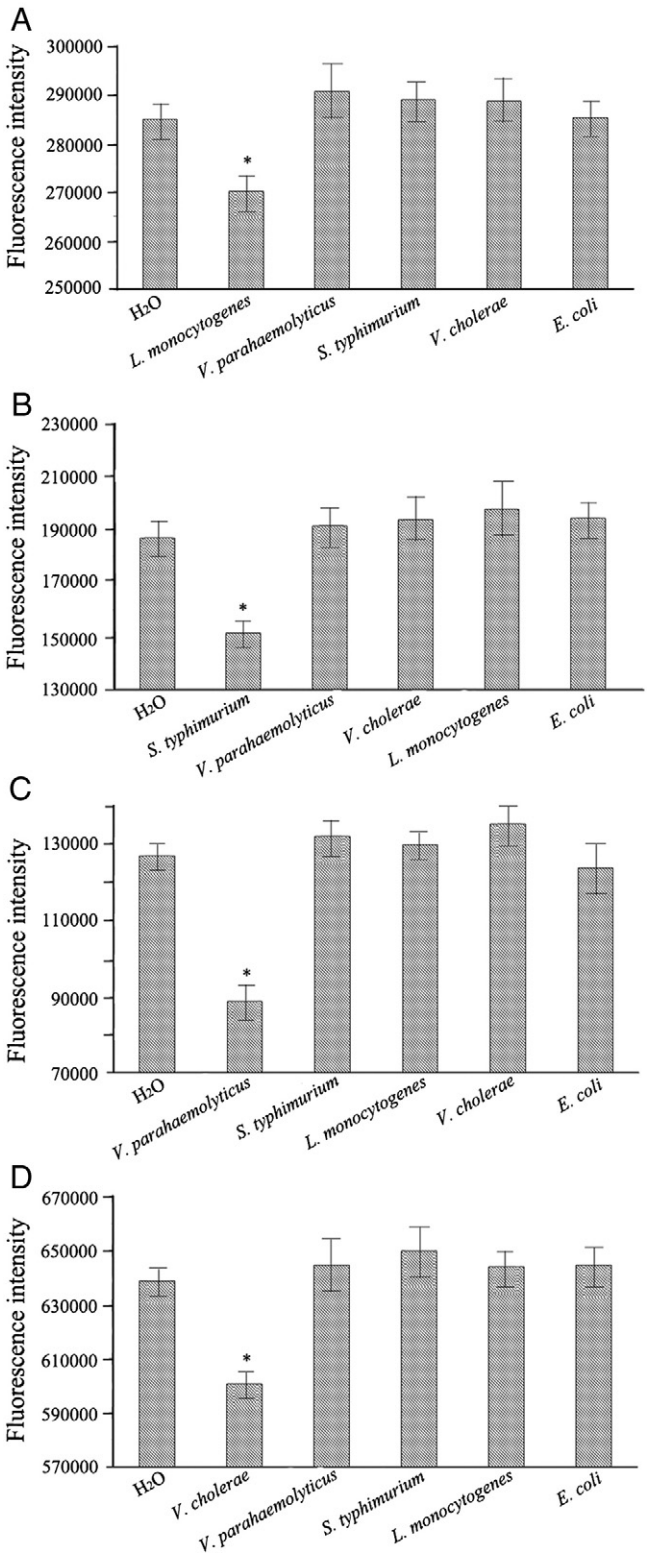

Fig. 1. Fluorescence values of reaction mixture. (A) Aliquots of 10 μL of 40 ng/mL DNA from *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus*, *V. cholerae* and *E. coli* were detected by 0.052 mg/mL Chro-*prfA*. (B) Aliquots of 10 μL of 60 ng/mL DNA from *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus*, *V. cholerae* and *E. coli* were detected by 0.026 mg/mL Chro-*invA*. (C) Aliquots of 10 μL of 10 ng/mL DNA from *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus*, *V. cholerae* and *E. coli* were detected by 0.026 mg/mL Chro-*toxR*. (D) Aliquots of 10 μL of 40 ng/mL DNA from *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus*, *V. cholerae* and *E. coli* were detected by 0.078 mg/mL Chro-*ompW*. Data were expressed as mean values ± SD of three separate experiments. The bars represent SD of mean values, and the asterisks indicate statistical significance ($p<0.05$).

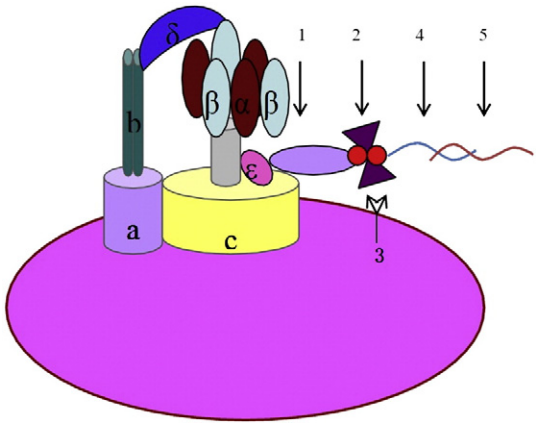

Fig. 2. Schematic diagram of F_0F_1-ATPase molecular motor biosensor. F_0F_1-ATPase within chromatophore was constructed as a molecular motor biosensor through ε-subunit antibody–biotin–streptavidin–biotin–AC5-Sulfo-Osu system. Based on the probe-DNA sequence-specific binding, the single strand target DNA is captured specifically by the F_0F_1-ATPase molecular motor biosensor. 1, ε-subunit antibody; 2, Streptavidin; 3, biotin; 4, probe; 5, single strand target DNA.

was constructed as a molecular motor biosensor through ε-subunit antibody–biotin–streptavidin–biotin–AC5-Sulfo-Osu system. Schematic diagram of the F_0F_1-ATPase molecular motor biosensor is shown in Fig. 2.

To confirm that F_0F_1-ATPase molecular motor biosensors were only in response to their target DNA specifically, DNAs from *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus*, *V. cholerae* and *E. coli* were detected by Chro-*prfA*, Chro-*invA*, Chro-*toxR* and Chro-*ompW*, respectively. It was found that only the wells containing target DNA and the chromatophores had an obvious decrease in fluorescence value (Fig. 1). It clearly indicates that only the target DNA can decrease the ATP synthesis activity specifically. In fact, the specificity is based on the probe-DNA sequence-specific binding. We used probes with length of 20–30 base pairs, which are long enough to ensure specificity during hybridization, yet short enough that resulting ligated bridges remain inflexible.

Compared to other pathogen detection technologies, our self-assembling nanodevice offers significant advantages that directly impact the limitations imposed by extensive detection times, target amplification, sequence specificity, and nonspecific binding. As well as its speed, high sensitivity and specificity make the method more powerful. In our system, the assay avoids the need for thermal cycling since capture of target DNA is performed at 37 °C. So the method is easy-to-use and quicker than PCR. The probe-DNA sequence-specific binding endows the method with high specificity, which overcomes the limitation of nonspecific binding of immunological hybridization methods. The capability of capturing single molecule makes the method to have lower detection limit. By the formula the minimum DNA amount $\times (6.02\times10^{23})/$(bps of bacterial genome $\times$ 660 dolton/bp), the numbers of CFU of *L. monocytogenes*, *S. typhimurium*, *V. parahaemolyticus* and *V. cholerae* that are needed to obtain the minimum DNA amount to be detected by the molecular motor biosensor are calculated approximately, and are 300, 180, 180 and 220, respectively. Because of these advantages above, the method has potential for use in single-molecule protein detection by functionalizing the gold nanorods and F_0F_1-ATPase with target-specific antibodies. It can be predicted that F_0F_1-ATPase molecular motor biosensor will be a convenient, quick, exact, and promising tool for detecting pathogenic particles including bacteria, virus, protein, and so on.

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