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FULL LENGTH ARTICLE

Highly sensitive detection of NT-proBNP by molecular motor

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Abstract F_oF_1-ATPase is an active rotary motor, and generates three-ATP for each rotation. At saturated substrate concentration, the motor can achieve about 10^3 r.p.m, which means one motor can generate about 10^5 ATP molecules during 30 min. Here, we constituted a novel nanodevice with a molecular rotary motor and a "battery", F_oF_1-ATPase and chromatophore, and presented a novel method of sandwich type rotary biosensor based on ε subunit with one target-to-one motor, in which one target corresponds 10^5 ATP molecules as detection signals during 30 min. The target such as NT-proBNP detection demonstrated that this novel nanodevice has potential to be developed into an ultrasensitive biosensor to detect low expressed targets.

Introduction

F_oF_1-ATPase is the ubiquitous enzyme that uses the transmembrane electrochemical potential to synthesize ATP in bacteria, chloroplasts and mitochondria. The holoenzyme can be divided into two rotary motors, Fo and F_1. F_1 motor consists of a crown type "stator" ($\alpha_3\beta_3$) and a eccentric "rotor" (γ), while Fo motor consists of a "stator" (a subunit) embedded in membrane and a ring channels "rotor" (c_n). The two "stators" are fixed by b_2 and δ subunits, while the two "rotors" are mechanically coupled by ε subunit. The membrane embedded Fo unit converts the proton motive force (p.m.f) into mechanical rotation of the "rotor", thereby causing cyclic conformational change of $\alpha_3\beta_3$ crown ("stator") in F_1 and driving three ATP molecules synthesis for each rotation at nearly 100% efficiency.[1–7]

In vitro, however, F_oF_1-ATPase must be reconstituted in polymersome and coupled with Bacteriochlorophyll to maintain its ATP synthesis, where the Bacteriochlorophyll converted the light energy into transmembrane p.m.f.[8] The polymersome with Bacteriochlorophyll was named chromatophore. The combination of F_oF_1-ATPase and chromatophore is a sophisticated nanomachine, in which

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chromatophore function as a "battery" to power the rotary motor F_oF_1-ATPase, as well as the "battery" can remotely be recharged by light. Furthermore, the combination of FoF1-ATPase and chromatophore can conveniently be prepared by the phototrophic bacterium, in which the cells were disrupted by sonication on ice. Each chromatophore vesicle of 100 nm diameter contains on average one F_oF_1-ATPase.[9]

Additionally, kinetics simulation[10] and nanoporous membrane experiment[11] have demonstrated that the motor can achieve about 10^3 r.p.m at saturated substrate concentration and the chromatophore can processively power the motor for more than one hour once the "battery" was recharged by light,[12–14] which means one motor can generate about 105 ATP molecules during 30 min.[15]

On the other hand, NT-proBNP is the amino-terminal fragment of the prohormone of BNP (brain natriuretic peptide), which is secreted mainly by atrial or ventricular myocardial cells, essentially in response to ventricular wall stress. The biological actions of BNP include diuresis, vasodilatation, reduction of systemic and regional (renal and cardiac) sympathetic tone, myocardial relaxation, anti-hypertrophic and anti-fibrotic effects and cytoprotection of cardiomyocytes. In clinical practice, BNP and NT-proBNP have been introduced because of their stability in biological specimens and their utility in the management of suspected or established heart failure (diagnosis, prognosis and guided medical treatment). That is, NT-proBNP is the biomarker of cardiac impairment.[16] However, the current sensitivity of NT-proBNP detection is 220 pg/ml.[17] There is an urge to have a high sensitive detection of NT-proBNP due to its low expression in early cardiac impairment. Here, we constituted a nanodevice with the combination of F_oF_1-ATPase and chromatophore to detect the NT-proBNP, a biomarker of early diagnoses of heart failure.

Results

As Fig. 1 shows, when target NT-proBNP was captured between antibody1 and antibody2, the motor embedded in chromatophore can be linked to the coverslip surface, while the free motors can be washed away. The number of captured motors is equal to that of NT-proBNP because of there is only one ε subunit in F_oF_1-ATPase motor. The amount of ATP generated by F_oF_1-ATPase, thus, is determined by the number of motors and time of synthesis based on the same storage energy in chromatophore. Here, the motor function as an amplifier in which the number of target NT-proBNP is amplified to 10^5 times ATP molecules during 30 min. The detector is able to be sensitive to 15 pg/ml of NT-proBNP as shown in Fig. 2. Therefore, the active nanodevice has a potential to be developed into a dynamic biosensor.

Materials and methods

Cell lines and reagents

Thermomicrobium roseum wa0073 (ATCC27502) was purchased from ATCC (USA). The luciferase/luciferin ATP detection kits were purchased from Promega Corporation (USA). ADP, (+)-biotin N-hydroxysuccinimide ester and NeutrAvidin were purchased from Sigma–Aldrich (St. Louis, USA). The microplate luminometer was a Centro XS3 LB 960 (Germany).

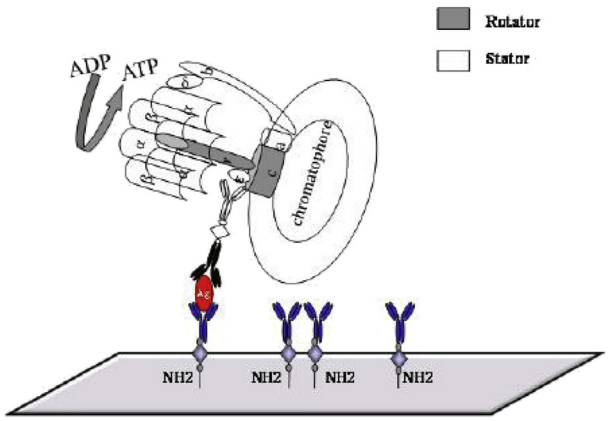

Figure 1 Sandwich type biosensor with one target-to-one motor. The red target, NT-proBNP has two sites, the one is employed to be fixed to the coverslip by antibody1 and avidin; while another site is used to be linked to ε subunit of motor by antibody2, avidin and antibody of ε complex. This immunoassay shows that the number of targets equals to that of motors.

Preparation of chromatophore containing the F_oF_1-ATPase

The chromatophore containing the F_oF_1-ATPase was isolated and purified according to our previous published

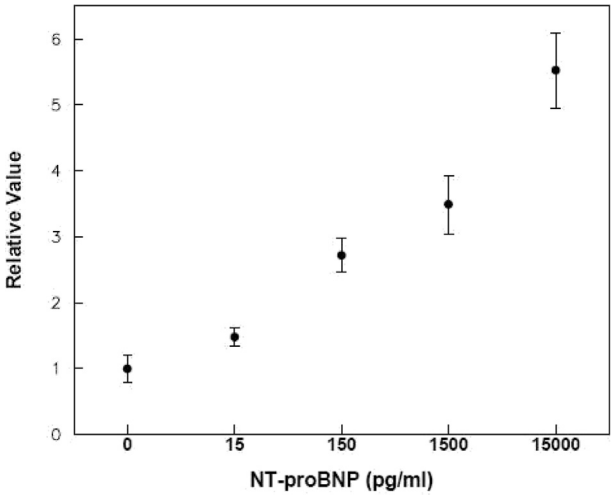

Figure 2 The relation between the relative measured value and NT-proBNT concentration. The light density emitted from luciferase-luciferin at different NT-pro-BNP concentration is normalized into the relative value by that of buffer. The light density was determined by the ATP concentration and measured by luciferase/luciferin ATP detection kit. The measured value increases monotonically with the NT-proBNP concentration. The limiting sensitivity achieved 15 pg/ml.

protocols.[14,18] *Thermomicrobium roseum* was cultured at 60 °C for 24 h, and the cells were collected by centrifugation at 4000 rpm for 20 min. The pellets were resuspended in 20 ml buffer A (pH 6.0), containing 20 mM Tricine-NaOH (pH 6.0), 2 mM $MgCl_2$, 100 mM NaCl, and 10% glycerin (v/v), and sonicated for 3 min. The lysate was centrifuged at 8000 rpm with R20A2 rotor for 30 min at 4 °C, and the supernatant was collected and centrifuged at 40,000 rpm for 90 min at 4 °C. The precipitate containing the chromatophores was resuspended in buffer B (pH 8.0), with 20 mM Tricine-NaOH (pH 8.0), 2 mM $MgCl_2$, 100 mM NaCl, and 10% glycerin (v/v), and stored at −80 °C for further use.

Preparation of monoclonal antibodies of ε-subunit

The ε-subunit was expressed and purified as in Ref. 19. The ε-subunit monoclonal antibodies were prepared according to the method for monoclonal antibody production procedure in Ref. 20, purified by precipitation with 33% $(NH_4)_2SO_4$ at 4 °C for 12 h, and the IgG parts were separated using Sephadex G-200 and stored at −20 °C before use.

NT-proBNP detection

The NT-proBNP (from the Dapbiotech. company, Beijing) was incubated with antibody 24C5 for 1 h at 37 °C, followed by three times washings, then 1 μl Ab-BNP2 antibody-conjugated chromatophore was added and incubated for 30 min at 37 °C. After three times washings, the ATP synthesis reaction was initiated and reacted for 60 min at 37 °C, then the ATP concentration was assayed using luciferase/luciferin ATP detection kits.

ATP synthesis assay

The ATP synthesis activity of F_oF_1-ATPase within the chromatophores was determined using the luciferin-luciferase method. 30 μl luciferase/luciferin working solution was added into ATP synthesis well. The chemiluminescence signals displayed in microplate luminometer were recorded immediately. The YS101 type high sensitive detector of chemiluminescence was manufactured by Yishang Innovation Technology Co., Ltd. The relative light density emitted from luciferase-luciferin at different NT-pro-BNP concentration is shown in Fig. 2 in which the light density of buffer is normalized. The results indicate that the measured value increases monotonically with the NT-proBNP concentration, and the sensitivity of detection was lower than 15 pg/ml.

Conclusions

We have developed a novel nanodevice constituted with a rotary motor and a "battery", F_oF_1-ATPase and chromatophore. The former can processively rotate at about 10^3 r.p.m for more than one hour once the latter was recharged by light. If the nanodevice is captured by a target such as NT-proBNP and processively rotate for 30 min, the number of targets will be amplified by 10^5 ATP molecules.[15] The sensitivity of the detection was lower than 15 pg/ml, which is more sensitive than the current sensitivity 220 pg/ml.[17] This method has potential to be developed into an ultrasensitive biosensor to detect low expressed targets such as NT-proBNP.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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