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A novel biosensor regulated by the rotator of F_0F_1-ATPase to detect deoxynivalenol rapidly

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ABSTRACT

A novel biosensor (immuno-rotary biosensor) was developed by conjugating deoxynivalenol (DON) monoclonal antibodies with the "rotator" ε-subunit of F_0F_1–ATPase within chromatophores with an ε-subunit monoclonal antibody–biotin–avidin–biotin linker to capture DON residues. The conjugation conditions were then optimized. The capture of DON was based on the antibody–antigen reaction and it is indicated by the change in ATP synthetic activity of F_0F_1–ATPase, which is measured via chemiluminescence using the luciferin–luciferase system with a computerized microplate luminometer analyzer. 10^{-7} mg/ml of DON can be detected. The whole detection process requires only about 20 min. This method has promising applications in the detection of small molecular compounds because of its rapidity, simplicity, and sensitivity.

1. Introduction

F_0F_1–ATPase is a ubiquitous membrane-bound holoenzyme in chloroplasts, bacteria, and in the mitochondria that have two components, a membrane-embedded F_0 ($a_1b_2c_n$) and a water-soluble F_1 ($\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$) [1]. These two parts are mechanically coupled by two stalks, a central stalk composed of γ and ε subunits that links to the c subunit ring and an outer stalk of δ and b_2 subunits that links $\alpha_3\beta_3$ to a subunit. F_0F_1–ATPase is a ubiquitous rotary motor that the electrochemical potential of protons (or sodium ions in some bacteria) across membrane drives to rotate the c-ring oligomer together with the γε complex in a clockwise direction from the F_0 side, forcing a cyclic conformational change in the $\alpha_3\beta_3$ crown ("stator") of F_1, which results in ATP synthesis from adenosine diphosphate (ADP) and inorganic phosphate (Pi) (Fig. 1) [2–5].

Previous investigations [5–10] have demonstrated that the holoenzyme activity can be regulated by external links on the "stator" β subunits and has great potential for designing rapid, free-labeled, sensitive, and selective biosensors. Since the "stator" β subunits conformational change is due to the "rotor" γε motivation, it will be more sensitive to bind the load to the rotor subunit, such as ε-subunit, to construct biosensors. However, whether the ATP synthetic activity of F_0F_1-ATPase can be regulated by external links on the "rotator" ε-subunit has never been studied.

Recent studies have suggested that the ε-subunit is necessary for high-yield and high-rate ATP synthesis. The ε-subunit binds onto the protruding part of the γ-subunit and provides a connection between the rotor parts of F_1 and F_0, and may stabilize the protruding part of the γ-subunit where the mechanical torque is applied [2,11]. It plays an important role in the regulation of ATP synthesis activity [12]. Single molecule assays have indicated that the activity of F_1 or F_0 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 enzyme (including F_0 and F_1), instead of chemical regulation, is still difficult to understand due to that the "rotor" of F_0 F_1 motor is mostly enwrapped by the "stator" $\alpha_3\beta_3$ crown and the exposed fraction of the "rotor" is also partly shaded by the b_2 subunit. [10]. In this study, we tried to investigate the optimized conditions of binding different loads to the ε-subunit of F_0F_1-ATPase and what will happen to the activity of the rotary motor if external complexes bind to the "rotor" ε-subunit. Due to the relationship between ATP synthesis activity and the loads to ε-subunit, F_0F_1 motors have potential as novel biosensors in the application of rapid and sensitive detection.

Deoxynivalenol (DON) is a mycotoxin produced largely by *Fusarium* fungi, which is the most common trichothecene that is frequently detected in cereal grains such as wheat, corn, and barley for human and animal consumption [13]. DON reduces yield and quality, and causes symptoms such as vomiting, diarrhea, and headaches upon human and animal ingestion [14]. It also possesses

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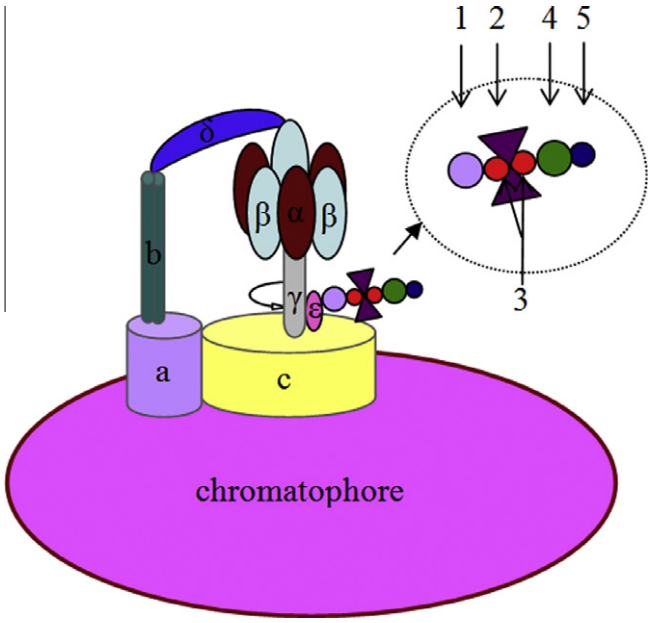

Fig. 1. The schematic illustration of immuno-rotary biosensor based on F_0F_1-ATPase. (1) ε-Subunit monoclonal antibody; (2) neutroavidin; (3) biotin; (4) DON monoclonal antibody; and (5) DON molecular.

significant cytotoxic activity, which may imply an immunosuppressive effect [15]. Currently, the maximum levels set by the US Food and Drug Administration and the European Commission for unprocessed durum wheat and maize are 1000 μg kg^{-1} and 1750 μg kg^{-1}, respectively [13]. To determine the DON levels in foods and feeds, several chromatographic methods and immunoassays have been developed for its detection, such as the thin-layer chromatography, high-performance liquid chromatography, gas chromatography [15], enzyme-linked immunosorbent assay, lateral flow immunoassay, and radioimmunoassay [16], which are time consuming, complicated, and require expensive equipment and reagents. Therefore, a novel simple and fast method is required for rapid DON detection.

F_0F_1–ATPase within chromatophores is an ideal biomaterial that can be developed into biosensors. This is the first report on a novel biosensor regulated by the rotator of F_0F_1–ATPase. The construction conditions of the novel biosensor were optimized. The ATP synthesis activity of the F_0F_1–ATPase regulated by external links on the ε-subunit has been studied in detail, and the residues of deoxynivalenol were detected rapidly using this novel rotary nanoscale biosensor.

2. Materials and methods

2.1. Chemicals and materials

Thermomicrobium roseum, wa0073 (ATCC27502) was purchased from ATCC, USA. ENLITEN Luciferase/Luciferin Reagent was purchased from Promega, USA. Aflatoxin B1, ADP, (+)-biotin *N*-hydroxysuccinimide ester, and neutroavidin were purchased from Sigma–Aldrich (St. Louis, USA). FITC was purchased from Molecular Probes (Eugene, Oregon, USA). Avidin, Alexa Fluor® 488 conjugate was purchased from Invitrogen, USA. DON was purchased from Fermentek Ltd., Israel. DON monoclonal antibodies were purchased domestically. The fluorometric luminometer reader was a Fluoroskan Ascent (Thermolabsystems, Finland). The microplate luminometer was a Centro XS3 LB 960 (Germany). All other analytically purified reagents were purchased domestically.

2.2. Preparation of chromatophores containing F_0F_1-ATPase

Chromatophores were prepared from the cells of *Rhodospirillum rubrum* according to Refs. [17,18].

2.3. Preparation of ε-subunit monoclonal antibodies

The ε-subunit was expressed and purified as in Ref. [19]. The ε-subunit monoclonal antibodies were prepared according to the method for β-subunit monoclonal antibodies in Ref. [10], purified by precipitation with 33% $(NH_4)_2SO_4$, and the IgG parts were separated using Sephadex G-200 and stored at −20 °C before use.

2.4. Preparation of FITC-labeled ε-subunit monoclonal antibodies and FITC-labeled DON monoclonal antibodies

FITC labeling was achieved by dialyzing the ε-subunit and DON monoclonal antibodies against a coupling buffer (90 mM $NaHCO_3$, 10 mM Na_2CO_3, and 126 mM NaCl, at pH 9.0). Up to 2 ml of dialyzed ε-subunit antibodies (0.5 mg/ml) and DON monoclonal antibodies (0.036 mg/ml) were mixed with 1.5 ml and 0.11 ml of FITC (0.1 mg/ml), respectively, for 8 h at 4 °C in coupling buffer. The mixtures were then dialyzed against PBS buffer (pH 7.4).

2.5. Preparation of biotinylated ε-subunit monoclonal antibodies and biotinylated DON monoclonal antibodies

The ε-subunit monoclonal antibodies were biotinylated by adding 2 μl of 0.5 mg/ml (+)-biotin *N*-hydroxysuccinimide ester into 20 μl of 0.5 mg/ml ε-subunit monoclonal antibodies and incubated at room temperature for 4 h. The redundant free (+)-biotin *N*-hydroxysuccinimide ester was removed thrice using dialysis in PBS. The biotinylated DON and ε-subunit monoclonal antibodies were prepared.

2.6. The immuno-rotary biosensor (IRB) was constructed as follows

Approximately, 15 μl of chromatophores (50 mg/ml) and 8 μl (0.5 mg/ml) of biotinylated ε-subunit monoclonal antibodies were mixed, diluted with 1 ml with PBS buffer, and then incubated at 37 °C for 60 min. The free ε-subunit monoclonal antibodies were washed away by centrifugation at 40,000×*g* for 20 min at 4 °C. The precipitate was resuspended in 500 μl of PBS buffer. Then, 7.5 μl (0.1 mg/ml) of neutroavidin was added and diluted into 1 ml with PBS buffer, and incubated at 37 °C for 10 min. The free neutroavidin was then washed away by 20 min centrifugation at 40,000×*g* at 4 °C. The precipitate was resuspended in 500 μl of PBS buffer. Then, 28 μl (0.036 mg/ml) of biotinylated DON monoclonal antibodies were added, diluted into 1 ml with PBS buffer, and incubated at 37 °C for 10 min. The free DON monoclonal antibodies were then washed away by centrifugation at 40,000×*g* for 20 min at 4 °C. The precipitate was resuspended in 500 μl of PBS buffer.

2.7. FITC fluorescence intensity detection

Up to 100 μl of FITC-labeled F_0F_1-ATPase molecular motor biosensor was used to detect the fluorescence intensity with a fluorometric luminometric reader (485 nm excitation and 518 nm emission). Each sample was assayed thrice.

2.8. ATP synthesis activity assay of F_0F1-ATPase within the chromatophores

The ATP synthesis activity of F_0F1-ATPase within the chromatophores was determined using the luciferin–luciferase method via a

microplate luminometer according to the instructions included with the ENLITEN Luciferase/Luciferin Reagent. Up to 10 μl of the F_0F_1-ATPase molecular motor biosensors and 30 μl of ATP synthesis buffer (containing 20 mM Tricin–NaOH pH 8.0, 2 mM $MgCl_2$, 2 mM Na_2HPO_4, and 0.5 mM ADP) were mixed together, and incubated at 37 °C for 10 min. After the reaction was terminated by adding 450 μl of PBS, 50 μl of dilution buffer was added to 30 μl of the luciferase/luciferin reagent, and was mixed. The chemiluminescence was then immediately detected using a microplate luminometer.

2.9. Detection of DON using the novel biosensor

The presence of DON was detected using the novel biosensor, as indicated by the change in fluorescence intensity through ATP synthesis by the F_0F_1-ATPase within the chromatophores. Then, 10 μl of the F_0F_1-ATPase molecular motor biosensors labeled with DON monoclonal antibodies were mixed with 10 μl of DON standard solution; after 10 min, the ATP synthetic activity of F_0F1-ATPase was determined.

2.10. Data analysis

The data are presented as means ± standard deviation. All experimental data were the average of at least 3–6 independent tests. ANOVA was carried out on the data using the SAS system for windows V8. The least significant difference (LSD) at the 5% confidence level was used for comparing treatments.

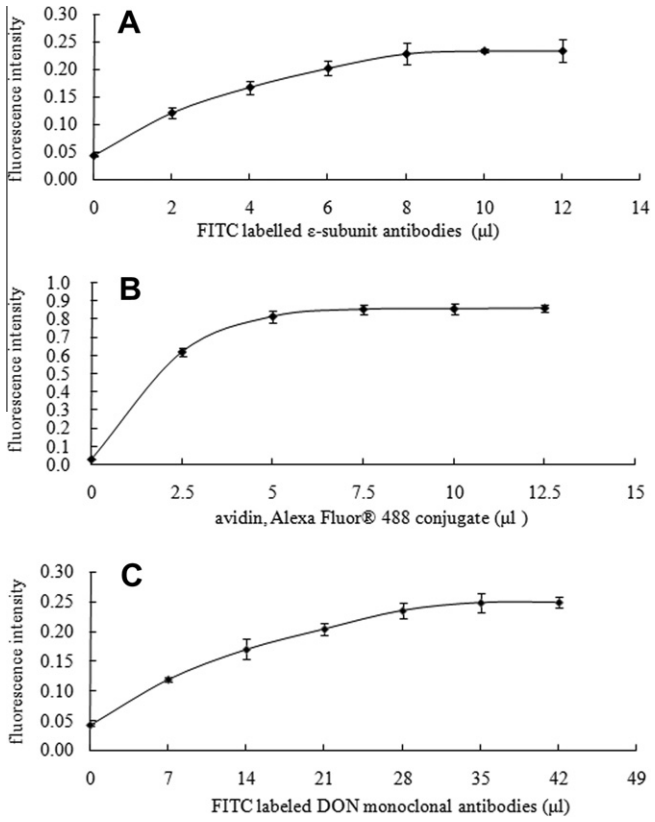

Fig. 2. (A) Intensity change of fluorescence intensity caused by the loading of FITC labeled ε-subunit monoclonal antibody onto F_0F_1-ATPase within the chromatophores. (B) Intensity change of fluorescence intensity caused by the loading of avidin, Alexa Fluor® 488 conjugate onto F_0F_1-ATPase within the chromatophores. (C) Intensity change of fluorescence caused by the loading of FITC labeled DON monoclonal antibody onto F_0F_1-ATPase within the chromatophores.

3. Results

3.1. Basic design of immuno-rotary biosensor based on F_0F_1-ATPase

Fig. 1 shows the basic design of the novel rotary biosensor based on F_0F_1-ATPase within a chromatophore. The DON antibodies were linked to the "rotator" ε-subunits of F_0F_1-ATPase within the chromatophores by the ε-subunit monoclonal antibody–biotin–neutroavidin–biotin–DON antibody system to capture DON residues. The activity of the F_0F_1-ATPase changed with the DON content loaded onto the biosensor, which was measured as chemiluminescence using the luciferin–luciferase system with a computerized microplate luminometer analyzer.

3.2. Optimization of the rotary biosensor conjugation conditions

The relationship between the changes in fluorescence intensities and various load quantities of the FITC-labeled ε-subunit monoclonal antibodies is shown in Fig. 2A. The FITC-labeled ε-subunit monoclonal antibodies were linked to F_0F_1-ATPase as described in Section 2. As shown in Fig. 2A, the fluorescence intensity increased with increasing loads of the FITC-labeled ε-subunit monoclonal antibodies. However, the trend proceeded slowly when the quantity of the FITC-labeled ε-subunit monoclonal antibodies was about 8 μl (0.5 mg/ml) because of the quantitative limitation of chromatophores. This result indicates that the ε-subunit monoclonal antibodies were successfully linked to the ε-subunit of F_0F_1-ATPase within the chromatophores. Therefore, 8 μl (0.5 mg/ml) of the ε-subunit monoclonal antibodies was the best choice for conjugation.

The average fold-increase in fluorescence intensity caused by the loading of avidin, Alexa Fluor® 488 conjugate onto the F_0F_1-ATPase within the chromatophores is shown in Fig. 2B. The biotinylated ε-subunit monoclonal antibodies were linked with the F_0F_1-ATPase as described in the Section 2, and then, the avidin, Alexa Fluor® 488 conjugate was linked. The increased fluorescence intensity curves of the avidin, Alexa Fluor® 488 conjugate remained steady when its quantity was about 7.5 μl (0.1 mg/ml). This might be caused by the quantitative limitation of chromatophores. Therefore, avidin was successfully linked with the ε-subunit of F_0F_1-ATPase within the chromatophores, and the final optimum quantity for avidin was 7.5 μl (0.1 mg/ml).

Fig. 2C shows the average fold-increase in fluorescence intensity caused by the loading of FITC-labeled DON monoclonal antibodies onto the F_0F_1-ATPase within the chromatophores. The biotinylated ε-subunit monoclonal antibodies were linked with

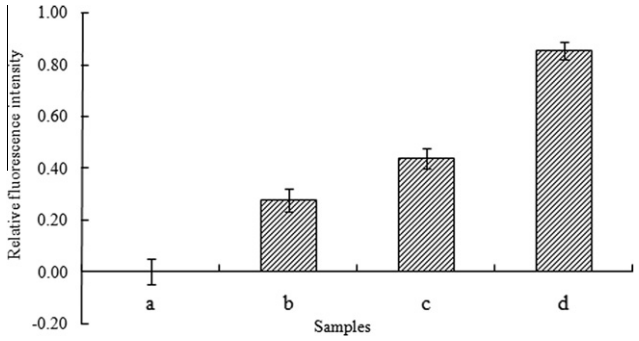

Fig. 3. Relative ATP synthesis activities of the F_0F_1-ATPase with different external links on the ε-subunit. (a) Fifteen microlitresl (50 mg/ml) of chromatophores; (b) 15 μl (50 mg/ml) of chromatophores binding to 8 μl (0.5 mg/ml) biotinylated ε-subunit monoclonal antibody; (c) 15 μl (50 mg/ml) of chromatophores binding to 7.5 μl (0.1 mg/ml) neutroavidin; (d) 15 μl (50 mg/ml) of chromatophores binding to 28 μl (0.036 mg/ml) biotinylated DON monoclonal antibody.

F_0F_1-ATPase as described in the Section 2, followed by neutroavidin, and then the FITC-labeled DON monoclonal antibodies. The fluorescence intensities stabilized when the amount of FITC-labeled DON monoclonal antibodies was 28 μl (0.036 mg/ml), which was caused by the quantitative limitation in chromatophores. This result indicates that the ε-subunit monoclonal antibodies were successfully linked with the ε-subunit of the F_0F_1-ATPase within the chromatophores and 28 μl (0.036 mg/ml) of DON monoclonal antibodies was optimum for conjugation.

3.3. ATP synthetic activity under different external links on the ε-subunits

The IRBs were constructed according to the optimized conditions. The ATP synthetic activity of the F_0F_1-ATPase was measured in each linkage step, which is shown in Fig. 3. To show the linkage effect, the activity of the native F_0F_1-ATPase was used as the control and was compared with the activity of the mixture. When the ε-subunit antibodies were bound, the ATP synthesis activity of F_0F_1-ATPase increased and its relative value was about 27% higher than that of the native form. If neutroavidin was joined to the ε-antibodies, the ATP synthesis activity increased to 44% higher than that of the native form. After the DON antibodies were linked to neutroavidin, the holoenzyme activity was activated with a relative value of 85% higher than that of the native form. The rate of florescence intensity change reveals a good correlation with the ATP synthetic activity and reflects the different load weight on F_0F_1-ATPase. These results are indicated in columns a, b, c, and d of Fig. 3. Therefore, various molecules can be detected directly via the change in fluorescence intensity depending on its load weight.

3.4. Detection of DON using novel biosensor

The DON residue was detected using the optimized IRBs. The sensitivity and detection range of the proposed method was determined. Table 1 shows the fluorescence intensity changes in the biosensors with different amounts of DON at 37 °C. The antibodies without DON loading were used as the control and the sensor was tested at DON concentrations ranging from 10^{-7} to 10^{-1} mg/ml. The statistical results clearly show a good correlation between the rate of fluorescence intensity change and the different DON content loaded into the F_0F_1-ATPase. As shown by the ANOVA, the novel rotary biosensors were able to detect not only the amounts of relatively abundant DON, but also the low levels DON because a significant difference was observed from 10^{-7} mg/ml to 10^{-1} mg/ml of DON compared with the control. The detection range was about seven orders of magnitude, which implies that this method can be used to quantitate DON at a wide range of concentrations.

Table 1
Detection results of DON using the optimal F_0F_1-ATPase biosensor.

DON (mg/ml)	*n*	Relative fluorescence intensity[a]
0	6	0.00167 ± 0.00028^{G}
10^{-7}	6	0.17768 ± 0.01367^{F}
10^{-6}	6	0.22904 ± 0.01848^{E}
10^{-5}	6	0.24273 ± 0.04115^{DE}
10^{-4}	6	0.26124 ± 0.00636^{D}
10^{-3}	6	0.30009 ± 0.01529^{C}
10^{-2}	6	0.34055 ± 0.01875^{B}
10^{-1}	6	0.46140 ± 0.00614^{A}

Means followed by different letters indicate significant differences among the different DON concentrations according to the LSD test at the 0.05 level. *n*, Number of independent replicates analyzed.

[a] Values are means ± standard deviation.

The specificity of the nanoscale biosensor was assessed by detecting aflatoxin B1, a toxin present in food and animal feed that is carcinogenic, mutagenic, teratogenic, and immunosuppressive to most animal species [20] simultaneously. The results show that the relative fluorescence intensity caused by loading 10 μl of aflatoxin B1 at 0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} mg/ml were 0.00167 ± 0.00028, 0.00165 ± 0.00032, 0.00169 ± 0.00057, 0.00162 ± 0.00076, 0.00171 ± 0.00014, 0.00165 ± 0.00037, 0.00167 ± 0.00083, and 0.00166 ± 0.00098 compared with the control, respectively. There was no significant difference compared with the control, which indicates that aflatoxin B1 does not have any direct effect on the biosensor.

4. Discussion

Although the "rotor" of F_0F_1 motor is mostly enwrapped by the "stator" $\alpha_3\beta_3$ crown and the exposed fraction of the "rotor" is also partly shaded by the b_2 subunit, ε-subunits has the potential to be linked with protein or probe complex for the location of ε-subunits is between the γ and b subunit. The activity of the F_0F_1-ATPase with different external links on the exposed "rotator" ε-subunits was studied and the results show that the ATP synthetic activity was activated with more numerous external links, which indicated that there was enough place between ε-subunits and b_2 subunit to contain the ε-subunit–avidin–DON antibody system. Previous studies have shown that holoenzyme activity is inhibited with external complexes that link to the exposed "stator" (β subunits) and is activated significantly by the binding of a large structure, such as a virus [10]. These findings indicate that as a "rotator", the ε-subunit may be more sensitive to the binding load than the β subunits. A possible reason was that the regulation role of ε-subunit was changed when probe system linked to it. Based on this principle, a novel method for the rapid detection of DON is presented by loading DON monoclonal antibodies onto the ε-subunits of F_0F_1-ATPase.

To investigate the biotin–avidin–biotin linkage of the DON monoclonal antibodies to F_0F_1-ATPase within the chromatophores to produce a novel biosensor for rapidly detecting DON, two parallel groups of samples, FITC-labeled and non-FITC-labeled antibodies were prepared. The FITC-labeled group was used to detect the fluorescence intensity to verify the binding of each part and the non-FITC labeled was used to detect the correlated ATP synthetic activity. To conjugate successfully the DON monoclonal antibodies onto the F_0F_1-ATPase within the chromatophores, the avidin levels were modulated. The PI of Avidin is about 10, which provided high non-specific binding, whereas the PI of neutravidin is about 6.3, which greatly decreases the non-specific binding [21]; therefore, neutravidin is the optimum choice for conjugation.

The ATP synthesis activity of the F_0F_1-ATPase, in which a series of external complexes were linked on the stator, exceeded the native levels. Using standard DON solutions, a working range between 10^{-7} and 10^{-1} mg/ml was obtained, which is two orders of magnitude more sensitive than the current method used for detecting DON [1], and can be done within 25 min without blocking, labeling, and washing. Furthermore, the specificity of the biosensor is perfect. To identify whether DON directly affects F_0F_1-ATPase activity, the activity of the native buffer incubated with DON was measured, which indicates that DON does not have any direct effect on holoenzyme activity. Therefore, the detection method based on F_0F_1-ATPase may be used as an alternative to the current method used for detecting DON in agricultural products.

Further studies are required to reveal the underlying mechanism of regulation and increase the speed at which the test can be performed and increase the test sensitivity to allow the detec-

tion of DON at low concentrations in agriculture products, which are now ongoing. Although the underlying mechanism of regulation is still unclear, F_0F_1-ATPase based rotary biosensors are a promising research tool that may be used for the ultrasensitive detection of small molecular compounds in the future.

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References

[1] H. Yagi, N. Kajiwara, H. Tanaka, T. Tsukihara, Y. Kato-Yamada, M. Yoshida, H. Akutsu, Structures of the thermophilic F_1-ATPase subunit suggesting ATP-regulated arm motion of its C-terminal domain in F_1, Proc. Natl. Acad. Sci. USA 104 (2007) 11233–11238.
[2] D. Okuno, R. Iino, H. Noji, Rotation and structure of F_0F_1-ATP synthase, J. Biochem. 149 (2011) 655–664.
[3] H. Noji, M. Yoshida, The rotary machine in the cell, ATP synthase, J. Biol. Chem. 276 (2001) 1665–1668.
[4] H. Imamuraa, K.P.H. Nhat, H. Togawa, K. Saito, R. Iino, Y. Kato-Yamada, T. Nagai, H. Noji, Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators, Proc. Natl. Acad. Sci. USA 106 (2009) 15651–15656.
[5] X.L. Liu, Y. Zhang, J.C. Yue, P.D. Jiang, Z.X. Zhang, F_0F_1-ATPase as biosensor to detect single virus, Biochem. Biophys. Res. Commun. 342 (2006) 1319–1322.
[6] Y.B. Cui, Z. Fan, J.C. Yue, Detecting proton flux across chromatophores driven by FoF1-ATPase using *N*-(fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl- sn-glycero-3-phosphoethanolamine, triethylammonium salt, Anal. Biochem. 344 (2005) 102–107.
[7] 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.
[8] 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.
[9] X.L. Liu, J.C. Yue, G. Yu, et al., Fabrication of F_0F_1-ATPase nanostructure on gold surface through dip-pen nanolithography, J. Nanosci. Nanotechnol. 8 (2008) 5753–5756.
[10] J. Cheng, X.A. Zhang, Y.G. Shu, J.C. Yue, F_0F_1-ATPase activity regulated by external links on β subunits, Biochem. Biophys. Res. Commun. 391 (2010) 182–186.
[11] Y. Rondelez, G. Tresset, T. Nakashima, Y. Kato-Yamada, H. Fujita, S. Takeuchi, H. Noji, Highly coupled ATP synthesis by F_1-ATPase single molecules, Nature 433 (2005) 773–777.
[12] B.A. Feniouk, T. Suzuki, M. Yoshida, The role of subunit epsilon in the catalysis and regulation of F_0F_1-ATPsynthase, Biochim. Biophys. Acta 1757 (2006) 326–338.
[13] Y. Xu, Z.B. Huang, Q.H. He, S.Z. Deng, L.S. Li, Y.P. Li, Development of an immunochromatographic strip test for the rapid detection of deoxynivalenol in wheat and maize, Food Chem. 119 (2010) 834–839.
[14] K. Fujita, M. Tsuta, M. Kokawa, J. Sugiyama, Detection of deoxynivalenol using fluorescence excitation–emission matrix, Food Bioprocess Technol. 3 (2010) 922–927.
[15] A.Y. Kolosova, S.D. Saeger, L. Sibanda, R. Verheijen, C. Van Peteghem, Development of a colloidal gold-based lateral-flow immunoassay for the rapid simultaneous detection of zearalenone and deoxynivalenol, Anal. Bioanal. Chem. 389 (2007) 2103–2107.
[16] T. Kadota, Y. Takezawa, S. Hirano, O. Tajima, C.M. Maragos, T. Nakajima, T. Tanaka, Y. Kamata, Y. Sugita-Konishi, Rapid detection of nivalenol and deoxynivalenol in wheat using surface Plasmon resonance immunoassay, Anal. Chim. Acta 673 (2010) 173–178.
[17] G.E. Zippora, K. Daniel, Selective extraction and reconstitution of F_1 subunits from *Rhodospirillum rubrum* chromatophores, Methods Enzymol. 126 (1986) 528–538.
[18] S. Philosoph, A. Binder, G.E. Zippora, Coupling factor ATPase complex of *Rhodospirillum rubrum*, J. Biol. Chem. 252 (1977) 8747–8752.
[19] T. Su, Y.B. Cui, X.A. Zhang, X.L. Liu, J.C. Yue, N. Liu, P.D. Jiang, Constructing a novel Nanodevice powered by δ-free F_0F_1-ATPase, Biochem. Biophys. Res. Commun. 350 (2006) 1013–1018.
[20] R.C. Garnerl, I. Dvorackova, F. Tursil, Immunoassay procedures to detect exposure to aflatoxin B_l and benzo(a)pyrene in animals and man at the DNA level, Int. Arch. Occup. Environ. Health 60 (1988) 145–150.
[21] H. Kobayashi, H. Sakahara, K. Endo, M. Hosono, Z.S. Yao, S. Toyama, J. Konishi, Comparison of the chase effects of Avidin, streptavidine, neutravidine, and avidine–ferritin on a radiolabeled biotinylated anti-tumor monoclonal antibody, Jpn. J. Cancer Res. 86 (1995) 310–314.