CdTe quantum dot-chromatophore conjugates as smart pH-sensitive probes to monitor ATP motor-mediated proton flux for simultaneous detection of viruses

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ABSTRACT

Here we described a novel approach of CdTe quantum dots fluorescence probes to improve the detection of proton flux through F_1F_0-ATP by ATP synthesis. It was found that both the CdTe quantum dots (535nm, 585nm) was attached on chromatophores, respectively, was not only dependent on each of them, but also the micro environment interfering was a little (about 5-6%) , if in the presence of 100 time gradient. CdTe quantum dots used as the smart pH probes monitoring proton flux driven by ATP synthesis for detection various virus simultaneously. Furthermore, a model of steady Double Diffused Layer of CdTe quantum dots on surface of chromatophores was proposed. it would be extended to optical coder detection of various viruses in late.

Keywords : CdTe quantum dots , F1F0-ATPase ; Biosensor ; proton flux indicator.

Introduction

The rapid growth and development in biodetection for rapid, selective, and sensitive is central to implement an effective response to viral infection. The current outbreak of avian influenza A was among poultry in all over the world, improving influenza surveillance would be important and urgent. Many methods can be used to detect the virus, including immunological assays, transmission electron microscopy and PCR are time consuming and require purifying the samples, which makes them inappropriate for fields diagnostics [1]. For such reasons, the design of immuno-rotary biosensor (IRB) based on rotary single F_0F_1-ATPase is described by our lab.[2].

Although it reported that the fluorescence probe F1300 labeled in inner chromatophores was used as a proton flux indicator, the organic dye in the present of many deficiencies for indicator proton flux, because the fluorescence quencher was difficulty to escape during the continuum monitor ATP motor-mediated proton flux. However, the Quantum dots are markedly differenced from those of organic dye, have been tested in most biotechnological applications as useful labels owing to their photos table, continuous absorption spectra, and efficient, narrow, tunable emission, base on the properties, Herein, we report a especial conjugation method utilizes a genetically the positive charge domains chromatophores assemble with the surface of negative charged CdTe quantum dots through electrostatic reaction. In this step the CdTe quantum dots can be equipped with function to traps proton. Furthermore, development (immuno-rotary biosensor, IRB) capture virus for rapid, selective, sensitive and optical coder detection individually was described.

Results

Fig.1 schematic of the specific conjugation of chromatophores to Qdots and immuno -rotary biosensor was constituted. Fig.2A showed the property of fluorescence spectra of the CdTe quantum dots labeled on surface of chromatophores with excitation at 470 nm and emission at 535 nm.

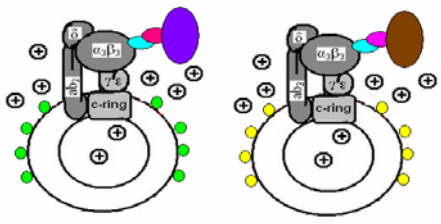

Fig.1 schematic of the specific conjugation of chromatophores to Qdots and immuno-rotary biosensor was constituted.

The fluorescence intensity of CdTe quantum dots-chromatophores (curve a) was higher than that of chromatophores as control (curve c). After the addition of 1mg/ml $CuCl_2$, the fluorescence intensity of chromatophores (curve a) was as lower as the control (curve b, vs. curve c), indicating that the CdTe quantum dots was strict attached on the surface of chromatophores.

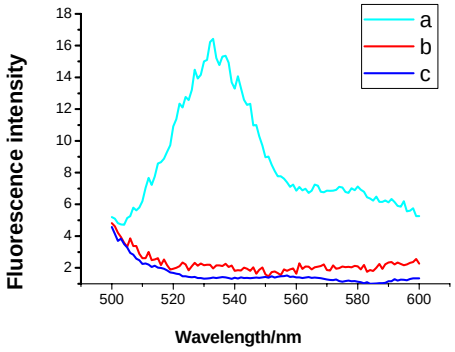

Fig.2A the fluorescence intensity of CdTe quantum dots-chromatophores

Fig.2 B showed that the fluorescence intensity increased by increasing pH values from 9.0 to 6.0 in solution with a linearity relationship between the pH values and intensity fluorescence (Fig.2B curve (a-f).

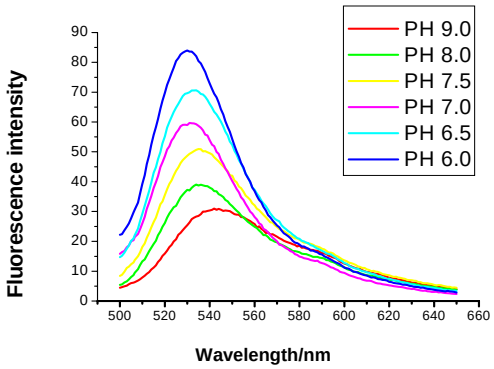

Fig.2 B the fluorescence intensity increased by increasing pH values

.Fig.3 showed that the proton transfer was in response to the activity of F_0F_1-ATPase by using CdTe quantum dots as indicator. The fluorescence intensity was increased from 38.5 unit to 54.7 unit in time course 15 min (curve a), when adding 2mM ADP. This fluorescence was increased due to the F_0F_1-ATPase synthesized ATP from ADP and Pi and pumped protons out of the vesicles. To further confirm that the fluorescence change was regulated by proton flux, the F_0 channel inhibitor DCCD was added to assay buffer incubation 30 min. and then 2mM ADP was adding, the fluorescence was only increased a little as shown in curve b, because the DCCD blocked the ATP synthesis-driven proton pump in F_0. Curve (c) was not adding ADP and Pi and without pumped protons out of the vesicles as control. This clearly showed that the CdTe quantum dots on surface chromatophores were suitable as pH probes monitor proton flux driven by ATP synthesis.

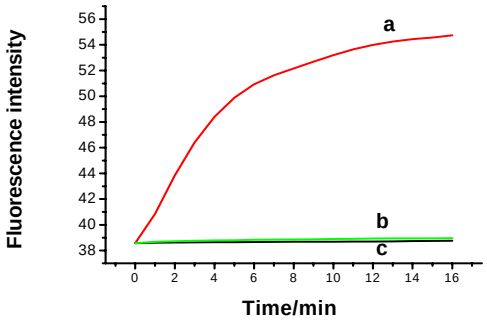

Fig.3 showed that the proton transfer was in response to the activity of F_0F_1-ATPase by using CdTe quantum dots as indicator.

Due to both CdTe quantum dots on the surface of chromatophores, there was a little sensitivity pH with environment buffer change, even the change ranges from pH 6 to pH 8 (data not showed). In order to confirm that was ability independent of each of the Electric Double Layer of Microenvironment of CdTe quantum dots on the surface chromatophores, therefore the pH poor buffer capacity solution of 0.1 mM tricine as microenvironment was chosen.

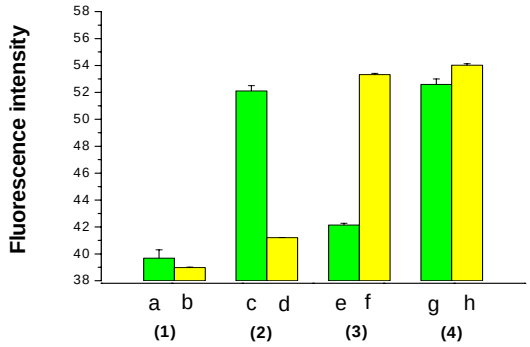

Fig. 4 the fluorescence intensity of the two types emission (535,585 nm) of CdTe quantum dots appear ability independent of each.

In order to confirm that was ability independent of each of the microenvironment of CdTe quantum dots on the surface chromatophores, therefore the pH poor buffer capacity solution of 0.1 mM tricine as microenvironment was chosen. Fig. 4 showed that the fluorescence intensity of the two types emission (535,585 nm) of CdTe quantum dots appear ability independent of each, while it was mixed in the same buffer. Fig.4 group (1) column (a, b) was two CdTe quantum dots-chromatophores (535,585 nm emission) which was loaded by antibody of β-subunit with antibody of H9 avian influenza virus, and antibody of β-subunit with antibody of MHV68, respectively, When ADP 2 mM added to initialize reaction, column (a) fluorescence increased from 37.78±0.67 to 39.67±0.62, and column (b):

fluorescence increased from 37.78±0.67 to 38.98±0.04, both was independent on each of them; however the group (2) column (c, d), the column (d) was the same as column (a), but the fluorescence change of column (d) was a few larger than column (a) (was about more 6.3%). Because in the present of the column (c), that the fluorescence from 37.78±0.67 increased to 52.11±0.39, means that the pH change from 8.0 to 6.0 (Fig.2 C), under thus microenvironment, may be a H^+ gradients 100 times than before, and inducing to the fluorescence change was a few larger than column (A). The group (3) column (e, f), the column (f) was the same as column (b), when the column (f) fluorescence increased from 37.78±0.67 to 53.32±0.09, means that the pH change from 8 to 6.0, thus microenvironment may be a H^+ gradients 100 times change than before, and induce fluorescence change of column (f) was a few larger than column (b) (was about more 5.6%). The group (4) of column (g, h) was fluorescence increased from 37.78±0.67 to 52.61±0.39, and to 54.02±0.11, respectively, compare to column c and e, the column (g, h) seem the same change of fluorescence than that of the column (c, e). From above, conclusion may be the CdTe-chromatophores (535,585 nm) independent on each of them if their pH change was the same (column g, h), but if in the presence of gradients 100 times, they would be not independent on each of them, but the influence degree was a little about 5-6%. Analyzing the experimental date, it not only find that the rate of H^+ diffusion, when from inside to outside was slower than that of when outside into inside, but also the outside H^+ induce in response to fluorescence of CdTe-chromatophores degree was very a little, Both of CdTe quantum-chromatophores (535, 585 nm) in the properties H^+ rate of diffusion and response to fluorescence independent on each of them was interested in the optical coder.

Discussion

The new significant accomplishment in our researches: (1) one of brand new biosensors (immuno-rotary biosensor) of F_0F_1-ATPase within chromatophore was constructed. The Capture of pathogen was based on antibody–antigen reaction. The detection of pathogen based on proton flux rate change driven by ATP-synthesis of F_0F_1-ATPase, which was indicated by fluorescence Qdots in chromatophores. (2) This biosensor extended for rapid, selective, sensitive and Qdots as smart pH meter and optical code for the biosensor.

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Reference :

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