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The Detection of cTn Ⅰ by The Aptamer Biosensor*

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Abstract A new aptamer biosensor for detection of cTn I was established. It can detect cTn I in samples. The surface of GC was modified with anode-oxidation, amidation and activation modification. We used EDC and NHS as activators. Due to aptamer's specificity for cTn I, we can detect the concentration of cTn I in sample solution. The linear range of the analytical signal is observed from 0.05～5 nmol/L. The lowest detected concentration is 0.05 nmol/L. The detection time is 5 min.

Key words aptamer, biosensor, cTn I
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The cardiac troponin (cTn) is a regulatory protein related with cardiac contractility variability. It consists of three subunits, cTn I, cTn T and cTn C. cTn I is a 21 ku single polypeptide chain[1]. In general, the serum cTn I level is lower than 0.3 μg/L in healthy human[2]. When the integrity of myocardial cell membrane is injured by ischemia or hypoxia, free cTn I can quickly enter into the blood. Moreover, the combined cTn I is gradually released into blood with the injury aggravating, which caused to the serum cTn I level rise within hours and remain elevated for longer period time [3-4]. Compared with non-tissue-specific biochemical markers including creatine kinase (CK), lactic dehydrogenase (LDH), myoglobin (Mb) and CK-MB, cTn I has a higher specificity in cardiac tissue. cTn I will be released into blood at the first time and remain a long time, when the cardiac muscle is damaged. Therefore, cTn I has become the preferred biomarker of early diagnosis of myocardial injury[5-6]. At present, the methods for cTn I determination are radioimmunoassay (RIA) [7], enzyme linked immunosorbent assay (ELISA) [8], chemiluminescence immunoassay[9]. They are costly, time-consuming, and requiring large instruments.

Aptamer biosensor is a novel type senor that aptamers are acted as recognition molecules immobilized on the surface of the glassy carbon electrode (GCE).[10] Aptamers can bind to the analyte in solution specifically and generate electrochemical signal. The current is related to the analyte concentration. Therefore, aptamer biosensor can be used to detect cTn I in sample. In recent years, more and more researches have been reported that aptamer biosensor possesses many advantages of specificity, stability, sensitivity, and low-cost, non-polluting[11-17].

The aim of this study is to establish a method of detecting cTn I for the future clinical diagnostics research. A novel aptamer biosensor for rapid cTn I determination was developed and illustrated in this paper.

1 Materials and methods

1.1 Materials and instruments

Bovine serum albumin (BSA) was purchased

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from Beijing Dingguo Biotech. Co., Ltd. 3-aminopropyltriethoxysilane (APTES), carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) were bought from Shanghai Sanland Cemegau International Co, Ltd. H_2O_2, $NH_3 \cdot H_2O$, $K_3Fe(CN)_6$ were purchased from Tianjin Yongda Chemical R & D center. The other reagents were all analytical grade.

The electrochemical experiments were undertaken on a LK2005A electrochemical workstation (Tianjin Lanlike Company) and a conventional three electrodes system. The glassy carbon electrode (GCE) was acted as working electrode, an Ag/AgCl electrode was acted as the reference electrode, and a Platinum electrode was applied as the counter electrode.

1.2 The working electrode modification

The GCE was polished with 0.05 μm Al_2O_3 and cleaned thoroughly. Then it was oxidized with immersing in 0.2 mol/L PBS (pH 6.0). Subsequently, it was immersed into hydrophilic solution of $NH_3 \cdot H_2O$: H_2O_2 : H_2O (1 : 1 : 10) at 72℃ for 20 min, so that the surface of the GCE was modified with hydroxyl groups. Next, the amino group attachment on the GCE was undertaken by reaction it with amination solution of APTES : H_2O (1 : 10) at 53℃ for 3h.

1.3 Immobilization of the aptamer

The amination electrode was activated with soaking in 0.01 mol/L EDC and 0.01 mol/L NHS. Then the aptamer was immobilized on the surface of the modified GCE by ultraviolet radiation for 2 h and heating 1 h at 80℃ . The electrode was rinsed with distilled water and dried naturally. Subsequently, 3 μl 1.5% BSA was added on the GCE surface at room temperature for 1 h to block the remaining active sites. Meanwhile, the bare GCE was used as negative control.

1.4 Detecting cTn I in samples

0.05 nmol/L～25 nmol/L cTn I PBS solution and cTn I serum solution were prepared, respectively. Then a series of cTn I PBS solution and cTn I serum solution were measured using the aptamer biosensor with cyclic voltammetry at potential of –0.1V～0.6V, respectively. A non-specific control was installed, simultaneously.

2 Results and discussion

2.1 The cTn I determination using aptamer biosensor

Figure 1 is the cyclic voltammetry curve of GCE before and after modification. The current decreased significantly after the GCE modification. It demonstrates that aptamers modified successfully.

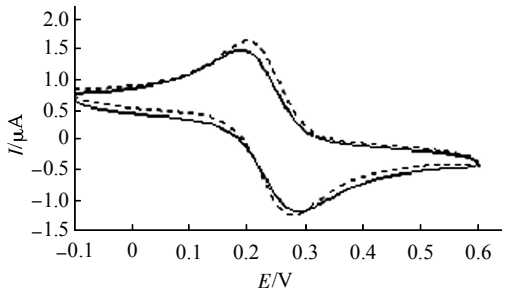

Fig. 1 Cyclic voltammogram curves of GC before and after modification

------ : Bare electrode; —— : Aptamers modified electrode.

A series of the cTn I concentrations was measured using the aptamer biosensor with cyclic voltammetry. Concentration-current relationship plots are shown in Figure 2～3. Line *a* in Figure 2 shows cTn I detecting with bare electrode in PBS solution. Line *b* in Figure 2 shows cTn I detection using the aptamer biosensor in PBS. Line *c* in Figure 2 shows cTn I detection using the aptamer biosensor in serum. Line *d* in Figure 2 shows BSA detection using the aptamer biosensor in PBS. It can be seen in Figure 2 that with the cTn I concentrations increasing, the current decrease. And the current changed to stabilize, when the cTn I concentration is higher than 5 nmol/L. This indicates that the aptamer binding to cTn I have been reached saturation. So the current is linear with the cTn I concentration, when the concentration is in the range of 0～5 nmol/L.

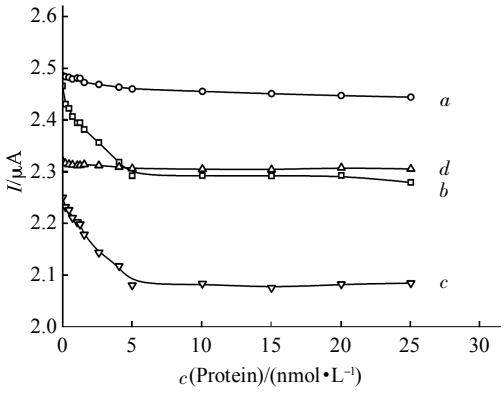

Fig. 2 Concentration-current plots of the aptamer biosensor for different cTn I concentrations in PBS solution and serum solution

a: The cTn I detecting with the bare electrode in PBS solution; *b*: The cTn I determination in PBS with the aptamer biosensor; *c*: The cTn I determination in serum with aptamer biosensor; *d*: The BSA determination in PBS with aptamer biosensor.

Results from Figure 3 confirm that the aptamer biosensor binds to cTn I specifically according to the current changed significantly(*b* and *c*). However, there is no significant current change in *a* and *d*. This shows that the bare electrode has no adsorption of cTn I, and the aptamer biosensor has no specificity of BSA.

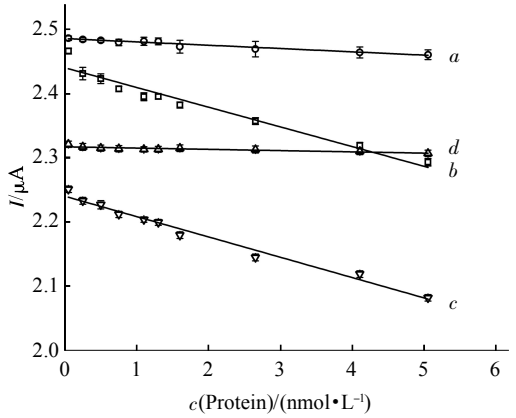

Fig. 3 Concentration-current plots of the aptamer biosensor when cTn I concentration is in the range of 0～5 nmol/L

a: The cTn I detection with the bare electrode in PBS solution; *b*: The cTn I determination in PBS with the aptamer biosensor; *c*: The cTn I determination in serum with aptamer biosensor; *d*: The BSA determination in PBS with the aptamer biosensor.

2.2 The specificity of aptamer biosensor

Curve *a* in Figure 4 shows the cTn I detection with the bare electrode in PBS solution, there is no significant current change. It is prompted that the bare electrode has no adsorption of cTn I. While the current changed significantly in curve *b* that is for the cTn I detection using the aptamer biosensor in PBS, which demonstrates that aptamers have a better adsorption of cTn I in PBS solution. And the linear relationship between the currents and the cTn I concentration is y=–0.034x+2.4398, r^2=0.9519.

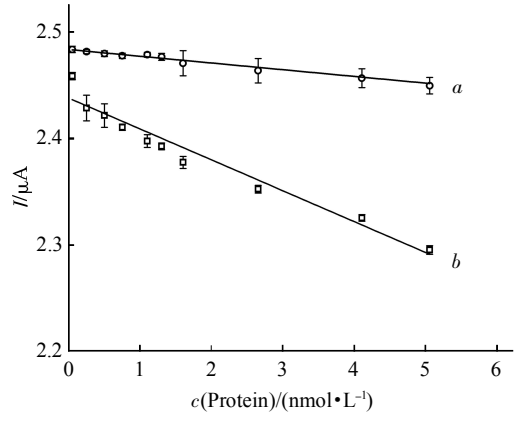

Fig. 4 Concentration-current plots of bare electrode and the aptamer biosensor for cTn Ⅰ detection in PBS

a: The bare electrode; *b*: The aptamer biosensor.

BSA is acted as structural analogues to analyze the specificity of the aptamers biosensor. In Figure 5, line *b* shows the BSA detection using the aptamer biosensor in PBS. With the protein concentration increasing, the current changed non-significantly. While the current changed significantly in line *a* that is for the cTn I detection using the aptamer biosensor in PBS. Figure 5 reveals that cTn I aptamer sensor only has specificity for cTn I, but not for BSA. And the response to BSA caused by nonspecifically adsorbed.

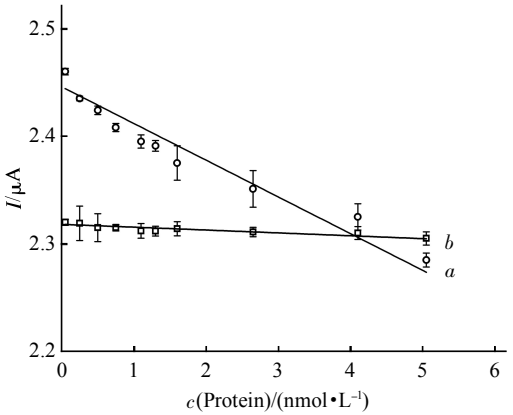

Fig. 5 Concentration-current plots of the aptamer biosensor for cTn Ⅰ and BSA in PBS solution

a: The cTn I determination; *b*: The BSA determination.

2.3 The cTn I determination in serum

We simulate the physical environment of cTn I determination. Figure 6 shows the cTn I detection

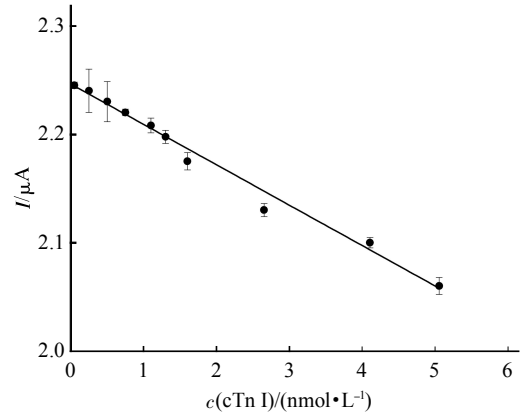

Fig. 6 Concentration-current plots of the aptamer biosensor for cTn Ⅰ in serum solution

using the aptamer biosensor in serum. It confirms that cTn I aptamer biosensor has a better specificity. And the linear relationship between the currents and the cTn I concentration is y=–0.0365x+2.2441, r^2=0.9734.

We prepared cTn I serum solution of four concentration gradient to confirm the accuracy of the aptamer biosensor. Comparing standard concentration (C_s) with detection concentration (C_d), the coefficient of variance (CV) was received as shown in Table 1. It can represent the accuracy of the aptamer biosensor. According to Table 1, detection concentration are similar to cTn I serum concentration prepared randomly, which confirms the practicality of the aptamer biosensor.

Table 1 Detection concentration of cTn Ⅰ from serum sample

Sample	C_s/(μmol•L^{-1})	C_d/(μmol•L^{-1})	C_a/(μmol•L^{-1})	SD	CV/%
1	0.80	0.875	0.071 53	0.053 033	6.33
2	1.90	2.056	0.129 44	0.110 309	5.58
3	2.60	2.442	0.181 02	0.111 723	4.43
4	3.50	3.699	0.233 81	0.140 714	3.91

3 Conclusions

The specificity of the aptamer biosensor binding to cTn I was confirmed. The variation of the cTn I concentration-current was obtained by cyclic voltammetry. The linear relationship between the cTn I concentration and the current is 0.05～5 nmol/L, and a detection limit of 0.05 nmol/L is achieved.

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核酸适配体生物传感器对心肌肌钙蛋白Ⅰ的检测研究*

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摘要　核酸适配体生物传感器是利用固定在电极表面的适配子与被测溶液中心肌肌钙蛋白Ⅰ(cTnⅠ)发生特异性结合，从而达到检测的目的．我们对玻碳电极进行阳极氧化、氨基化修饰，通过碳二亚胺盐酸盐(carbodiimide hydrochloride，EDC)、N-羟基琥珀酰亚胺(N-hydroxysuccinimide，NHS)活化作用将适配子结合在电极表面．cTnⅠ最佳检测范围是 0.05～5 nmol/L，最低检测限为 0.05 nmol/L，检测时间为 5 min．

关键词　核酸适配体，生物传感器，cTnⅠ
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