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Letter

Piezoelectric Immunoassay of Thyroidal Triiodothyronine*

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Abstract **A re-utilizable piezoelectric crystal (PC) immunosensor for thyroidal triiodothyronine (T3) has been developed. The crystal was coated with protein A and then reacted with T3 antibody. T3 could be detected in the range of 0.5×10^{-6} g/L to 10.0×10^{-6} g/L by the system. Crystal was regenerated by saturating with T3 and subsequent binding of a new anti-T3 antibody layer. The T3 assay could be repeated up to 5 times using each crystal.**

key words **piezoelectric immunoassay, crystal, thyroidal triiodothyronine (T3), immobilization, antibody, antigen**

0 Introduction

The first analytical application of a PC detector was reported by King in 1964[1]. For the next two decades, intensive researches were directed toward developing organic and inorganic coating for the detection and determination of various toxic agents in the environment and the workplace area[2,3]. Since biologically active materials, such as antibodies, enzymes, and antigens are highly specific, their uses as active coating have been deeply exploited, leading to a new class of PC biosensors in liquid and gas phase analysis[4]. In this paper, a strong complexation between protein A and the gold electrode[5] was exploited to construct a re-utilizable piezoelectric crystal. As a model, the crystal coated with protein A was applied for detection of T3. The immobilizing methods, the T3 concentration effecting resonant frequency shifts, the effect of thyroxine (T4), and the recovery of the electrode were also discussed.

1 Experimental

1.1 Apparatus

A quartz piezoelectric crystal (AT-cut) with a fundamental frequency of 9 MHz was purchased from Beijing 707 Plant with gold electrode (about 0.22 cm^2) on either side. The crystal was attached to one top of a groundglass tube with silicone rubber sealant. Only one face of the electrode was exposed to the solution (Fig. 1). Connection to the

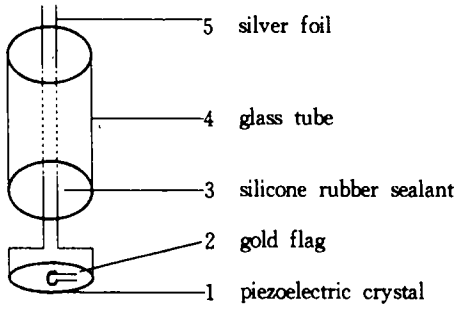

Fig. 1 Construction of the electrode

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external circuit was made by a silver foil glued to the electrode surface. An oscillator circuit was constructed from the crystal, and the resonance frequency was monitored by a frequency counter (CN3165, The Forth Radio Plant, Shijiazhuang, China) (Fig. 2).

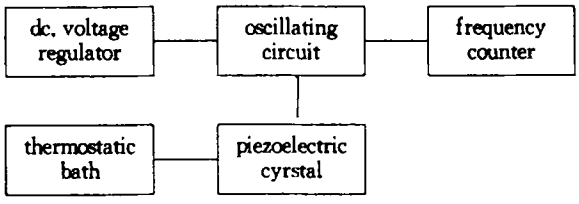

Fig. 2 Block scheme of experiment device

1.2 Materials

T3 was product of Sigma Co., and T3 antibody was provided by Shanghai Cell Institute. The antiserum was not diluted. Protein A was purchased from Shanghai Bioproduct Institute. Preparation of Protein A: 2.0 mg Protein A was added to the solution which was made up of 2.0 mL of 0.05 mol/L phosphate buffer solution (pH7.2) and 2.0 mL of 0.1 mol/L sodium acetate buffer solution (pH4.5)(containing 5% NaCl). HCl solution was used to modify the mixed solution near pH5.5. The Protein A stock solution (0.5 g/L) was stored at 4 ℃ PBS (phosphate buffered saline). Polyethyleneimine, glutaraldehyde, and other chemicals were analytically pure. The deionized water was used as solvent.

1.3 Immobilizing method

The crystal was soaked in 2.0 mol/L NaOH solution for 10 min, then rinsed with distilled water, and soaked in 4.0 mol/L HCl solution for 5 min, again rinsed with distilled water, then dried in the air. 5.0 mL of Protein A (0.5 g/L) was added by a syringe to a dry crystal and dispersed on the surface of the gold electrode. After the crystal was incubated at ambient temperature for 30 min, Protein A was tightly bound to the prepared gold electrode. The crystal was washed with 0.5 mol/L NaCl solution, 0.05 mol/L PBS, anddistilled water in sequence, then dried in the air. After drying, T3 antibody was added by a syringe onto the Protein A coated layer and dispersed on the surface, then incubated at 4 ℃ for 2～3 h. Finally, the electrode was rinsed with 0.5 mol/L NaCl solution, 0.05 mol/L PBS, and distilled water. The immobilizing electrode was stored in a refrigerator at 4 ℃. The resonance frequency changes in the immobilizing process were listed in Table 1.

Table 1 Observation of the immobilizing process frequency shift **Hz**

Crystal	1	2	3
Unmodified electrode	26 352	26 970	28 820
DWProtein A	26 070	26 721	28 494
T3 antibody	25 806	26 447	28 196

It can be seen from this table that the frequency of PC decreased after every immobilizing step, indicating a mass increase following each step.

1.4 Procedure

After the quartz PC was modified, T3 antigen was added by a syringe onto the T3 antibody layer and dispersed on the surface. The same volume of T3 was added from lower concentration to higher concentration. After reacting in the air for 30 min, the crystal was rinsed with 0.5 mol/L NaCl solution, 0.05 mol /L PBS, and distilled water in sequence. The frequency shift of each concentration was determined in the distilled water.

2 Results and discussion

2.1 Effect of immobilizing method on response sensitivity

Compared with polyethyleneimine method, protein A immobilizing antibody electrode had higher sensitivity, more simple procedures, and better stability. The results were shown in Table 2.

Table 2 Comparasion of immobilizing methods **Hz**

Matter	Unmodified (f_u)	Modified(f_m)	$\Delta f_1(f_m-f_u)$	Antibody coating (f_a)	$\Delta f_2(f_a-f_m)$
Protein A	30 915	30 673	−242	30 343	−330
Polyethyl-eneimine	33 780	33 226	−554	33 013	−213

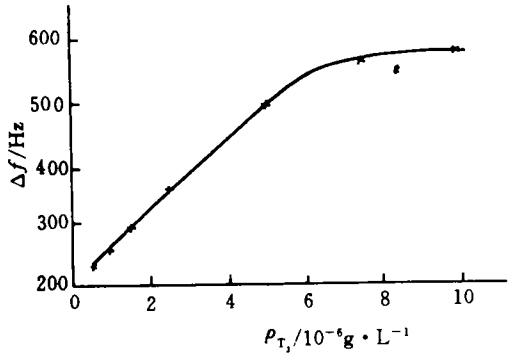

Fig. 3 Calibration curve for T3

2.2 Response of the modified PC for T3

When the anti-T3 modified PC was exposed to the solution, the frequency of PC decreased. For a T3 concentration of $(0.5\sim10)\times10^{-6}$ g/L, the frequency shifts (Δf)responded proportionally to the T3 concentration. The linear relation between Δf and the concentration of T3 was shown in Table 3.

Table 3 Correlation between resonant frequency changes and the concentration of T3

$C/10^{-6}$ g·L	0.5	1.0	1.5	2.5	5.0	7.5	10.0
$-\Delta f$/Hz	232	263	293	355	497	524	553

The detection limit was 1.0×10^{-7} g/L. No obvious frequency shift was observed below this concentration. In each measurement, the same volume solution was added, and it can diminish the influence of non-mass factors. The calibration curve of Δf(fnequency shifts) to T3 (the concentration of T3) was shown in Fig. 3.

2.3 Selectivity

The response of the modified PC for T4 was also studied with the same method as for studying the response for T3. After T4 was added to the crystal, there was no frequency changes observed. This suggested that T4 did not react with the anti-T3 immobilized on the crystal. It also implied that the linear response was caused by the special binding between T3 and its antibody.

2.4 Recovery of the electrode

A second antibody layer could be bound to the crystal which had been saturated with T3. Then the crystal could be recovered to detect T3 again. This process could be repeated up to five times. Thus, each PC was capably used approximately 5 times in succession to detect T3. However, the design of a piezobiosensor which is suited to multiple liquid-phase assays is still a formidable task.

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