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High sensitive detection of *presenilin-1* point mutation based on electrochemiluminescence

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Abstract **Electrochemiluminescence (ECL) is a high-sensitive detection method with broad biological applications. Ruthenium (Ⅱ) tris (bipyridyl) ($Ru(bpy)_3^{2+}$) and tripropylamine (TPA) are most commomly used combination of the reactive species in ECL. The redox of $Ru(bpy)_3^{2+}$ with excess TPA at the surface of an electrode produces a highly efficient and stable light emission due to a rapid cycle of the reactions. This rapid, highly sensitive and accurate method has been applied to gene quantification. In this work, an ECL detection system is designed and applied in detection of *presenilin-1* (*PS-1*) gene mutation. The results show that given the same polymerase chain reaction (PCR) cycle number, the ECL intensities from the digested and the undigested wild-type samples are nearly identical, but the difference in ECL intensities between the digested and the undigested mutant samples is distinctive. This detection technology connects PCR with ECL and allows a reliable discrimination between the wild-type and the mutant genes.**

Keywords: electrochemiluminescence, point mutation, *presenilin-1* gene.

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Electrochemiluminescence (ECL), in contrast to the conventional chemiluminescence (CL), is a chemiluminescent reaction of species generated electrochemically at an electrode surface. Ruthenium (Ⅱ) tris (bipyridyl) ($Ru(bpy)_3^{2+}$) and tripropylamine (TPA) are used as reactive species in ECL. $Ru(bpy)_3^{2+}$ is oxidized at the surface of a working electrode, forming a strong oxidant, $Ru(bpy)_3^{3+}$. Simultaneously, TPA, in large molar excess in the solution, is oxidized at the electrode surface to form a cation radical, $TPA^{+\cdot}$, which rapidly and spontaneously loses a proton to form a radical reductant, $TPA^{\cdot}$. The oxidant and the reductant react to form an excited-state ruthenium complex, $Ru(bpy)_3^{2+*}$, and other inactive by-products. The energy used for formation of the excited state arises from the large difference in electrochemical potentials of the $Ru(bpy)_3^{3+}$ and the $TPA^{\cdot}$. The excited-state $Ru(bpy)_3^{2+*}$ decays through a normal fluorescence mechanism, emitting a photon at 614 nm (see Fig. 1). This process regenerates the original form of the $Ru(bpy)_3^{2+}$, which can be recycled in future reactions. Given an adequate data collection time, each $Ru(bpy)_3^{2+}$ molecule can emit many photons that can be detected as an integrated signal. This new technology has many distinctive advantages over other detection systems: no radioisotopes are used; detection limits are extremely low (200 fmol/L); the dynamic range for quantification extends over six orders of magnitude; the labels are extremely stable compared with those of most other chemiluminescent systems; and measurement is simple and rapid, requiring only a few seconds[1−3]. Since Kenten[4] first used ECL in DNA probe assay, this method has been widely used in DNA quantification.

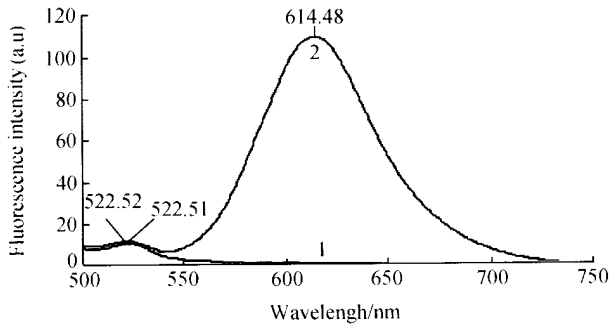

Fig. 1. The fluorescence emission spectrum of $Ru(bpy)_3^{2+}$. 1, The fluorescence emission spectrum of DMF using an excitation wavelength of 456 nm; 2, the fluorescence emission spectrum of $Ru(bpy)_3^{2+}$ dissolved in DMF using an excitation wavelength of 456 nm.

Majority of the early-onset familial Alzheimer's disease (FAD) are caused by mutations in the *presenilin-1* (*PS-1*) gene[5−8]. Campion et al.[9] have discovered that the mutation at codon 235 of the *PS-1* gene will cause FAD at a very young age (ranged 29 — 35 years-old). The Leu235Pro mutation is a *PS-1* mutation associated with the youngest age of AD onset. It, thus, may play an important role in effecting the *PS-1* function[9]. We may diagnose FAD by detecting this point mutation.

Here we describe a rapid, highly sensitive and accurate method which connects ECL with PCR for detection of the *PS-1* Leu235Pro mutation. It provides a reliable method to discriminate the wild-type and the mutant genes.

1 Materials and methods

(ⅰ) Templates and primers

(1) Wild-type template: A 108-base fragment within the exon 8 of *PS-1* gene, including the codon 235:

ACTCCAGCAG GCATATCTCA TTATGATTAG TGCC-CTCATG GCCCTGGTGT TTATCAAGTA CCTCCCTG-AA TGGACTGCGT GGCTCATCTT GGCTGTGATT T-CAGTATA.

(2) Mutant template: A 108 base-fragment within the exon 8 of *PS-1* gene, including the mutated codon 235: ACTCCAGCAG GCATATCTCA TTATGATTAG TGCC-CTCATG GCCCCGGTGT TTATCAAGTA CCTCCCTG- AA TGGACTGCGT GGCTCATCTT GGCTGTGATT T- CAGTATA

(3) Primer A: 5′-Ruthenium-ACT CCA GCA GGC ATA TCT CAT TAT - 3′

(4) Primer B: 5′-Biotin-TAT ACT GAA ATC ACA GCC AAG ATG A - 3′

The above templates and primers were all synthesized by Shanghai Shenggong.

(ii) Chemicals. Taq DNA polymerase was purchased from Promega. dNTP and *Hpa* II (10 U/μL) were from Shanghai Shenggong.

(iii) Equipment. The electrochemiluminescence detection instrument was customer-built in our laboratory. Fig. 2 is a diagram of the essential components of the instrument. The heart of the instrument is the electrochemical reaction cell, containing a working electrode, a counter electrode and a reference electrode. The working electrode (disk) and the counter electrode (mesh) are made from platinum. The reference electrode is an Ag/AgCl thread. A potentiostat (Sanming Fujian HDV-7C) applies various voltages to the electrodes. An optical fiber bandle receives the light emitted during the ECL reaction and conducts it to an ultrahigh sensitivity single photon counting module (Channel Photomultiplier, Perkinelmer MP-962). The signal from the single photon counting module is amplified and discriminated. The TTL pulses are counted in an integral form by the Labview software through a multi-function acquisition card (Advantech PCL-836). The computer controls the signal collection.

(iv) PCR amplification. Two genotype templates were amplified under the same condition. Pre-denaturation: 2 min at 95℃. Cycling: 94℃ and 55℃ for 45 s each, and 72℃ for 50 s. Extension: 2 min at 72℃. The samples were subjected to 25, 30 and 35 cycles respectively. All amplification reactions were carried out in a total volume of 50 μL.

(v) *Hpa* II digestion. 2 μL of *Hpa* II (20 units), 2 μL of buffer and 6 μL of sterile water were added to 10 μL of samples from each PCR product. The samples were digested for 60 min at 37℃ and 20 min at 65℃.

(vi) ECL detection. The instrument employs ECL for detection which utilizes $Ru^{2+}(bipy)_3$-chelates as a label in combination with universal streptavidin-coated paramagnetic microparticles. The digested and the undigested samples were added to the electrochemical reaction cell in turn after they had been incubated with streptavidin microbeads. Then the luminescence signal was detected.

2 Results and discussions

The basic principle of the assay is outlined in Fig. 3. Briefly, a DNA fragment spanning the mutation is amplified with a biotin-labeled 3′ primer and a ruthenium-labeled 5′ primer. After amplification, the sample is split into two aliquots. One aliquot is digested with *Hpa* II that cuts the fragment only if the mutation is present, and thus, removes the ruthenium-labeled 5′ part of the fragment from the biotinylated 3′ part. The other aliquot remains unaltered. Via streptavidin, the biotinylated DNA is bond to paramagnetic beads, thus, is kept in the reaction cell, while all other components are washed away. The amount of ruthenium bound to the biotinylated DNA is determined for both samples by measuring the ECL generated by the ruthenium complex in the reaction cell. The

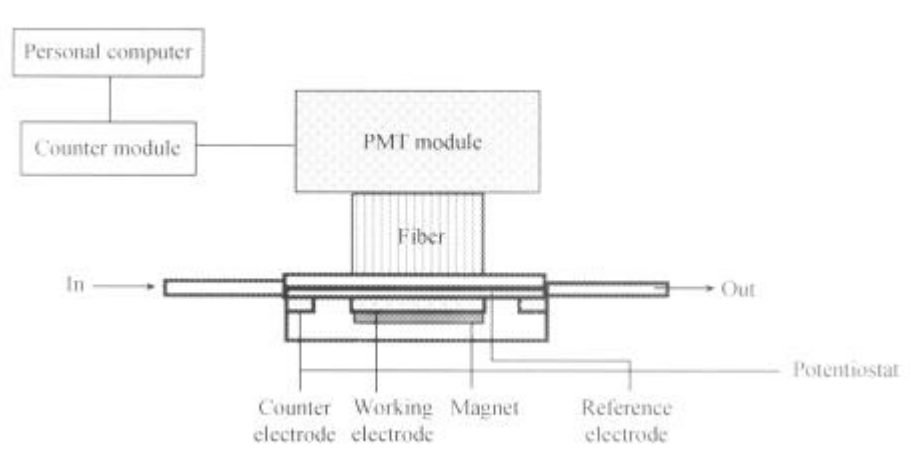

Fig. 2. A diagram of the essential components of the instrument.

genotype is determined by comparing the signal intensities between the digested and the undigested samples.

(ⅰ) DNA digestion with *Hpa*Ⅱ. The assay is based on the appearance of *Hpa*Ⅱ restriction site (5′ C*CGG 3′). The 108-bp fragment amplified from mutant templet can be cut by *Hpa*Ⅱ, generating a 44-bp and a 64-bp fragments, thus removing the Ru-label from the biotinylated part of the fragment. If wild-type templet is amplified, *Hpa*Ⅱ will not cut the fragment and the Ru-label is not removed.

(ⅱ) ECL detection. Fig. 4 demonstrates the assay results of the mutant samples. Curve 1 shows the result of the amplified digested sample after 30 cycles. Curves 2, 3, 4 show the results of the amplified undigested samples after 25, 30 and 35 cycles, respectively. Results show that the ECL intensities of the amplified undigested samples after 25, 30 and 35 cycles are 47, 176 and 212 cps, respectively. And the ECL intensitiy of the amplified digested sample after 30 cycles is about 7 cps, which is close to the signal from contral. So we consider that the ECL signal of digested sample is negligible.

Table 1 shows the ECL intensities of different cycles between the wild-type and the mutant samples. The data show that, after the same PCR cycle number, the ECL intensities of the digested and undigested wild-type samples are nearly identical, but the difference of ECL intensities between the digested and undigested mutant samples is significantly different. Given the distinctive difference that among the signal intensities, we consider that the point mutation can be detected using ECL method. Thus, ECL detection of PS-1 gene mutation may provide a new means for FAD diagnosis.

3 Conclutions

In this report, ECL, combined with PCR and the

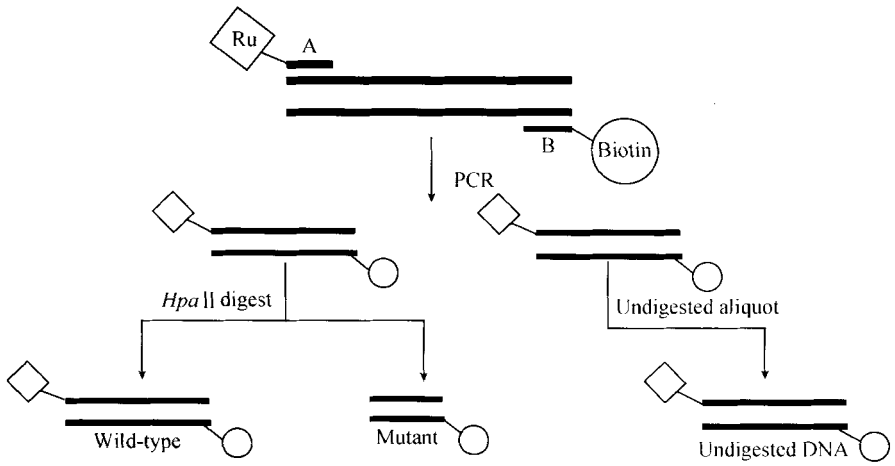

Fig. 3. The principle of PS-1 mutation assay. A, 5′primer; B, 3′primer; ◇, $Ru^{2+}(bipy)_3$-chelate; ○, Biotin.

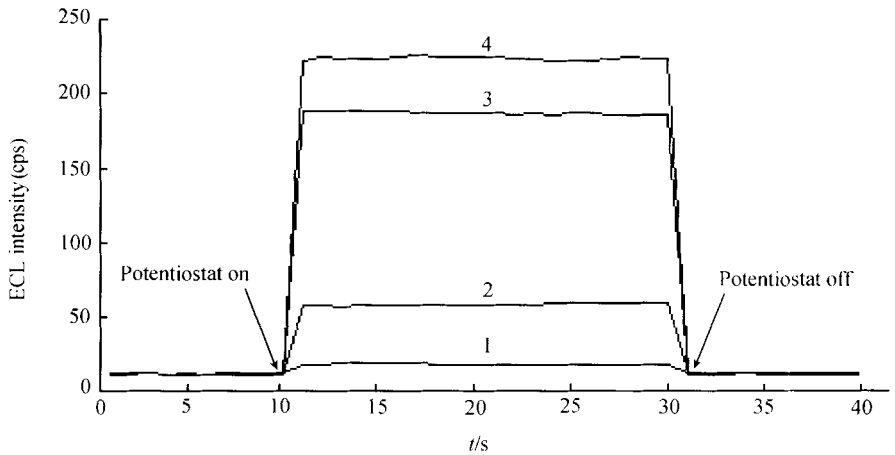

Fig. 4. The assay results of the mutant samples. 1, ECL signal of the amplified digested sample after 30 cycles; 2, 3 and 4, ECL signals of the amplified undigested samples after 25, 30 and 35 cycles, respectively.

Table 1 ECL intensities of different cycles between the wild-type and the mutant samples

Cycles	ECL intensity(cps)			
	undigested wild-type sample	digested wild-type sample	undigested mutant sample	digested mutant sample
25	46.0±2.0	48.0±2.4	47.0±1.6	5.0±0.4
30	178.0±6.0	175.0±5.0	176.0±6.0	7.0±1.0
35	214.0±8.0	209.0±6.4	212.0±7.0	6.0±0.7

restriction endonuclease technology, was used to detect point mutation of coden 235 of *PS-1* gene *in vitro*. The results show that this method can reliably discriminate the wild-type and the mutant genes with high sensitivity, speed, accuracy and easiness of operation. The method supplies a new approach for detecting the point mutation of FAD-related genes. It may be applied in the early diagnosis of Alzheimer's disease.

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Effects of pattern shape on adaptation of dLGN cell

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Abstract **Pattern adaptation is one of the fundamental sensory processes in the visual system. In this study, we compared pattern adaptation induced by two types of sinusoidal drifting grating in dLGN cells of cat. The two types of grating have the same parameters (e.g. spatial frequency, temporal frequency and contrast) except their pattern shapes, one of which is normal grating and the other annular grating. The results suggested that the annular grating elicited stronger response and stronger pattern adaptation than the normal grating. This is consistent with the adaptation and aftereffect to the two types of drifting gratings seen in psychology and may reflect the subcortical neural mechanism underlying these psychological phenomena.**

Keywords: cat, dLGN, pattern adaptation, normal grating, annular grating.

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Pattern recognition and pattern adaptation are important functions of the visual system on information processing, but it is still unclear which level of the visual pathway they initiate. It is widely agreed that the cells processing the complicated information of object shape (e.g. the contour of the object, the orientation of one line) are located in areas 17 and 18 of the visual cortex and the senior cortex[1−4]. De Weerd and his colleagues (1994) studied the cell responses in visual cortex and subcortex of cat using focal brain lesions, and found that, when areas 17 and 18 were damaged, the animal was still able to recognize the object shape. However, when the subcortical inputs to these areas were damaged, the ability of recognition was abolished. This suggested that the subcortex is related to the shape recognition function[5]. Nothdurft and his colleagues' study (1990) in cat dLGN cells revealed that if the shape of dots constructing a pattern was different, the responses would be different, and the differences were more obvious when the cell was stimulated by the edge of the patterns[6]. Shou et al. first reported that the dLGN cells had pattern adaptation, and the pattern adaptation was not due to the feedback from visual cortex[7].

The psychological experiments indicate that the adaptation and the aftereffect induced by annular grating are stronger than those induced by normal grating[3]. In