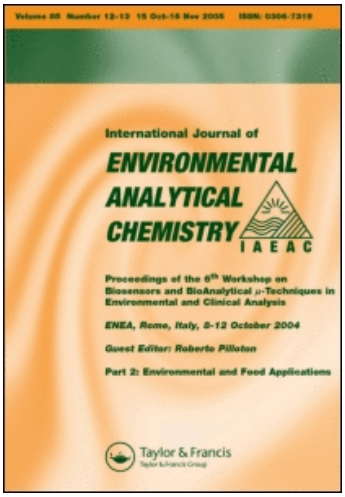

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Detection of DNA/DNA hybridization by electrogenerated chemiluminescence

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Detection of DNA/DNA hybridization by electrogenerated chemiluminescence

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Oligonucleotides labelled at 5′ with $Ru(bpy)_3$ were hybridized to complementary DNA that had been previously immobilized on a glassy carbon electrode by anodic oxidation. The hybrids emitted detectable electrogenerated chemiluminescence upon application of 10–50 ms pulse(s) of 2.3 V. Pre-accumulation of the $Ru(bpy)_3$ label at the electrode was found to enhance light emission as compared to the label in free solution. Since the intensity of the emitted light was greatly influenced by the electrode material, gold, platinum, and various carbon based materials (glassy carbon, graphite, carbon fibre, and composite plastic/carbon materials) were tested. The light generated at glassy carbon electrodes resulted at least two orders of magnitude higher than any other material tested.

Keywords: Nucleic acids; ECL; Electrochemiluminescence; Ruthenium

1. Introduction

Organometallic ruthenium(II) complexes such as $Ru(bpy)_3$ [=Tris(2,2′-bipyridyl) ruthenium(II)] have been extensively studied for the ability of generating electrochemiluminescence (ECL) at room temperature in aqueous buffered solutions, and in the presence of dissolved oxygen and other impurities. The anodic oxidation of $Ru(bpy)_3^{2+}$ in the presence of tri-n-propylamine produces ECL, following a reaction whose mechanism is still debated [1]. ECL detectors have been reported to be used successfully in small volumes, as their light output, unlike that of an electrochemical detector, is not affected by the presence of background electrical noise. In this work a procedure was developed to label DNA with $Ru(bpy)_3^{2+}$ and the potential use of $Ru(bpy)_3$ generated ECL for the sensitive detection of DNA/DNA hybridization was investigated.

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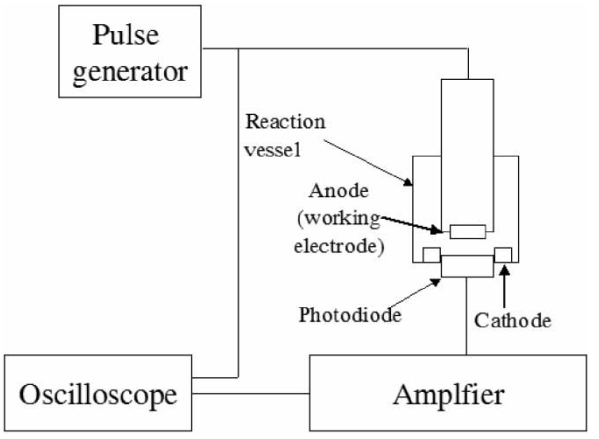

Figure 1. Experimental set-up.

2. Experimental

The experimental set-up used in this work is outlined in figure 1. In initial experiments, a single 10 ms pulse of 2.3 V was sent to an electrode in 300 μL of PBS buffer containing 4% tri-n-propylamine. The labelled DNA was either in solution or hybridized to another DNA immobilized on the electrode surface. The excitation of $Ru(bpy)_3^{2+}$ generated light which was detected by a photodiode, amplified and sent to an oscilloscope. In further experiments a cooled CCD camera was used to detect the light generated by a series of 50 ms pulses at 400 ms intervals.

For DNA labeling with Ru(bpy)3, an 18mer oligonucleotide was synthesized with an amine at its 5′ end; 100 nmol nucleic acids were reacted overnight with a 50× molar excess of bis(2,2′-bipyridine)-4′-methyl-4 carboxybipyridine-ruthenium N-succinimidyl ester-bis (hexafluorophosphate) in 100 mM sodium carbonate buffer, pH 9. The labeled DNA was then precipitated with the addition of six volumes of ethanol and recovered by centrifugation.

Covalent attachment of DNA to glassy carbon was obtained by anodic oxidation of 5′ aminated DNA. For this purpose, cyclic voltammetry from 0 to 1.9 V to 0 V at 20 mV s^{-1} toward a pseudo reference platinum electrode was performed in 200 μL of Britton and Robinson buffer with the addition of 2 nmol aminated DNA, according to Deinhammer *et al.* [2].

3. Results and discussion

The DNA labeled as described in the experimental section emitted detectable ECL when added in concentrations in the μM range to the electrochemical cell. The intensity of the light emission was found to be highly dependent on the material used for the fabrication of the electrodes. Several different materials were tested to find the most appropriate for biosensor fabrication.

In table 1, the limits for the detection of labeled DNA in a solution of 0.3 mL with different materials as working electrode are reported. Due to much higher sensitivity

Table 1. Amounts of $Ru(bpy)_3$ labeled DNA detected in a volume 0.3 mL using different materials as working electrode.

Electrode material	Detection limit
Gold	2 nmol
Platinum	2 nmol
Graphite ink	> 5 nmol
Polystyrene/Glassy carbon powder	> 5 nmol
Polystirene/Carbon fiber	500 pmol
Glassy carbon	5 pmol

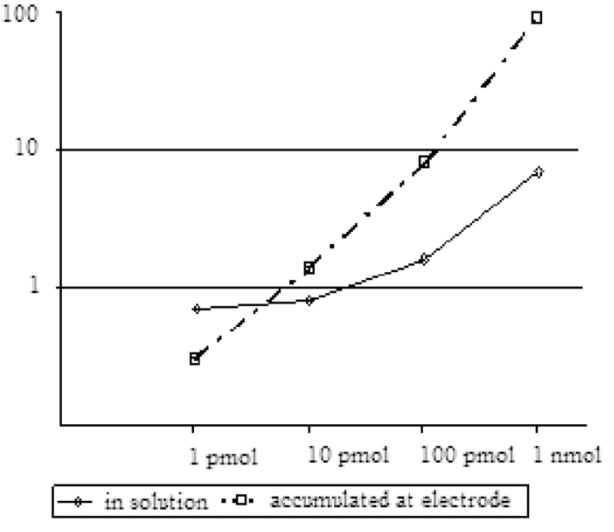

Figure 2. Light generated in a 300 µL cell at a 10 mm^2 glassy carbon electrode by a $Ru(bpy)_3$-labeled oligonucleotide either in solution or preaccumulated at the electrode.

of glassy carbon (GC) electrodes, further work was carried out only with GC electrodes. Pre-accumulation of the $Ru(bpy)_3$ label at the electrode (as occurs following hybridization to the immobilized oligonucleotide) was found to enhance light emission as compared to the label in free solution. The ECL generated by $Ru(bpy)_3$ in solution varied depending on the shape of the cell and electrode area, and did not depend linearly on the concentration. Conversely, as shown in figure 2, the light generated by $Ru(bpy)_3$ labelled DNA pre-accumulated at the electrode is proportional to the label amount. Thus, this system has potential as real time hybridization detector, because the gradual accumulation due to hybridization of labelled DNA at the electrode may result in signal increase if the electrode area and buffer volume are chosen properly. However, to achieve detectable dynamics a smaller cell and higher sensitivity would be required.

Oligonucleotides labelled at 5′ with $Ru(bpy)_3$ were hybridized in 2× SSC to complementary DNA immobilized on glassy carbon electrodes. After extensive washing, the hybrids were revealed by bringing the electrode to a positive potential with a pulse of 10 ms, and the generated light was detected by a photodiode. Detectable signals were recorded with this method when the hybridization solution contained at least

10 pmol of complementary Ru-labelled oligonucleotide. However, the kinetics of the hybridization was slow, possibly because the immobilization of the first helix was not limited to the 5′ end but involved DNA secondary amines, thus constraining the DNA. Since the $Ru(bpy)_3$ is not consumed in the reaction, to enhance sensitivity a detection strategy including prolonged excitation of the label was attempted. The light signal was detected by a cooled CCD camera. Interrogation times as long as 10 min were used with minimal reduction of the light signal strength in time, and significant increase of the sensitivity of the detector. The detection limit was better than 1 pmol DNA for labelled DNA in solution. In conclusion, ruthenium generated ECL has great potential for the development of biosensors and advanced methods for DNA detection. Dynamic detection of DNA is possible due to the enhanced signal generation by short pulses on labeled molecules preaccumulated at the electrode. Electrode derivatization and ECL generation/detection can be carried out efficiently as long as glassy carbon is used as the electrode material. The methods for DNA and electrode modification used here are rapid, easy and well suited for industrial preparation. However, although techniques for glassy carbon soft lithography have been reported [3], this material is expensive and poorly suitable for mass production.

References

[1] W. Miao, J.P. Choi, A.J. Bard. *J. Am. Chem. Soc.*, **124**, 14478 (2002).
[2] R.S. Deinhammer, M. Ho, J.W. Anderegg, M.D. Porter. *Langmuir*, **10**, 1306 (1994).
[3] O.J.A. Schueller, S.T. Brittain, G.M. Whitesides. *Sensors & Actuators A*, **72**, 125 (1999).