

PROJECT TITLE

Electrochemiluminescent biosensor for detection of auto-antibodies associated with celiac disease

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DESCRIPTION

Overview:

The overall objective of the project is to develop a generic platform capable of detection of auto-antibodies associated with celiac disease with electrochemiluminescent (ECL) transduction.

Background and State of the Art:

Electrogenerated chemiluminescence (also called electrochemi-luminescence and abbreviated as ECL) is a process whereby species generated at electrodes undergo high-energy electron-transfer reactions to form excited states that emit light. ECL has become a very powerful analytical technique and been widely used in the areas of, for example, immunoassay, food and water testing, and biowarfare agent detection,¹ highlighting the use of ECL as a powerful tool for ultrasensitive biomolecule detection and quantification. There are commercially available clinical chemistry analyzers such as the Elecsys 1010 and 2010 by Roche Diagnostics which are prohibitively expensive for developing regions. Thus, high-throughput, miniaturized biosensors based on ECL technology capable of multiplexed detection with high sensitivity, low detection limit, and good selectivity and stability continue to attract the interest of the research community.

Electrochemiluminescence (ECL) of tris(2,2'-bipyridyl)ruthenium(II) ($\text{Ru}(\text{bpy})_3^{2+}$) is a well-known detection method that provides high sensitivity with low background through generation of an optical signal triggered by an electrochemical reaction.² To trigger the optical signal, a sacrificial amine (usually tripropylamine, TPA) is oxidized at the electrode surface generating a radical that reduces the Ru(II) complex to Ru(I) which is further transformed into a Ru(II) excited state that generates the luminescence. In comparison with traditional laser-induced luminescence detectors, the instrumentation for ECL detection is substantially less complicated and less expensive as the excitation laser and optical filters are eliminated. Moreover, because the ECL reaction only occurs close to the surface of an electrode, the sample requirement is quite small. This makes ECL an ideal detection method for microdevices.³

Celiac disease is a gluten-sensitive enteropathy that affects as much as 1% of the population and patients with celiac disease should maintain a lifelong gluten-free diet, in order to avoid serious complications and consequences. Therefore, it is essential to diagnose celiac disease at the earliest possible conjecture.

¹ Miao, W. *Chem. Rev.*, **2008**, *108*, 2506.

² *Electrogenerated Chemiluminescence*; Bard, A. J., Ed.; Dekker: New York, **2004**.

³ Cao, W.; Liu, J.; Yang, X.; Wang, E. *Electrophoresis* **2002**, *23*, 3683.

Project Contribution and Methodology:

Auto-antibody detection will be based on immobilized antigens (gliadin, tissue transglutaminase (tTG)) that are recognized by the corresponding IgA or IgG auto-antibody and detected with ruthenium-bipyridyl labeled antibodies. The detecting conjugates will be prepared by covalent coupling of commercially available $[\text{Ru}(\text{bpy})_2(\text{bpy}'\text{-COONHS})^{2+}]$ (NHS: N-hydroxysuccinimide ester) with the amino groups of the antibody. Additionally, the $[\text{Ru}(\text{bpy})_2(\text{bpy}')^{2+}]$ moiety will be linked to gold nanoparticles bearing biotin groups, which will react with previously prepared streptavidin/ detecting antibody conjugates (Figure 1).

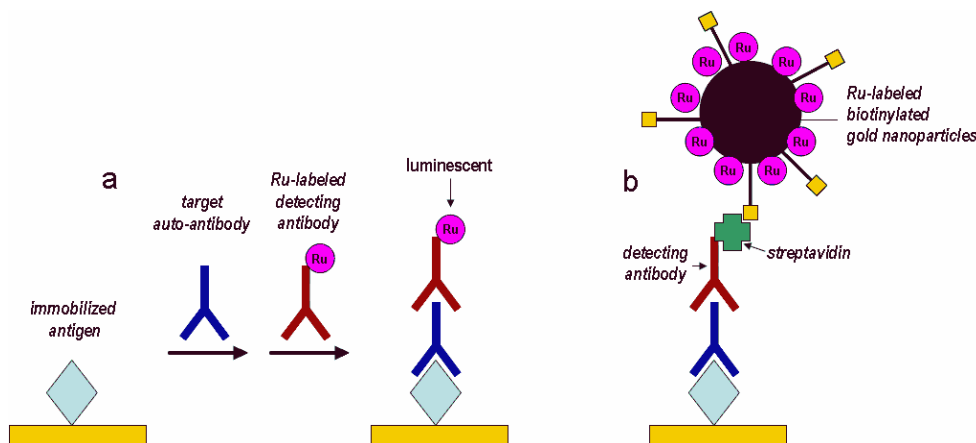


Figure 1. Sandwich assays for the ECL detection of auto-antibodies associated with CD using Ru-labelled detecting antibody (a) $[\text{Ru}(\text{bpy})_3^{2+}]$ -labelled biotinylated nanoparticles (b).

Evaluation of surface chemistry methodologies

The biosensor surface will be optimized in terms of achieving maximal sensitivity while reducing non-specific interactions. Strategies based on pure or mixed carboxylic acid terminated SAMs for the covalent immobilization of gliadin and tTG will be tested in conjunction with the use of blocking agents.

Electrochemical techniques such as amperometry, voltammetry and electrochemical impedance spectroscopy are the techniques of choice to characterize the prepared surfaces.

Evaluation of ECL detection of auto-antibodies associated with CD

A sandwich assay format will be used for the detection of IgA and IgG auto-antibodies associated with CD (Figure 1). The corresponding antigen (gliadin, tTG) will be covalently immobilized on the gold surface, followed by auto-antibody capture and detection using a Ru-labeled detecting antibody (Figure 1a). Strategies for ultrasensitive detection based on the use of $[\text{Ru}(\text{bpy})_3^{2+}]$ -labelled biotinylated nanoparticles will also be developed (Figure 1b). A comparison of the ECL biosensor performance with commercially available anti-gliadin and anti-tTG ELISA assays will be carried out. Initially, auto-antibodies will be detected in pure form (i.e. from buffered standard solutions) but the sensor will be clinically evaluated and validated using anti-gliadin and anti-tTG in real patient serum samples provided by King's College London.