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Selective Electrochemiluminescent Sensing of Saccharides using Boronic Acid-Modified Coreactant

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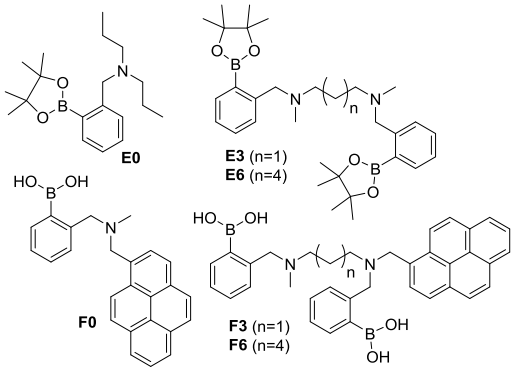
We report a strategy for modulating the electrogenerated chemiluminescence (ECL) response by integrating a boronic acid to the chemical structure of coreactants. Excellent selectivity for D-glucose was achieved by tuning the linker length of a bis-boronic acid amine coreactant.

The discovery of electrogenerated chemiluminescence (ECL) in aqueous media has resulted from intense fundamental research on the mechanisms of the phenomenon[1-2] and has led eventually to important bioanalytical applications including commercialized immunoassays for clinical diagnostics.[3-4] The corresponding ECL process is based on a reaction cascade initiated at an electrode surface between a tandem system composed of a luminophore and a sacrificial coreactant species. The luminophore reaches the excited state by the highly exergonic reactions with the electrogenerated coreactant radicals. It relaxes then to the ground state by emitting ECL light which is the analytical signal. During the process, the luminophore is regenerated, whereas the coreactant is consumed by the electrochemical reactions. The amount of light generated directly depends on the concentration of the luminophore but also of the coreactant. The luminophore has been used as ECL labels in numerous bioassays[5] or as molecular probes with different receptor sites.[6-11] For example, ruthenium and iridium complexes have been modified with crown ether moiety to measure different metal cations.[6-9] In this case, the role of the coreactant is just to generate efficiently electrochemically suitable radicals with adequate redox potentials and life-times. Another enticing possibility is to detect and to quantify any ECL-active coreactant treated thus as an analyte as its concentration will directly influence ECL intensity. An example of the ECL detection of coreactants is the determination of oxalate and peroxydisulfate. Another class of analytes that may act as coreactants is amines. ECL assays for amine-based compounds find many analytical applications because amine groups are present in a large variety of pharmacologically important compounds such as antibiotics, anti-histamines, opiates, *etc*.[5, 12-13] However, a major limitation is that the analytes have to be electro-active and also able to generate strong reducing or oxidizing radicals to bring the luminophore to the excited state. So it limits strictly the nature of the possible molecules to detect.



Scheme 1 Chemical structures of the boronic acid based coreactants **E0**, **E3** and **E6** and Fluorescent boronic based saccharide sensors **F0**, **F3** and **F6**.[14]

As an alternative strategy, we propose to confer recognition properties to the coreactant by integrating a receptor site to its chemical structure (Scheme 1). The recognition of the target analyte would then modulate its electrochemical properties and the resulting ECL emission of the luminophore. Here, we develop an approach using a boronic acid as the receptor group.

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We chose the boronic acid receptor group as our first target due to its proven abillity to bind saccharides in water.[15]

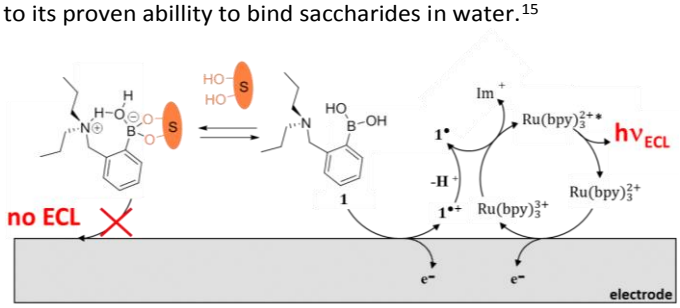

Fig. 1 Influence of the binding equilibrium of phenylboronic acid to the saccharides (either D-fructose or D-glucose) on the ECL mechanism of the system $Ru(bpy)_3^{2+}$/**E0**. Im^+ is the iminium product. [Under the measurement conditions the pinacol protecting group is removed.]

Model systems providing high ECL efficiency in water consist of the luminophore $Ru(bpy)_3^{2+}$ with coreactants such as tri-*n*-propylamine (TPrA) or 2-(dibutylamino) ethanol.[16-18] The pursuit of efficient ECL reagents is an intense area of investigation and several groups have studied the ECL mechanism using amine coreactants.[19] In general, tertiary amines produce ECL emission more efficiently than secondary amines and primary amines, following this order.[5] Recently, it has been reported that increasing the electrochemical oxidation rate of amines leads to higher ECL efficiency. For instance, ECL emission is amplified with amines bearing electron-donating groups.[18] In addition, aromatic amines, such as the pyridine ring of NAD^+, the coenzyme of the dehydrogenase enzyme class, do not produce chemiluminescence or ECL with the ruthenium luminophore.[20] By contrast, NADH, the reduced form of the coenzyme, may generate ECL emission because the aromaticity of the pyridine ring is destroyed and the aliphatic tertiary amine group undergoes ECL emission. Herein, inspired by the remarkable structure/reactivity relationship of this very efficient biomolecule, we designed and prepared a series of amine-based coreactants (**E0**, **E3** and **E6**) integrating boronic acid function as receptor units (Scheme 1). For the first time, we demonstrated that the recognition of the saccharide modifies both the structure and the reactivity of the coreactant and thus the resulting ECL emission (Fig. 1). With the presented approach, ECL generation depends on target molecules that are electro-inactive and do not to change the conformation of the luminophore as classically performed with molecular probes. Moreover, differential selectivity for D-glucose and D-fructose is achieved by tuning the number of boronic acid groups and the spacer length.

To explore the usefulness of the above strategy, we selected the prototypical $Ru(bpy)_3^{2+}$ complex as a luminophore and modified tertiary amines as oxidative-reduction coreactants because such a model system leads very efficiently to strong ECL emission.[16] As a test case, we designed and prepared compound **E0** which is similar to the TPrA structure but containing a boronic acid as recognition unit. **E0** and the other coreactants shown in Scheme 1 (**E3** and **E6**) were synthesised through the alkylation of their corresponding amine using 2-(2-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (Schemes S1 and S2).

Once isolated, **E0** electrochemical and ECL properties were investigated by using cyclic voltammetry in PBS solution that contained 10 µM of $Ru(bpy)_3^{2+}$ and 0.2 mM of **E0** (Fig. 2). The coreactant concentration was in excess in comparison to the luminophore, as very classically employed in ECL experiments because it is consumed during the ECL process. As shown in Fig. 2, irreversible oxidation of **E0** occurred at 0.77 V *vs.* Ag/AgCl/KCl and the oxidation wave is shifted to less anodic potentials by 100 mV in comparison to the model TPrA reagent (Fig. S1a). It indicates that the phenylboronic acid group is a better donor than ethyl group. This donor effect makes the molecule much easier to oxidize and can also stabilize the radical cation by charge delocalization.

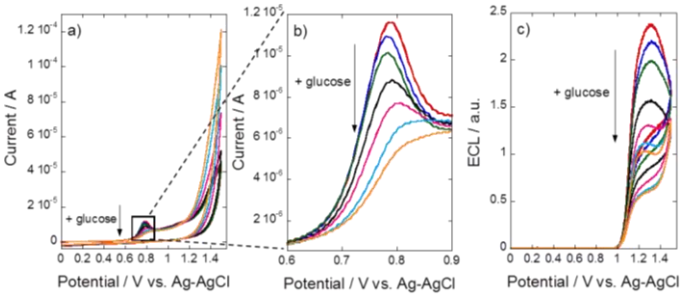

Fig. 2 Effect of D-glucose addition on electrochemical oxidation of **E0** and on the corresponding ECL response. (a-b) Cyclic voltammetry and (c) ECL signal of a PBS solution (pH 7.4) containing 10 µM $Ru(bpy)_3^{2+}$, 0.2 mM **E0** and different concentrations of D-glucose. The arrow indicates increasing concentrations of D-glucose (0, 0.5, 1, 10, 50, 100 and 200 mM). Experiments were performed on glassy carbon (GC) electrode at a scan rate of 0.1 V s^{-1}.

ECL emission occurred at the oxidation potential of $Ru(bpy)_3^{2+}$ (Fig. 2c), as for TPrA in the same experimental condition (*i.e.* low $Ru(bpy)_3^{2+}$ concentration). It follows an oxidative-reduction mechanism where both the luminophore and the coreactant are oxidized (Fig. 1). After a deprotonation step, the cation radical gives a highly reducing neutral radical that react with the oxidized $Ru(bpy)_3^{3+}$ to generate the excited state of the luminophore. This one relaxes *in fine* to the ground state by emitting ECL light. In comparison to TPrA, ECL response with **E0** is divided by a factor 2 (Fig. S1b). Indeed, the nature of substituents attached to nitrogen or α-carbon on the amine coreactant can also affect the ECL efficiency[5, 21] and it has been reported that aromatic-substituted amines that can conjugate the radical intermediates consistently lead to lower ECL emission response.[22]

The addition of D-glucose caused a notable effect on both the voltammetric wave and the ECL response (Fig. 2). It resulted in a dramatic decrease of the oxidation current along with its shift to more anodic potentials (Fig. 2b). Correspondingly, ECL intensity decreases progressively with the addition of D-glucose. Under these conditions, the effects of D-fructose were qualitatively similar to the ones of D-glucose but with a much stronger decrease of both anodic wave and ECL intensity for a given concentration (Fig. S2). Indeed, the oxidation wave almost vanished and the ECL peak decreased by 57% with 50 mM of D-

fructose (Fig. 3a). The same limit values for the ECL intensity and for the current were reached with 200 mM of D-glucose than with 50 mM of D-fructose. Such a difference was expected because the complexation constant of phenylboronic acid with D-fructose is much higher than with D-glucose (110 dm^3 mol^{-1}).[15, 23]

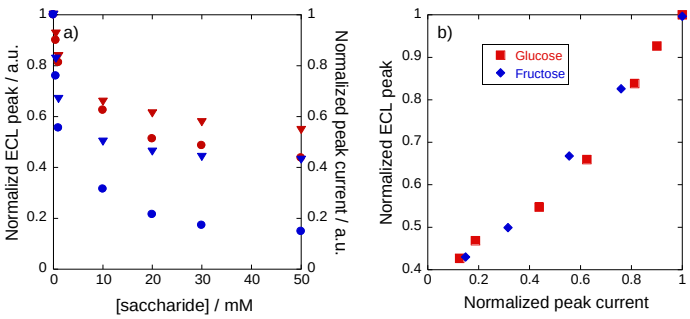

Fig. 3 a) Variation of the normalized ECL peak intensity (triangles) and of the oxidation peak current (dots) for the system $Ru(bpy)_3^{2+}$/**E0** as a function of the concentration of D-glucose (red markers) or of D-fructose (blue markers). b) Correlation between the ECL signals and the peak current corresponding to the **E0** oxidation for the addition of different concentrations of D-glucose or of D-fructose.

To check that the observed decrease was related to the complexation of the saccharides by the phenylboronic acid from **E0** and not to some photochemical or quenching effects, we recorded first the ECL spectra of $Ru(bpy)_3^{2+}$ with increasing concentrations of D-fructose (Fig. S3). Identical ECL and photoluminescent spectra were obtained showing that the photochemical properties of the luminophore were not affected neither by D-fructose nor D-glucose. Then we investigated the electrochemical and ECL signals of the model $Ru(bpy)_3^{2+}$/TPrA in presence of D-fructose (Fig. S4) and the effects of D-fructose addition led to minute changes. Finally, to determine the rate controlling step in the global ECL process, we plotted the variations of the normalized ECL peak (*i.e.* final readout signal) as a function of the normalized peak current (*i.e.* first step in the ECL process) with increasing concentration of D-fructose or D-glucose (Fig. 3b). One can observe unambiguously that both signals are cross-correlated for both saccharides. Which demonstrates that the variation of the ECL intensity is governed by the oxidation of the coreactant. The electrochemical step, and more specifically the heterogeneous oxidation of **E0**, controls the efficiency of the ECL process. In other words, the decrease of the ECL intensity observed with increasing concentration of saccharides is due to the direct oxidation of the **E0** at the electrode surface when it binds the saccharides.

The oxidation behavior of tertiary amines at glassy carbon electrodes has been examined in aqueous solutions.[17, 24-26] It is known that oxidation of a tertiary amine occurs when it is in the deprotonated form.[17] When protonation of the tertiary amines becomes important at acidic pH, its oxidation peak decreases in intensity and shifts toward more positive potentials.[17] As illustrated in Fig. 1, the complexation of the saccharide by **E0** induces a solvent-inserted B-N bond which behaves in a very similar fashion to protonation of the nitrogen atom.[15, 27] Therefore, heterogeneous oxidation of the coreactant in the presence of saccharide becomes a very inefficient process that limits drastically the ECL signal. However, even at high D-fructose concentrations where almost all the **E0** is bound, we still observe an ECL signal. Since ECL is obtained at the oxidation potential of $Ru(bpy)_3^{2+}$, the remaining ECL intensity resulted probably from the electrocatalytic mechanism where **E0** is homogeneously oxidized by the electrogenerated $Ru(bpy)_3^{3+}$.

To further assess our approach and to improve the selectivity, we designed and prepared new symmetric tertiary bis amino reagents bearing boronic acid end-groups and having linkers with different lengths (Scheme 1). Previous, research has demonstrated that saccharide selectivity can be achieved in systems with two boronic acid groups.[14-15] We investigated first the properties of the compound **E6** with a hexyl linker between both amino groups. The cyclic voltammogram in Fig. S1a is dominated by the irreversible oxidation waves of the amino moieties at the same potential as TPrA. The peak current is increased by approximately 2.1-fold indicating that both amino groups are simultaneously oxidized. However, the ECL signal is 15% lower with **E6** than with TPrA (Fig. S1b), even if two amino groups were oxidized and may participate to the ECL process. This low ECL response is probably related to the lower stability of the electrogenerated dication radicals. The addition of D-fructose or D-glucose induces the decrease of the oxidation current and the shift of the corresponding anodic wave, as reported for **E0** (Fig. S5), which resulted in a decrease of the ECL response in the presence of both saccharides (Fig. S5-S6). To further investigate the properties of **E6**, additional competitive assays were performed. They clearly showed that D-fructose and D-glucose caused notable modification of the current and of the ECL signal (Fig. S7). The detection limits of D-glucose and of D-fructose for **E6** were 20 µM and 10 µM, respectively (Fig. S8). Initially, it was somewhat surprising to us that **E6** could not discriminate between D-fructose and D-glucose and detect them selectively. However, from our previous fluorescence work for **F6** the measured binding constants for D-fructose and D-glucose are very similar 784±44 and 962±70 dm^3 mol^{-1}.[14] Further electrochemical experiments were performed on to investigate the influence of the linker length on the selective recognition of both saccharides. Fig. 4a presents the results obtained with **E3** where the linker between both amino group and the phenylboronic acid moieties is shorter. The cyclic voltammogram showed that the oxidation process of **E3** is more complex exhibiting a first anodic wave at 0.87 V *vs.* Ag/AgCl/KCl and a second at 0.95 V *vs.* Ag/AgCl/KCl (Fig. 4a).

The fact that both waves are partially separated indicates that the electronic communication between both amino groups is not negligible, in contrast to what was observed for **E6**. Adding D-glucose induces the decrease of the current for both oxidation waves as well as the parallel decrease of the ECL response (Fig. 4b). Remarkably, an invatriant voltammetric signal and ECL response (Fig. 4 c-d) was observed for D-fructose, allowing discriminating between both saccharides (Fig. 5). While this result seems quite strange, our observations with similar fluorescence systems[14] can help explain the observed selectivity. Since, the binding of **F3** with D-fructose is

significantly impaired when compared with the model system **F0** evidenced by a 4-fold reduction in binding for D-fructose from 395±11 dm^3 mol^{-1} for **F0** to 95±9 dm^3 mol^{-1} for **F3**. However, for D-glucose the binding is enhanced 2-fold from 44±3 dm^3 mol^{-1} for **F0** to 103±3 dm^3 mol^{-1} for **F3**.[14]

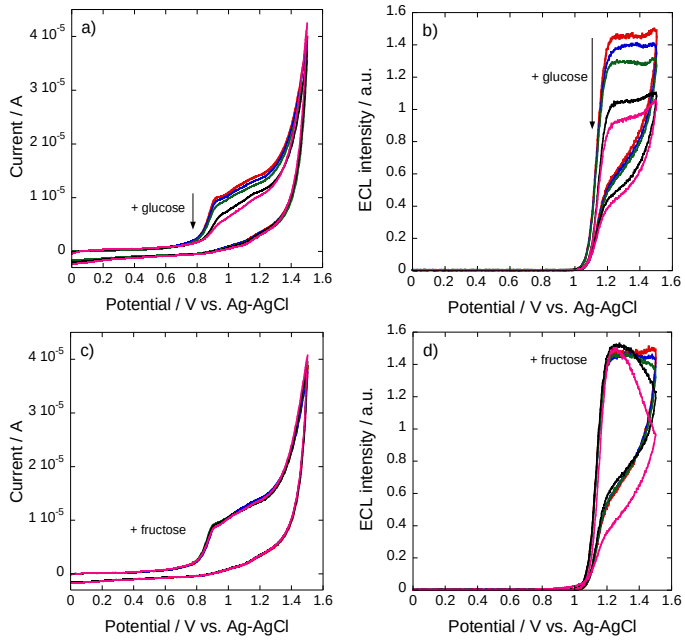

Fig. 4 Effect of (a-b) D-glucose or (c-d) D-fructose addition on electrochemical oxidation of **E3** and on the corresponding ECL responses. Cyclic voltammograms and ECL signals of a PBS solution (pH 7.4) containing 10 µM $Ru(bpy)_3^{2+}$, 0.1 mM **E3** and different concentrations of D-glucose or of D-fructose. The arrow indicates increasing concentrations of D-glucose or of D-fructose (0, 0.5, 1, 10 and 50 mM). Experiments were performed on glassy carbon (GC) electrode at a scan rate of 0.1 V s^{-1}.

The proportional decrease of the ECL readout signal with the D-glucose concentration allowed for facile quantification. Finally, the correlation of the ECL variation with the current for **E3** confirmed that the oxidation step of the coreactant is the one determining the efficiency of the ECL process.

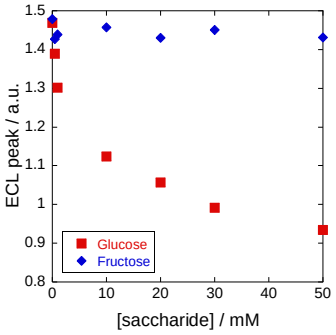

Fig. 5 Variation of the ECL peak intensity for the system $Ru(bpy)_3^{2+}$/**E3** as a function of the concentration of D-glucose or of D-fructose.

In summary, we have developed a saccharide selective electrogenerated chemiluminescence (ECL) system that functions in aqueous media. To our knowledge this is the first report of a sensing strategy based on the recognition of the target analyte by the ECL coreactant which directly impacts the CL readout. We achieved excellent D-glucose selectivity by varying the linker length of a bis-boronic acid amine coreactant. Our approach allows manipulating the ECL readout signal with an additional parameter and not by playing only with the structure of the luminophore and the electrode potential. The simplicity of the system ensures that it will find many applications from biological and chemical sensing to incorporation in molecular logic circuits.

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