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A supramolecular approach to the selective detection of dopamine in the presence of ascorbate†

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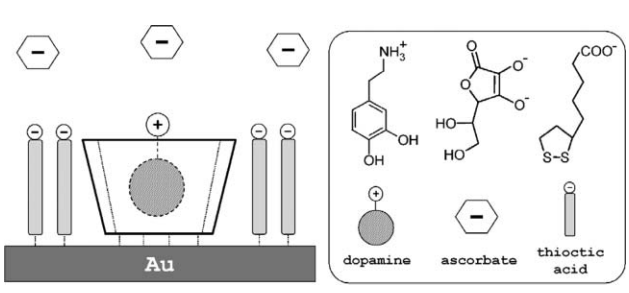
Dopamine can be detected selectively in the presence of ascorbate at a gold electrode modified by a β-cyclodextrin/ thioctic acid mixed monolayer.

Dopamine (3,4-dihydroxyphenethylamine, DA) is a very important neurotransmitter and its determination by electrochemical methods has been the subject of intensive investigation in the past.[1] One of the major problems experienced in the determination of DA is the interference of ascorbic acid (AA), which is oxidized at a similar potential and is present *in vivo* at a concentration 100–1000 times that of DA.[2] Several approaches using chemically- and physically-modified electrodes have been reported so far to circumvent these difficulties. Ion-exchange membranes such as Nafion[3] or poly-(aniline)s,[4] or self-assembled monolayers (SAMs) of charged thiols[5–9] have been used as electrode modifiers. Most of these rely on electrostatic interactions to resolve the oxidation peaks of DA and AA based on the fact that they are oppositely-charged at physiological pH, achieving peak resolutions in the range of 0.2–0.4 V. However, resolution is not perfect and it limits the applicability of these methods.

On the other hand, SAMs consisting of immobilized molecular receptors such as cyclodextrins (CD) are known.[10–12] Despite their great potential for sensing applications, most reports have dealt with physicochemical studies of adsorbate–electrode interactions using model compounds to confirm interfacial recognition rather than to address practical problems.[10–12] In this context, we have developed a novel strategy to detect DA in the presence of AA that consists of the modification of gold electrodes with a mixed monolayer of βCD and thioctic acid (TA). This provides the electrode with a molecular recognition motif (the CD) in addition to the electrostatic attraction of the TA (Scheme 1). Since CD forms stronger inclusion complexes with DA derivatives[13] than with AA[14] ($K_{DA} \sim 2000\ M^{-1}$, $K_{AA} = 130\ M^{-1}$), its presence in the monolayer in combination with TA is expected to increase the selectivity and sensitivity for the determination by inducing a cooperative effect based on simultaneous electrostatic and host–guest interactions.

The modified electrodes‡ were prepared by immersing a clean gold bead[15] in a 1 mmol L^{-1} DMSO solution of *heptakis*-6-thio-6-deoxy-βCD[10] (Au/CD), followed by immersion in a 1 mmol L^{-1} ethanol solution of TA also containing 5 mmol L^{-1} of 1-adamantanol (Au/CD + TA). Reductive desorption experiments of the mixed monolayer gave $\Gamma_{CD} = 3.0 \times 10^{-11}$ mol cm^{-2} and $\Gamma_{TA} = 5.1 \times 10^{-11}$ mol cm^{-2}; that is, the TA : CD molar ratio in the monolayer is about 1.7 although CD covers about 70% of the electrode surface.



Scheme 1

As expected, DA and AA gave indistinguishable signals at a bare gold electrode ($E \sim 0.2$ V *vs.* Ag/AgCl) in both cyclic (CV) and square wave (SWV) voltammetry (Fig. 1A and 1B, trace a). At the Au/CD electrode both signals decrease in intensity ($\sim 30\%$) due to the partial blocking of the electrode surface by the adsorbed CD molecules (Fig. 1A and 1B, trace b). Interestingly, when TA is present in the monolayer the DA signal intensity increases by about 20% with respect to the Au/CD electrode, while the AA signal is almost suppressed and shifted to more positive potentials (Fig. 1A and 1B, trace c). This means that TA blocks the access of AA to the electrode surface, presumably by electrostatic repulsion.

The voltammetric behavior of DA and AA is consistent with their impedance responses obtained at the modified electrodes (Fig. 2, Table 1). As can be seen, the Au/CD + TA electrode is responsive to the analyte present in solution as indicated by the R_{CT} values obtained. The R_{CT} value of DA decreases slightly when TA

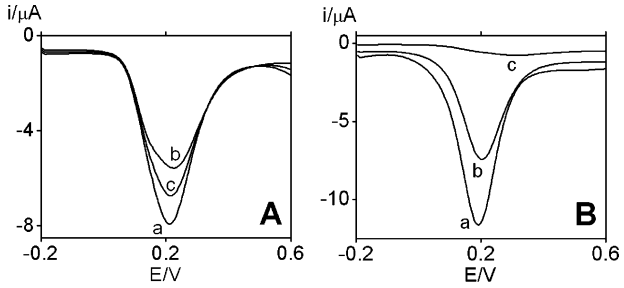


Fig. 1 SWV of (A) 1 mmol L^{-1} DA and (B) 1 mmol L^{-1} AA at bare gold (a), Au/CD (b) and Au/CD + TA (c) electrodes. Supporting electrolyte: phosphate buffer pH 7.0 (0.1 mol L^{-1}). Scan rate: 0.1 V s^{-1}.

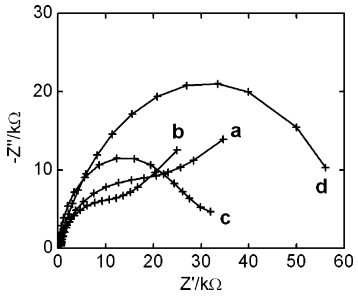


Fig. 2 Complex impedance profiles for DA at Au/CD (a), DA at Au/CD + TA (b), AA at Au/CD (c) and AA at Au/CD + TA (d), electrodes. Supporting electrolyte: phosphate buffer pH 7.0 (0.1 mol L^{-1}). Frequency range: 1 kHz to 0.1 Hz.

† Electronic supplementary information (ESI) available: Langmuir plot for DA (Fig. S1) and optimized geometries for the CD–DA and CD–AA inclusion complexes (Fig. S2). See http://www.rsc.org/suppdata/cc/b4/b407792j/

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Table 1 Charge transfer resistance (R_{CT}) values for DA and AA at the modified electrodes

	DA[a]	AA[a]
Au/CD	28.6	31.0
Au/CD + TA	19.4	62.5

[a] Values in kΩ. The experimental values were recorded at 1 mmol L^{-1} analyte concentration and fitted to an equivalent circuit to obtain R_{CT}.

is present at the monolayer. In contrast, a two-fold increase in the R_{CT} value of AA was observed at the Au/CD + TA electrode, indicating a stronger blocking effect to charge transfer. The electrochemical behavior of AA can be rationalized considering that the negatively-charged TA repels AA, blocking its diffusion towards the electrode and retarding the subsequent electron transfer. As expected, the opposite effect is obtained for DA.

A saturation behavior of the peak intensities as a function of DA concentration was observed at the Au/CD + TA electrode, indicating that the probe is actually bound to the receptor (Fig. S1). Treatment of the data using the Langmuir isotherm gave an interfacial association constant $K_{DA} = (6400 \pm 300)$ L^{-1} mol, a value 4-fold higher than that obtained for a CD-only monolayer (1600 ± 200 L^{-1} mol). The maximum surface coverage of DA also increased when TA was present in the monolayer with $\Gamma_{DA}/\Gamma_{CD} = 0.83$ and $\Gamma_{DA} = 2.5 \times 10^{-11}$ mol cm^{-2}. In contrast, the current changes observed for AA at the Au/CD + TA electrode with increasing concentrations (up to 0.01 mol L^{-1}) were so small that an accurate evaluation of its interfacial parameters was not possible. This confirms the discriminating function of the immobilized CD receptors in allowing the electrode to recognize DA only, even in the presence of AA.

The function of CD in the monolayer is also evidenced by sensitivity ($\Delta i/\Delta c$) measurements obtained from calibration plots recorded at +0.22 V and low analyte concentrations ($< 10^{-4}$ mol L^{-1}). At the Au/CD + TA electrode, DA is detected with a sensitivity of 0.70 mA L mol^{-1}, a value about 2-fold higher than those previously reported.[4,8] This increased sensitivity results from the presence of the CD cavities that provide the electrode with a higher active surface towards DA oxidation. Blocking of the CD cavities with 1-adamantanol reduces the sensitivity to 0.03 mA L mol^{-1}. This latter value is indicative of some permeability of the TA monolayer towards DA, as has been observed previously,[6] although it is evident that most of the current components result from CD-bound DA. In the case of AA, the sensitivity at the Au/CD + TA electrode was two orders of magnitude lower than that of DA and addition of 1-adamantanol did not give any significant change in the sensitivity value.

Quantum mechanical calculations performed for CD–DA and CD–AA inclusion complexes suggest that DA is deeply embedded in the CD cavity but AA does not penetrate at all, only interacting by H-bonds with the outer OH groups of CD (Fig. S2). This contrasting binding behavior of DA and AA can be explained by the higher size, charge and hydrophilicity of the AA molecule that reduce to a great extent its interaction with the hydrophobic CD cavity.

Another important difficulty that has been previously encountered in DA determination is the reaction that takes place between oxidized DA and AA.[1] In other words, DA-quinone is reduced by AA thus consuming DA in a side-reaction not detected electrochemically. Fig. 3 shows the CV of DA at the Au/CD + TA electrode in the absence and in the presence of a 10-fold excess of AA. As can be seen, both oxidation and reduction waves are clearly visible for DA with a peak-to-peak separation of 160 mV at 0.1 V s^{-1}. This denotes that oxidized DA is not consumed by AA, probably because the DA-quinone form is retained in the CD cavity and protected from interaction with AA (although continuous potential cycling produced a slow deterioration of both signals). This is further evidence of the discriminating function of immobilized CDs. On the other hand, the presence of AA in the solution adds a low anodic current to the DA response (4% at +0.22 V), in agreement with the results obtained with AA alone.

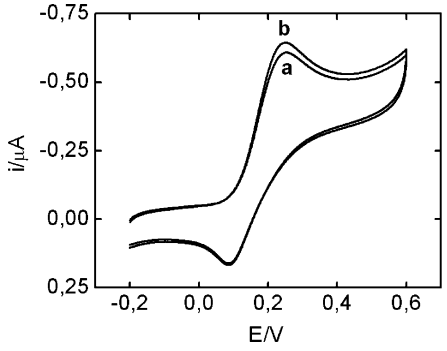

Fig. 3 CV obtained at the Au/CD + TA electrode for (a) 0.1 mmol L^{-1} DA and (b) a mixture of 0.1 mmol L^{-1} DA and 1 mmol L^{-1} AA. Supporting electrolyte: phosphate buffer pH 7.0 (0.1 mol L^{-1}). Scan rate: 0.1 V s^{-1}.

In conclusion, a novel strategy to selectively detect dopamine in the presence of ascorbate using a doubly-modified gold electrode with a molecular receptor and an anionic adsorbate is presented. The use of a combination of host–guest and electrostatic interactions is a novel aspect allowing a more rational design of analytical strategies that could find application in neuroscience. Studies in this direction are currently underway.

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Notes and references

‡ CV, SWV and impedance measurements were performed on a CH Instruments electrochemical workstation. Potentials were referenced to Ag/AgCl and nitrogen saturated phosphate buffer pH 7 (0.1 mol L^{-1}) was used as the supporting electrolyte. Reductive desorption experiments were carried out in degassed 0.75 mol L^{-1} KOH at 10 mV s^{-1} scan rate. Since the cathodic desorption wave of TA (−0.95 V) strongly overlapped with that of CD (−0.88 V), Γ_{TA} was determined by subtracting the charge passed under the mixed monolayer peak with that of the CD-only monolayer considering the rupture of 7 and 2 Au–S bonds for CD and TA respectively. Impedance measurements were performed in the frequency range of 1 kHz to 0.1 Hz with an alternating current amplitude of 5 mV. Data analysis was carried out using the commercially available program EQUIVALENT CIRCUIT written by B. A. Boukamp (University of Twente, The Netherlands).[16]

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