



Attachment of an aromatic dendritic macromolecule to gold surfaces

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Abstract

Surface immobilization of dendrons and dendrimers presents an exciting opportunity for creating a wide variety of functionalized polymeric architectures suitable for the immobilization of biomolecules. Dendritic molecules contain multifunctional groups that can be efficiently modified to control the properties of the resulting polymers. We are developing strategies to generate a highly functionalized surface using multifunctional and rigid dendrons immobilized onto different substrates. In this paper, electrochemical methods and scanning probe microscopy were used to explore the immobilization of a dendritic macromolecule (3,5-bis(3,5-dinitrobenzoylamino)benzoic acid) or (D-NO₂) onto gold electrodes. D-NO₂ adsorbs spontaneously by dipping the metal surface in dendron solution and also via grafting of cystamine covalent attached to gold electrode. Reduction of this layer generates the hydroxylamine product. The resulting redox-active layer exhibits a well-behaved redox response for the adsorbed nitroso/hydroxylamine couple.

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1. Introduction

Self-organized molecular layers combining both structural and functional control could be a key concept for the development of materials and provide a promising route for constructing new nanodevices and optimizing nanosensors. Dendrons and dendrimers are precise quantized, three-dimensional nanostructure that offer such control and are of great interest to both nano and polymer scientists as building blocks due to their unique, architecturally driven, macromolecular properties [1,2]. By having a high density of functional groups on their surfaces, dendrimers and dendrons are suitable for the role of spacer and linker between surfaces of solid supports and biological macromolecules [3,4]. Bioreactive surfaces prepared

using dendron thiols with a high density of amine groups is useful for the immobilization of biological macromolecules in biosensors, such as the fabrication of DNA microarrays and protein chips [5].

In a previous work, the benefits using dendrons as structure modifiers in the synthesis of hybrid and amphiphilic structural polymers were demonstrated [6]. In addition, we presented an alternative pathway for the synthesis of hybrid linear-dendritic copolymers [7] using (3,5-bis(3,5-dinitrobenzoylamino)benzoic acid) or (D-NO₂). The presence of an aromatic amine as constituent of a rigid fragment (three aromatic rings) appears to be responsible for the decrease of the amine nucleophilicity and basicity, which enhances the availability of amine groups to rapid and efficient derivative reactions [7]. In addition to the aromatic backbone another reason for the change in basicity/nucleophilicity of the amine groups on surfaces is because attaching nitro groups for example to carbon surfaces has been shown to alter the pK_a due to different solvation

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and the relative hydrophobicity/hydrophilicity of the surface [8,9]. In the present work, the attachment of D-NO₂ onto gold electrodes is proposed. Cyclic voltammetry and scanning tunneling microscope (STM) were used to characterize the derivatised surface. Attachment of the dendron was followed through the observation of the electrochemical signal of reporting groups such as the nitro group.

2. Experimental

2.1. Materials

N,N-Dimethyladetamide (DMAc, Tedia) was dried over 4 Å molecular sieves; and 3,5-diaminobenzoic acid (Aldrich) was purified by recrystallization from water. D-NO₂ was obtained using 3,5-diaminobenzoic acid and 3,5-dinitrobenzoyl chloride in DMAc [7]. The rest of commercially available chemicals were reagent grade and were used without further purification. Water was purified with a Millipore Milli-Q system.

2.2. Electrochemical measurements

Electrochemical measurements were performed with an Autolab electrochemical analyzer and a conventional three-electrode system, comprising a gold working electrode, a platinum foil as counter-electrode, and a Ag/AgCl 3.0 M NaCl electrode (from BAS) as reference. Nitrogen gas was used to deaerate all aqueous solutions before use.

Preparation of working electrode: 1.6 mm-polycrystalline gold (Bioanalytical System Inc. (BAS), Lafayette, IL) were polished with 1.0, 0.3 and 0.005 mm alumina slurry on microcloth pad, rinsed with water and ethanol and sonicated (10 min) in distilled water. They were subsequently activated by repeatedly scanning the potential between the hydrogen evolution and the oxygen evolution regions in 1 M H₂SO₄, rinsed with water and dried in N₂ flux.

2.3. Scanning tunneling microscopy (STM)

STM measurements were carried out with a commercial instrument (Molecular Imaging PicoScan 5) at room temperature in air. STM tips were prepared from Pt–Ir (70:30) wire (0.25 mm) by the mechanical cutting method. STM images were taken in constant-current mode at a scanning rate of 1 Hz. The Au(111) single crystal electrode (10 mm diameter, Accumet) was annealed in a hydrogen flame before each experiment and cooled in N₂ flux.

2.4. Attachment of D-NO₂ by self-assembly technique

The cleaned gold electrode was incubated in a dimethylsulfoxide solution containing 10 mM of D-NO₂ for times varying to 5 min up to overnight. Following the modification, gold electrodes were subsequently rinsed with copious volumes of ethanol and water, and were employed immediately after preparation.

2.5. Covalent modification of gold electrode by using cystamine's strategy

A gold electrode was prepared as reported in 2.2 and modified by immersion into an aqueous 20 mM solution of cystamine for 2 h [10]. Then, the electrode was placed into a DMAc solution containing 20 mM of D-NO₂ acid chloride for 14 h at 0 °C without stirring. After the modification, the electrode was rinsed with copiously DMAc, ethanol and water, and was employed immediately after preparation.

3. Results and discussion

Fig. 1A shows cyclic voltammograms recorded for a dendronized gold electrode after the spontaneous attachment of an aromatic polyamide dendron (first generation, D-NO₂). The initial potential was set at 0.2 V. The electrochemical reduction of the derivatised surface yields an irreversible cathodic wave at −0.5 V during first scanning toward negative potentials. When the scan direction is reversed only one oxidation wave at *ca.* 0.1 V is observed with its corresponding reduction wave at 0.0 V. This redox couple, centered at 0.05 V, appears if the cathodic switching potential reaches −0.5 V. In dotted line, the bare gold

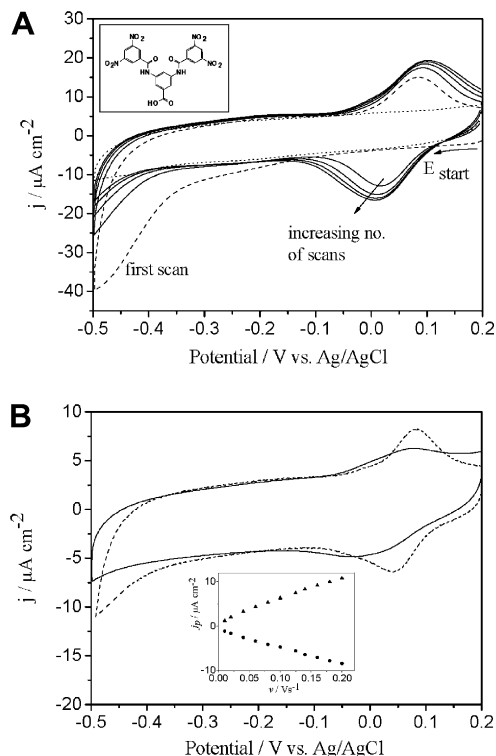


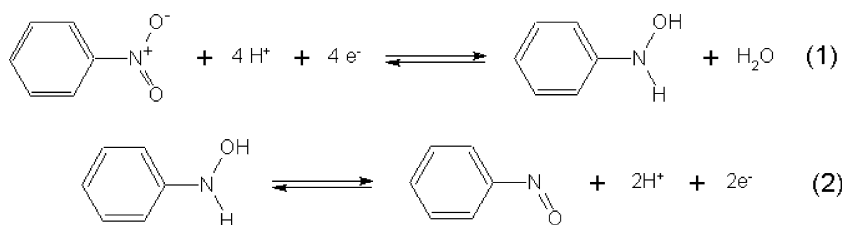
Fig. 1. Cyclic voltammograms at 0.1 V s^{−1} for an Au electrode modified with D-NO₂ (by self-assembly, 16 h) in 0.1 M phosphate buffer (pH 7). (A) First (dashed) and subsequent (solid) scans. Bare electrode: dotted line. Inset: structural formula of D-NO₂. (B) Comparison between second (dashed) and 50th (solid) scans. Inset: plots of anodic (▲) and cathodic (●) peak currents measured after 50 scans as a function of *v*.

electrode profile is shown. No Faradaic processes were observed between -0.40 and -0.15 V either for the bare or the D-NO₂-modified gold electrode. It is interesting to notice that capacitive current does not decrease with the presence of the dendron.

The electrochemical response is qualitatively similar to those previously reported by Casero et al. [11] for 5,5'-dithiobis(2-nitrobenzoic acid) adsorbed onto gold electrodes in phosphate buffer and the electrochemical voltammograms of gold electrodes in 1 mM *p*-nitrobenzoic acid in 0.1 M HClO₄ [12]. Comparison with the literature, and “fingerprinting” by comparison of the voltammetry of nitrobenzene and nitrosobenzene physisorbed onto different substrates [13–15], reveals that the voltammetric response observed is indeed characteristic of the electrochemical reduction of an aromatic nitro compound and is consistent with the general mechanism shown in Scheme 1.

By analogy, the strong cathodic wave at -0.5 V corresponds to the four-electron reduction of each nitro moiety to the corresponding aryl-hydroxylamine (reaction 1). On the subsequent positive-going sweep, the pair of anodic and cathodic peaks can be attributed to the two-electron oxidation/reduction of the aryl-hydroxylamine/aryl-nitroso moieties (reaction 2). Comparing the charge passed during the first cycle to about -0.5 V (0.033 mC/cm²) with that associated with the wave at 0.1 V (0.010 mC/cm²), is about twice the value anticipated from the ratio of stoichiometries of reactions 1 and 2, suggesting that only about 60% of the charge passed gives rise to the reversible redox couple. The difference in charge is likely due to generation of non-redox active secondary products, as is often found in these types of transformation [11].

The second and subsequent voltammograms for dendron modified gold electrodes give rise to essentially the



Scheme 1. The electrochemical reduction of an aryl-nitro moiety in aqueous solution illustrate by the generic example of nitrobenzene itself.

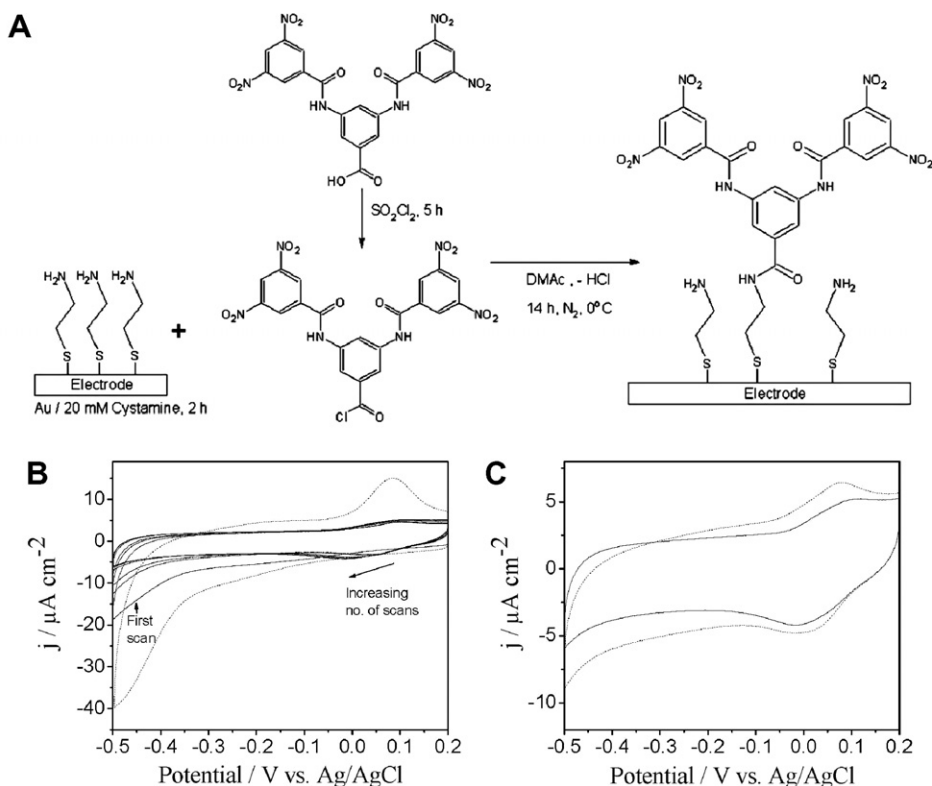


Fig. 2. D-NO₂ immobilization via grafting of cystamine covalently attached to gold electrode. (A) Scheme of the basic strategy of attachment. (B) Cyclic voltammograms at 0.1 V s⁻¹ for modified gold electrode in 0.1 M phosphate buffer (pH 7) (solid lines). First scan obtained for a dendron modified gold electrode without cystamine (dashed) is shown for comparison. (C) Cyclic voltammograms at 0.1 V s⁻¹ for a dendron (dashed line) and a cystamine/dendron modified gold electrode (solid line) in 0.1 M phosphate buffer (pH 7) after 50 scans.

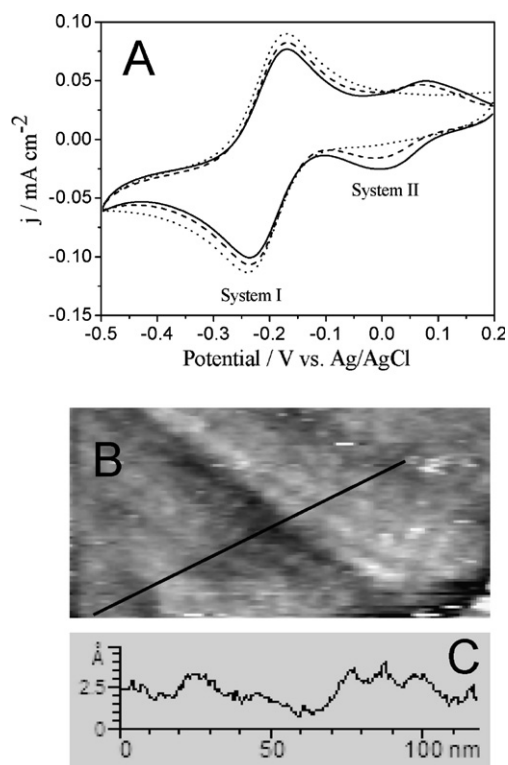


Fig. 3. (A) Cyclic voltammograms at 0.1 V s^{-1} for an Au electrode in 0.1 M phosphate buffer (pH 7) containing 2 mM $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$. Bare electrode (dotted) and Au incubated in D-NO_2 solution for 2 h (dashed) and 24 h (solid). (B) $150 \times 75 \text{ nm}^2$ STM image of $\text{Au}(111)$ surface after incubation in D-NO_2 solution for 24 h. $E_{\text{bias}} = -0.8 \text{ V}$. (C) Profile diagram recorded along the solid line in (B).

same electrochemical behaviour. Fig. 1B shows the second and 50th cycle recorded in 0.1 M phosphate buffer (pH 7), whereas the reduction wave decreases to the same size than the oxidation wave while shifted 90 mV in the negative direction. There is an increase of the irreversibility of the process evidenced by the increase of the difference between the anodic and the cathodic peak potentials. The insert illustrates the scan rate dependence of the peak current density. Both the cathodic peak current, $j_{\text{p,c}}$, and the anodic peak current, $j_{\text{p,a}}$, are proportional to the sweep rate v indicating that the molecules left on the surface constitute a surface-confined redox pair.

Furthermore, the nitroso/hydroxylamine couple is interesting due to its general use in the electrocatalytic oxidation of nicotinamide adenine dinucleotide (NADH) and related materials as well as in biosensor application [11,16,17] and the electroanalytical exploitation of nitroso-phenyl modified substrate for the quantification of thiols [18].

On the other hand, a covalent attachment of D-NO_2 via cystamine was achieved (Fig. 2A). At the first scanning toward negative potentials, the irreversible cathodic wave at -0.5 V is slightly observed but the electrochemically almost-reversible two electron, two proton oxidation (reaction 2) forming the aryl-nitroso species is clearly shown (B). The stabilized voltamperometric profiles after 50 scans for

dendronized gold electrodes with or without cystamine shows the same electrochemical behaviour but exhibit also a decrease in the capacitive current region due to the presence of a low-dielectric cystamine layer (C).

Cyclic voltammetry was used to evaluate the blocking property of the layer-coated electrodes using diffusion controlled redox couples as probes. With this purpose we chose $\text{Ru}(\text{NH}_3)_6^{3+}/\text{Ru}(\text{NH}_3)_6^{2+}$ couple which undergoes a simple charge transfer process with a high rate constant. Fig. 3 shows the cyclic voltammograms of bare Au and D-NO_2 modified Au electrodes in 2 mM $\text{Ru}(\text{NH}_3)_6^{3+} + 0.1 \text{ M}$ phosphate buffer solution (pH 7) (A). The bare Au electrode shows a reversible voltammogram for the redox couple (system I) indicating that the electron transfer reaction is completely diffusion controlled. A small decrease in the charge density of system I for modified electrodes is observed. This behavior goes together with the redox response of the aryl-hydroxylamine/aryl-nitroso moieties (system II) exhibiting a correlation with the decrease of charge in system I and the increase of charge of system II among the increase of the incubation time. In spite of the electrochemical response, we can see from STM images that layers of dendrons lead to effective coverage of gold surfaces (B). Self-assembled layers of D-NO_2 form patterned surfaces with nanometer-sized features (7 nm) and in long-range order. The cross section profile exhibits a layer with electronic corrugation of *ca.* 0.1 nm and a gold step with electronic corrugation of 0.25 nm (C).

4. Conclusions

The synthesis of a dendritic macromolecule and its immobilization onto gold electrodes was achieved. D-NO_2 adsorbs onto gold electrode surfaces spontaneously by dipping the metal surface in dendron solution and via grafting of cystamine covalently attached to gold electrode. Reduction of this layer generates the hydroxylamine product with a coulometric yield that is estimated to be on the order of 60%. The resulting redox-active layer exhibits a well-behaved redox response for the adsorbed nitroso/hydroxylamine couple.

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