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Surface-confined redox-active monolayers of a multifunctional anthraquinone derivative on nanoporous and single-crystal gold electrodes

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ABSTRACT

We have investigated self-assembled molecular monolayers (SAMs) of a novel thiol-bearing anthraquinone derivative AQ(OMe) on nanoporous gold (NPG) electrode surfaces. We employ cyclic voltammetry, electrochemical impedance spectroscopy and X-ray photoelectron spectroscopy (XPS). Single-crystal Au(111)-electrode surfaces area used as a reference. XPS exhibits S2p3/2 peaks at 161.1 and 162.1 eV, verifying well-defined AQ(OMe) Au-S bound SAMs. The mid-point potential (E1/2) of the SAMs shifts negatively with increasing pH with a slope of −55 mV pH−1, indicative of a 2e2H redox reaction. The anthraquinone group exhibits quasi-reversible behavior over a wide range of pH values (from 2.8 to 9.0) and a sigmoidal electron transfer (ET) rate constant (kapp) with a maximum value at pH 5.5, which is likely to be close to the isoelectric point. The electrochemically addressable surface coverage, determined from the voltammetric peak areas, decreases with increasing pH. The apparent surface coverage on NPG is more than 13 times higher than that on Au(111), attributed mainly to the larger surface area and different structural packing modes, and possibly also to favorable terrace edges on NPG, but – somewhat unexpectedly – with little effect on kapp. The new anthraquinone derivative offers perspectives for gentle immobilization and efficient mediated interfacial electrochemical ET of redox proteins and other complex biomolecules.

1. Introduction

Self-assembled molecular monolayers (SAMs) of alkanethiols on gold surfaces, based on Au-S bonding, with tailored terminal functional groups, offer an excellent approach to the control of electrode surface properties [1–3]. SAM-functionalized surfaces are also platforms for gentle bioelectrochemical immobilization of proteins/enzymes [3–7]. The terminal groups of the SAM can thus provide molecular binding via “gentle”, i.e. non-covalent electrostatic, hydrophobic/hydrophobic and hydrophilic/hydrophilic interactions, thus mitigating leakage of the enzyme [8–11]. Electrochemical techniques, particularly cyclic voltammetry (CV), are highly suitable to explore the SAMs, but alkanethiol SAMs themselves have no voltammetric features other than reductive or oxidative desorption, requiring redox probes such as hexacyanoferrate (II)/(III) or other metal complexes to reveal other surface properties [12–15]. Irreversible reductive desorption at highly negative potentials offers efficient evaluation of the surface coverage of non-redox SAMs, but it is essentially a destructive process [16,17]. Introduction of redox-active thiol-based SAMs would thus be highly convenient for exploring the quality of SAMs, as well as electrochemical electron transfer (ET) [14,18–20].

As an important quinone class, anthraquinone derivatives have been widely studied [21–23], also with respect to flow redox batteries due to well-tailored redox potential windows [21]. A metal-free redox capacitor can be fabricated by introducing quinone derivatives as supporting electrolyte, and efficient charge carriers [23]. Anthraquinone derivatives are also facile electron acceptors or donors, promoting mediated bioelectrocatalysis of FAD-dependent glucose dehydrogenase [22]. The electrochemistry of anthraquinone derivatives is a core example of proton-coupled ET (PCET), undergoing sequential or concerted two-electron/two-proton transfer [18,24,25]. It is generally accepted that quinone derivatives undergo “ideal” [17], fully synchronous PCET, i.e. hydrogen atom transfer, in buffered aqueous solutions [26,27]. The rationale is that synchronous PCET involves little charge separation and
therefore both lower environmental reorganization and bypassing of unstable semiquinone intermediates [17]. The electrochemistry of especially surface-confined anthraquinones on carbon surfaces was comprehensively studied by Compton and associates [26–28], who demonstrated a linear relationship between peak potential and pH, with a slope of ca. −59 mV pH⁻¹, indicative of a reversible Nernstian redox reaction [26].

While redox SAM electrochemistry on gold electrodes has been broadly reported [14,29], PCET of Au surface-confined anthraquinone derivatives has rarely been studied. Nanoporous gold (NPG) possesses a large surface area, good biocompatibility, and high chemical stability, thus representing a versatile and relatively novel electrode substrate [9,30]. Dealloyed NPG derived from etching alloys with strong acid gives very clean surfaces, providing an attractive SAM platform [31]. In the present work, we have designed and synthesized a new thiol-bearing anthraquinone derivative, AQ(OMe) (1-amino-4-((3-mercapto-5-(methoxycarbonyl)phenyl)amino)-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid), (Fig. 1a), and studied the electrochemistry of AQ(OMe) SAMs on NPG in aqueous buffers, with single-crystal Au(1 1 1)-electrodes as a reference system. We found that the surface coverage of the AQ(OMe) SAM on NPG is about 13 times higher than that on Au(1 1 1), but, notably, with similar interfacial ET rate constants. Both the ET rate constant and the electrochemically addressable AQ(OMe) SAM coverage are sensitive to pH.

The new anthraquinone derivative is also designed for use as a molecular linker for electrochemically active biomolecules such as redox (metallo)proteins and enzymes, and as an immobilized redox mediator for the biomolecules in a well-defined microenvironment [32]. In addition to the electrochemical anthraquinone core, the molecule has several functional groups suitable for protein linking, Fig. 1a. The molecule is thus equipped with a thiol group for linking to the electronically “soft” Au surface; the thiol group is attached via a flexible linker which allows favourable surface orientation. The carboxylate and amine groups allow immobilization of bioelectrochemically active redox proteins and enzymes, while the sulfonate group, existing as a zwitterion with the ortho-positioned amine group, provides high aqueous solubility. For the present investigation we esterified the carboxylic acid with methanol to reduce the number of proton transfer sites, facilitating mapping of the pH profile of the electrochemical coverage and kinetics patterns.

2. Experimental

2.1. Reagents

Nitric acid (HNO₃, 70%) was purchased from Fisher Scientific, UK, acetic acid (CH₃COOH, 100%) from Merck, Germany, ethanol (C₂H₅OH, 100%) from VWR International SAS, France. Sulfuric acid (H₂SO₄, 95–97%), hydrochloric acid (HCl, 37%), citric acid (HOC(COOH)(CH₂COOH)₂, ≥99.5%), potassium hexacyanoferrate(III), (K₃[Fe(CN)₆]), ≥99.0%), sodium acetate (CH₃COONa, ≥99.0%), sodium mono-hydrogen phosphate dodecahydrate (Na₂HPO₄⋅12H₂O, ≥99.0%), sodium phosphate monobasic monohydrate (NaH₂PO₄⋅H₂O, ≥99.5%), sodium hydroxide (NaOH, ≥97.0%), and potassium chloride (KCl, ≥

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Fig. 1. (a) Schematic illustration of the preparation of NPG and NPG-AQ(OMe); (b) XPS survey spectra; (c, d) high-resolution spectrum of (c) Au4f, and (d) S2p of AQ(OMe) SAM.
99.0%) were all from Sigma-Aldrich, USA. Sodium acetate buffer (NaAc, pH = 3.6, 4.2 and 5.0), McIlvaine buffer (McBp, pH = 5.5) and phosphate buffer (PBS, pH = 7.0 and 9.0) were prepared by mixing solutions of acetic acid and sodium acetate, citric acid and NaH₂PO₄ and Na₂HPO₄, respectively, with a final concentration of 100 mM. AQ(OMe) was synthesized according to the procedure described in the Supporting Information. All solutions were prepared with Milli-Q water (18.2 MΩ cm) and all chemicals used as received.

2.2. Preparation of AQ(OMe) SAMs

Au/Au alloy leaves (12-carat, 100-nm thick, Eytzinger, Germany) were etched in concentrated HNO₃ at 30 °C for 30 min to fabricate a NPG film with an average pore diameter of ca. 30 nm [33,34]. After rinsing with Milli-Q water, the NPG film was physically anchored on a polished glassy carbon electrode (GCE) and air-dried overnight. Cyclic voltammetry (CV) was conducted to activate the NPG electrode in 1 M H₂SO₄ solution for 15 cycles in a potential range from −0.3 to 1.45 V vs. Ag/AgCl (sat. KCl) at 50 mV s⁻¹. The ratio of the real surface area, measured by integrating the reduction peak of gold oxide with an empirical charge density of 386 μC cm⁻² for a Au monolayer, to the geometric area (0.1256 cm⁻²) constitutes the roughness factor (Rₗ) [9]. The NPG film used had Rₗ = 7. Au(1 1 1) electrodes were prepared and characterized by the method of Hamelin [35]. The electrodes were electropolished in 0.1 M H₂SO₄ at 10 V for 10 s, cleaned several times in 1 M HCl and Milli-Q water to remove the gold oxides formed, and annealed at 860 °C for 8 h. Prior to use, the Au(1 1 1)-electrodes were quenched by hydrogen gas.

AQ(OMe) SAMs were formed by immersing activated NPG or quenched Au(1 1 1) electrodes into 1.5 mM AQ(OMe) 2:1 v/v ethanolic solution [37,38] (Fig. S1). A single peak at 399.2 eV appears in the high-resolution N1s spectrum, reflecting the amino groups in the AQ(OMe) SAM [37]. Two peaks (S5, S6) at 162.9 and 163.5 eV, respectively, can be assigned to unbonded thiol [37]. The S2p spectrum is dominated by the aromatic C – C bond at 160.1 and 163.5 eV, reflecting the fact that the NPG-AQ(OMe) is engaged in a Nernstian reaction with equal number of electrons and protons (here 2). The redox potential of NPG-AQ(OMe) is shifted negatively with increasing pH, strongly indicative of a PCET process. The middle potential (E₁/₂) and the formal redox potential (E°) can be obtained from the anodic and cathodic peaks. Fig. 2b shows a linear relationship between E₁/₂ and pH with a slope of −55 mV pH⁻¹, close to the ideal −59 mV pH⁻¹, reflecting the fact that the NPG-AQ(OMe) is engaged in a Nernstian reaction with equal number of electrons and protons (here 2) [26,39] over the whole pH range [40].

The high-resolution N1s spectrum consists of two S2p peaks at 163.5 and 164.1 eV, respectively, and a higher binding energy peak at 163.9 eV, attributed to oxidized sulfur species [36]. The S2p spectrum is dominated by the aromatic C – C bond at 160.1 and 163.5 eV, reflecting the fact that the NPG-AQ(OMe) is engaged in a Nernstian reaction with equal number of electrons and protons (here 2).

3. Experimental measurements

Electrochemical measurements were recorded using an Autolab PGSTAT12 instrument (Eco Chemie, Switzerland) and 100 mM buffer, pH 2.8–9.0 at room temperature (ca. 20 °C). The AQ(OMe) SAM-modified electrode, a Pt wire, and a Ag/AgCl electrode (sat. KCl) served as working, counter and reference electrode, respectively. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were carried out for the same buffer solutions containing 5 mM K₃[Fe(CN)₆] with an applied potential of 0.22 V vs. Ag/AgCl (the midpoint redox potential of K₃[Fe(CN)₆]) and a frequency range of 0.1–10 kHz. All electrolytes were degassed with argon for at least 30 min and an argon atmosphere maintained above the solution throughout the experiments.

The chemical compositions of the AQ(OMe) SAM-modified Au electrodes were characterized by X-ray photoelectron spectroscopy (XPS, ESCALABMKII, Thermo Scientific, USA) using a BioNavis gold chip. High-resolution spectra were calibrated against the C1s spectrum with C=C/C = C at 284.6 eV.

3.3. Results and discussion

3.1. XPS studies

AQ(OMe) monolayers on NPG (average pore size: 30 nm; Fig. S1) were prepared simply by soaking (Fig. 1a). Fig. 1b shows XPS survey spectra with peaks of the elements C, N, O, S and Au. The high-resolution XPS spectra provide detailed information about the chemical and electronic states of S and the Au-S bond in the SAMs. The high-resolution Au 4f spectrum, Fig. 1c, shows two distinct peaks at 84.1 and 87.8 eV, attributed to metallic and S-bonded Au, respectively. The high-resolution S2p spectrum consists of a 2p₃/2/2p₁/₂ doublet with a 2:1 area ratio and an energy separation of 1.2 eV [36,37]. Two S2pₓ/₂ peaks (S1, S2) at 161.1 and 162.1 eV, Fig. 1d, are assigned to the AQ(OMe) SAM while S3 and S4 at 162.9 and 163.5 eV, respectively, can be attributed to unbonded thiol [37]. The S2p spectrum is dominated by Au-S bonded AQ(OMe) and substantiates the theory that a well-defined AQ(OMe) Au-S bond SAM has been formed. Two peaks (S5, S6) at higher binding energies are assigned to oxidized sulfur species [36]. The high-resolution Cls spectrum shows peaks at 284.6 and 288.6 eV, assigned to the aromatic C–C/C = C and –O–C = O species, respectively [38], Fig. S2. A single peak at 399.2 eV appears in the high-resolution N1s spectrum, reflecting the amino groups in the AQ(OMe) SAM [37].

3.2. Electrochemistry of NPG-AQ(OMe) SAMs at variable pH

Prior to the electrochemistry of SAMs, the effect of dioxygen of AQ (OMe) was assessed, disclosing an increasing reduction signal in ambient atmosphere (Fig. S3), indicating that the electrochemical characterizations must be conducted in an Ar-saturated atmosphere. The CVs of NPG-AQ(OMe) shown in Fig. 2 are stabilized after several scans, at a scan rate of 20 mV s⁻¹ in the pH range 2.8–9.0. The pair of redox peaks is assigned to the anthraquinone/anthrahydroquinone couple. The redox potential of NPG-AQ(OMe) is shifted negatively with increasing pH, strongly indicative of a PCET process. The mid-point potential (E₁/₂) and the formal redox potential (E°) can be obtained from the anodic and cathodic peaks. Fig. 2b shows a linear relationship between E₁/₂ and pH with a slope of −55 mV pH⁻¹, close to the ideal −59 mV pH⁻¹, reflecting the fact that the NPG-AQ(OMe) is engaged in a Nernstian reaction with equal number of electrons and protons (here 2) [26,39] over the whole pH range [40].

The peak separation (ΔEₚ) (Fig. S4a) also gives the interfacial electrochemical ET rate constant (kₑapp). Most ΔEₚ values are smaller than 120 mV, indicative of a "quasi-reversible" behavior (Fig. S4a), pH clearly affects ΔEₚ and thus kₑapp, with the minimum ΔEₚ value at pH 5.5. kₑapp was obtained from the Laviron equations:

\[ ΔE_p = \left(\frac{4RT}{nF}\right) \times \ln \left(\frac{1}{2m}\right) \]  

\[ ΔE_p = \frac{nFv}{RTk_{app}} \]  

where R is the gas constant (8.314 J mol⁻¹ K⁻¹), T the temperature (298 K), F the Faraday constant (96,485 C mol⁻¹), n the number of electrons transferred (here 2), v the scan rate (V s⁻¹), and m is determined from ΔEₚ. Eqs. (1) and (2), kₑapp shows a sigmoidal pH dependence, Fig. 2c, reaching a plateau of 0.13 ± 0.01 s⁻¹ at pH 5.5. The redox peaks can, however, also be affected by other factors, such as intermolecular interactions in the SAMs, as well as electrolyte interactions [41].

We take the peak current density difference jₓ – jₓ as a measure of the strength of the redox wave, relevant to the amount of active AQ(OMe) on the surface. The separate values of jₓ or jₓ are not representative due to the asymmetric CV profiles (Fig. 2a), jₓ – jₓ shows a downward trend with increasing pH beginning around pH 4, Fig. S4b. In addition, the SAM surface coverage (Γ', mol cm⁻²) is a useful parameter, frequently obtained by reductive desorption of the surface monolayers. The electrochemically addressable surface coverage (Γ') of the redox-active SAMs can be extracted from the anthraquinone anodic/cathodic peak areas [41]. Background-subtracted anodic peaks were integrated to assess Γ' of the AQ(OMe) SAM on the NPG surface. Γ' scaled to the geometric area (A, cm²) can be expressed as:

\[ Q = \int \text{Idt} = \int \text{idv} \left(\frac{E}{F}\right) \]  

\[ Γ' = \frac{Q}{nFA} \]
where \( Q \) is the consumed charge (C).

\( \Gamma' \), as determined from reduction of the quinone moiety follows a similar declining trend with increasing pH, as \( j_a - j_c \), Fig. 2d. Assuming that the actual amount of AQ(OMe) in the SAM is unchanged over the whole pH range, this indicates that the amount of addressable AQ(OMe) SAM in PCET follows the stoichiometric proton concentration for quinone reduction [26]. The electrochemical stability of the AQ(OMe)
SAMs is another important issue, especially in relation to the use of the SAMs as a mediator in bioelectrocatalysis. The voltammograms were found to remain stable over at least 12 cycles at different pHs (pH 3.6, 5.5 and 9.0), Fig. S5.

To further understand the pH pattern of the SAM-modified NPG electrode [42], a common redox probe, negatively charged Fe(CN)₆³⁻, was used (Fig. 3). One merit of Fe(CN)₆³⁻ is that its redox potential is separate from the AQ(Ome) midpoint potential. Fe(CN)₆³⁻ shows clear redox waves at both the bare NPG and NPG-AQ(Ome), with a midpoint potential of 0.22 V (Fig. 3a-d) largely independent of pH. It has been reported that the redox signal of Fe(CN)₆³⁻ is significantly blocked on dense monolayers or multilayer modified electrodes [19,30]. A reversible redox signal is clearly found, Fig. 3, which means that the Fe(CN)₆³⁻ signal is only weakly blocked. The attenuated peak current density and larger peak separation of NPG-AQ(Ome) compared with bare NPG, most clearly seen at pH 3.6, imply that a loosely packed AQ(OMe) sub-monolayer is formed. Electrostatic repulsion between Fe(CN)₆³⁻ and the surface thus seems to be of minor importance, which is again due to the weakly charged AQ(OMe) SAM at low pH [42].

Fig. 4. c. AQ(OMe) thus seems to organize optimally at pH 5.5 for interfacial ET for both adsorbed AQ(OMe) and diffusing Fe(CN)₆³⁻ at pH 5.5 but then increases slightly, to 244.3 Ω, at pH 9.0. This overall trend largely follows the k_app Pattern, Fig. 2c. AQ(Ome) thus seems to organize optimally at pH 5.5 for interfacial ET for both adsorbed AQ(Ome) and diffusing Fe(CN)₆³⁻.

The pH profiles offer clues as to the particular functional AQ(OMe) groups engaged in protonation equilibria. The sulfonate group is fully deprotonated over the whole pH range. pKₐ values for the primary amino group adjacent to the –SO₃ and the linking secondary amino group are not available, but we can note, first, that pKₐ of secondary diatomic amines is low, say pKₐ ≈ 1, pKₐ ≈ 4 for anthracene-1-amine, and pKₐ ≈ 8 for anthraquinone itself [43]. Secondly, following both classical [44] and more recent analysis [45], the electrostatic effect of adjacent charged groups, here –SO₃, can shift pKₐ for a primary proton-transferring group, here the amino group, to higher values by up to several units, supporting the hypothesis that this latter group most likely controls the pH profiles shown in Fig. 2. The –SO₃ group remains negatively charged, the other groups neutral, and overall AQ(OMe) is taken from an electrostatically neutral state to a singly negatively charged state, as the pH is taken from low to high values.

3.3. Comparison of AQ(Ome) SAM voltammetry on NPG and Au(111) electrodes

The electrochemical behavior of AQ(Ome) SAMs on the Au(111) electrode in MCB buffer, pH 5.5 was investigated as a reference. E₁/₂ and ΔEₚ on Au(111) are identical to those on NPG, Fig. 4a. The much higher NPG-AQ(Ome) surface area exhibits correspondingly higher values of the electrochemical double-layer capacitance and the AQ(Ome) surface coverage than Au(111). CVs of Au(111)-AQ(Ome) and NPG-AQ(Ome) SAMs at scan rates from 10 to 200 mV s⁻¹, based on the geometric dependence of the peak current density on the scan rate [39,46], are shown in Fig. S6a,b. The coverage Γₐ (normalized to the geometric area) of the AQ(Ome) SAMs on Au(111) and NPG, both independent of the scan rate (ν, V s⁻¹), was obtained using the Laviron equation:

\[ j = \frac{n^2F^2}{4RT} Γₐ \nu \]  

Γₐ on NPG is more than 13 times higher than on Au(111), Fig. 4b, based on the geometric area and accords with the values determined from Eq. (3). Notably, this is twice the NPG roughness factor (which is only 6.6) and reflects the fact that the high surface areas lead to a denser monolayer than on Au(111). This is further rooted in the abundance of well-defined single-crystal Au(100) and Au(110) domains on the NPG surfaces [30,47]. Previous efforts have found that the SAM loading of thiol-based molecules (cysteines) and packing modes of Au(100) and Au(111) are quite similar (Fig. 4b). This implies that the PCET kinetic is even further mean either that there is a tendency to multi-layer formation of electrochemically active AQ(Ome), or that surface sites inactive in Au oxidation are active in AQ(Ome) voltammetry.

Somewhat unexpectedly, the apparent rate constants, k_app on NPG and Au(111) are quite similar (Fig. 4b). This implies that the PCET kinetics are little affected by the different facets, kinks, and other nanoscale structures on the porous NPG surface. A recent theoretical and computational study of electron transfer between Au nanoparticles and a probe redox molecule (ferrocene) showed that the electron transfer rate constant is in fact expected to be quite sensitive to particular surface structural elements (facets, edges, top, etc.) [49]. It seems that these kinetic features are somehow coarse-grained (“smeared out”) over the NPG surface. CVs of NPG-AQ(Ome) at varying scan rates are shown in Fig. S7.
4. Conclusions

We have introduced a new multifunctional redox-active anthraquinone derivative, AQ(OMe), designed to secure at the same time efficient Au-S bonding to Au surfaces, and gentle, non-covalent linking and forming. We further investigated the voltammetric and EIS responses of AQ(OMe) SAMs on both well-defined nanoporous and single-crystal gold surfaces over a wide pH range. The pH dependence of $E_{1/2}$ shows a linear dependence on pH with a slope of $-55$ mV pH$^{-1}$, consistent with 2e2H PCET. $k_{app}$ displays a sigmoidal pH profile, reflecting a protonation equilibrium, primarily of the core primary amino group. Notably, the electrochemically addressable $I^*$ of AQ(OMe) SAMs on NPG also decreases conspicuously, caused by the reduction in the amount of stoichiometric protons in the reduction process as the pH increases. The AQ(OMe) coverage on nanoporous gold exceeds the coverage on a single-crystal gold electrode by more than 13-fold, but with little effect on $k_{app}$. In conclusion, the new anthraquinone design with our accompanying spectroscopic and electrochemical molecular SAM mapping demonstrates that gold electrodes modified by this new molecular class could become new combined linkers and mediators in future bioelectrocatalysis applications.

CRediT authorship contribution statement

Xiaomei Yan: Data curation, Methodology, Writing - original draft. Charlotte Udlahl Hansen: Data curation, Methodology, Writing - review & editing. Fangyuan Diao: Data curation. Katrine Qvortrup: Conceptualization, Methodology, Writing - review & editing, Resources. David Tanner: Conceptualization, Resources, Writing - review & editing. Jens Ulstrup: Conceptualization, Formal analysis, Writing - review & editing. Xinxin Xiao: Data curation, Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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