

Immobilization of DNA on an Aluminum(III) Alkanebisphosphonate Thin Film with Electrogenerated Chemiluminescent Detection

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The immobilization of DNA on an electronically conductive surface is of interest both in studies of DNA itself and in various applications. For example, in scanning tunneling microscopy (STM) (or other scanning probe techniques), the DNA must be firmly affixed to a surface to minimize the effects of tip interactions during scanning.¹ Immobilization of DNA on an electrode is also used to produce biosensors, e.g., those that can recognize the base sequence on a complementary strand of DNA.² In this latter application, a single strand of DNA must be strongly attached to the electrode surface in a manner that still permits access to the bases and allows hybridization with a complementary DNA strand in solution.

The strategy employed in this study was to prepare, on a smooth gold substrate, a well-ordered film that contained metal centers, e.g., Al(III), that would interact strongly with the phosphate groups of DNA. Films of this type have been described previously.³ Previous studies have also demonstrated binding of DNA to crystal (insulator) surfaces via the phosphate groups to Ca²⁺ and Mg²⁺ centers.⁴ In the present study, the small amounts of DNA that were adsorbed on the surface were identified by intercalation into double-stranded (ds) DNA of a transition metal chelate, Ru(phen)₃²⁺, which can be made to emit light when oxidized electrochemically in the presence of a suitable coreactant, in an approach previously used with solution phase DNA.⁵ Film formation and DNA adsorption were also monitored with a quartz crystal microbalance (QCM).

The technique for the fabrication of the aluminum alkanebisphosphonate (Al₂C₄BP) film by sequential adsorption/reaction steps followed previous practice³ and is illustrated in Figure 1a. 4-Mercaptobutylphosphonic acid (MBPA) was adsorbed on a gold film (2000 Å) sputtered on top of a 50 Å Cr layer on a silicon wafer.⁶ The phosphonic acid-terminated surface was rinsed thoroughly with EtOH, dried in a stream of nitrogen, and then immersed in a 5 mM Al(NO₃)₃ solution. Following rinsing with water, it was immersed in 5 mM bisphosphonic acid, H₂O₃-P(CH₂)₄PO₃H₂, rinsed with water, and then immersed in 5 mM Al(NO₃)₃ solution. As shown in previous studies of similar films, this produces a self-assembled film with a surface exposing many Al(III) binding sites.⁷ Calf thymus ds-DNA was immobilized on the surface by immersing it in a solution of DNA (1.9 mM

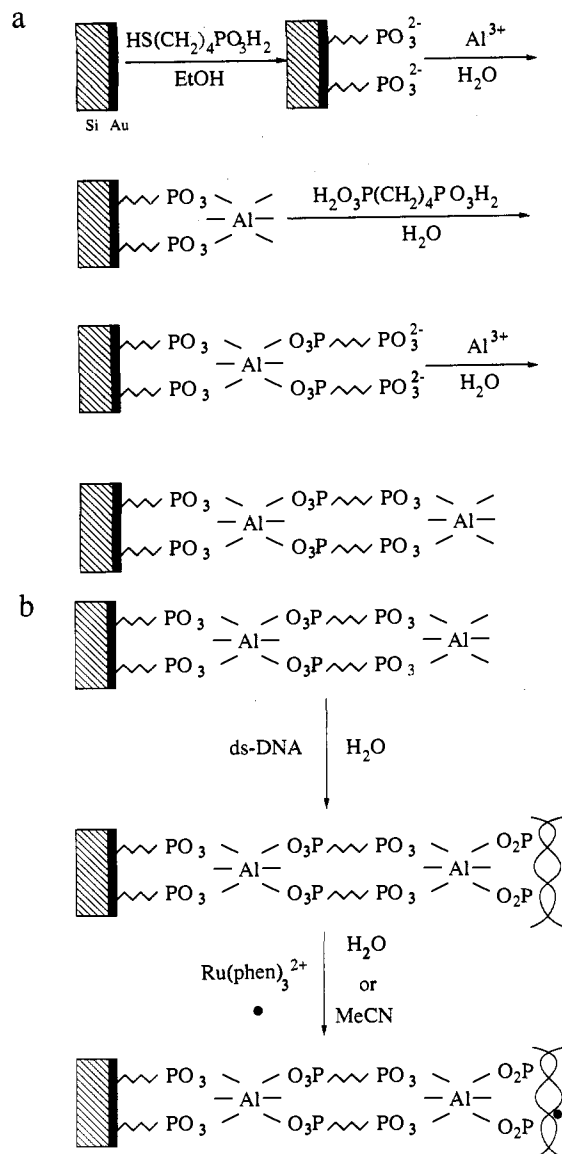


Figure 1. Schematic representation of (a) sequential steps in Al₂(C₄BP) film formation on the gold surface supported on a silicon wafer and (b) immobilization of DNA on the film and interaction of Ru(phen)₃²⁺ with the DNA.

in nucleotide phosphate, NP) for 4 h (Figure 1b). The film was then rinsed three times with 4-mL portions of deionized water and then immersed for 4 h in either an aqueous 0.56 mM Ru(phen)₃Cl₂ solution or a 0.12 mM Ru(phen)₃(ClO₄)₂ solution in MeCN. As shown in previous studies, Ru(phen)₃²⁺ associates with ds-DNA⁸ and can be detected through its electrogenerated chemiluminescence (ECL).⁵ Alternatively, the film could be soaked in a mixed ds-DNA (1.9 mM NP) and Ru(phen)₃Cl₂ (0.12 mM) solution for 4 h to produce the adsorbed layer.

ECL was produced by scanning the potential of the electrode following film formation, DNA adsorption, and Ru(phen)₃²⁺ association, from 0 to 1.6 V vs a saturated calomel electrode (SCE) while it was immersed in a solution of 0.19 M phosphate buffer (pH 7) containing 0.13 M tri-*n*-propylamine (TPrA). Typical ECL transients, detected with a single-photon-counting apparatus, are shown in Figure 2. As proposed in previous studies,^{5,9} emission arises from the energetic electron-transfer

(7) We have also prepared analogous films with Zr(IV) and La(III) centers. Preliminary experiments indicate a much smaller extent of immobilization of ds-DNA on these films.

(8) Barton, J. K. *J. Biomol. Struct. Dyn.* 1983, 1, 621.

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(1) Engel, A. *Annu. Rev. Biophys. Biophys. Chem.* 1991, 20, 79.

(2) See, e.g.: Su, H.; Kallury, M. R.; Thompson, M.; Roach, A. *Anal. Chem.* 1994, 66, 769 and references therein.

(3) Yang, J. C.; Aoki, K.; Hong, H.-G.; Sackett, D. D.; Arendt, M. F.; Yau, S.-L.; Bell, C. M.; Mallouk, T. E. *J. Am. Chem. Soc.* 1993, 115, 11855 and references therein.

(4) (a) Rhodes, D.; Klug, A. *Nature* 1980, 286, 573. (b) Tullius, T. D.; Dombroski, B. A. *Science* 1985, 230, 679.

(5) Carter, M. T.; Bard, A. J. *Bioconjugate Chem.* 1990, 1, 257.

(6) Film preparation: Si wafers were soaked in trichloroethylene for 30 min, rinsed twice with 2-PrOH and then water, and dried in N₂ before sputtering of Cr and Au at 10⁻² Torr. The Au surface was cleaned in hot chromic acid (saturated K₂Cr₂O₇ in 90% H₂SO₄) for 10 s, rinsed with copious amounts of water, and then immersed in 0.5 mM MBPA in EtOH for about 24 h. The surface was then thoroughly rinsed with EtOH, dried with N₂, and immersed alternately in 5 mM Al(NO₃)₃, 5 mM bisphosphonic acid, and 5 mM Al(NO₃)₃, taking 4 h for each immersion, with washing with water between each step.

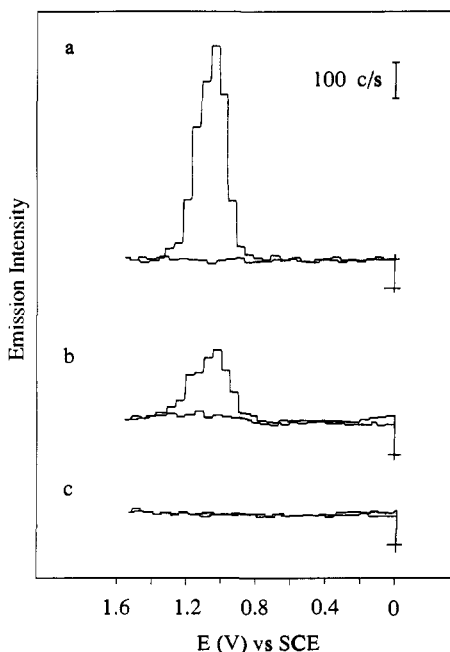
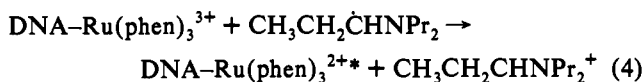
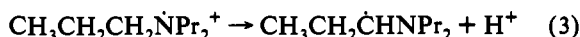
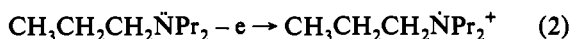
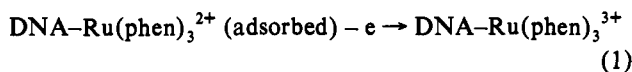


Figure 2. ECL emission-potential transients at the $\text{Al}_2(\text{C}_4\text{BP})/\text{DNA-Ru}(\text{phen})_3^{2+}$ electrode in 0.19 M phosphate buffer, pH 7, containing 0.13 M TPrA. (a) First, (b) second, and (c) third scans. (Scanning was halted and the solution stirred after each scan.) Scans were initiated at 0 V and were directed toward more positive potentials. Scan rate, 50 mV/s.

reaction between electrogenerated $\text{Ru}(\text{phen})_3^{3+}$ and an intermediate in the oxidation of TPrA:



The emission intensity decreased on a second scan, and none was seen on a third, suggesting loss of $\text{Ru}(\text{phen})_3^{3+}$ from the film and diffusion into the bulk solution. In a control experiment, an electrode with a film of $\text{Al}_2\text{C}_4\text{BP}$ that had not been treated with DNA was soaked for 4 h in either an aqueous 0.56 mM $\text{Ru}(\text{phen})_3^{2+}$ or a 0.12 mM $\text{Ru}(\text{phen})_3^{2+}$ in MeCN solution and then rinsed with MeCN and water. This electrode showed no ECL emission upon potential sweep in the same TPrA solution described above, demonstrating that adsorption of $\text{Ru}(\text{phen})_3^{2+}$ on the $\text{Al}_2\text{C}_4\text{BP}$ film does not occur.¹⁰ The ability to generate $\text{Ru}(\text{phen})_3^{3+}$ electrochemically in this ECL experiment demonstrates that the $\text{Al}_2\text{C}_4\text{BP}$ film and the DNA layer do not prevent heterogeneous electron-transfer reactions.

To obtain further evidence for $\text{Al}_2\text{C}_4\text{BP}$ film formation and DNA immobilization, experiments were undertaken with a gold-coated quartz crystal and a QCM.^{3,11} The gold was repeatedly

(9) Leland, J. K.; Powell, M. J. J. *Electrochem. Soc.* **1990**, *137*, 3127.

(10) A previous study (Xu, X-H.; Bard, A. J. *Langmuir* **1994**, *10*, 2409) has shown that Ru chelates will adsorb on unmodified gold and produce ECL under these conditions.

(11) Buttry, D. A. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1991; Vol. 17, p 1.

Table 1. Frequency and Mass Changes on a QCM Plate for Film Growth, DNA Immobilization, and $\text{Ru}(\text{phen})_3^{2+}$ Binding

	Δf^a (Hz)	Δm (ng)	$10^9 \Gamma^h$ (mol/cm ²)
after immersion in ^a			
MBPA ^b	-70	137	1
$\text{Al}(\text{NO}_3)_3^c$	-27	53	1 ⁱ
C_4BPA^d	-145	283	2
$\text{Al}(\text{NO}_3)_3^c$	-25	49	1 ⁱ
ds-DNA ^e	-24	47	0.3 ^j
$\text{Ru}(\text{phen})_3^{2+ f}$	-18	35	0.1

^a Immersions were sequential in order given from top to bottom; bare surface was gold; bare crystal frequency, 6,011 329 MHz. ^b 0.5 mM MBPA in EtOH for ~24 h. ^c 5 mM aqueous $\text{Al}(\text{NO}_3)_3$ for ~4 h. ^d 5 mM phosphonic acid for 4 h. ^e Calf thymus DNA (3.8 mM NP) for 4 h. ^f 0.24 mM $\text{Ru}(\text{phen})_3^{2+}$ in MeCN for 4 h. ^g The variation and drift of the signal over the series of measurements was about ± 1 Hz. ^h Assuming roughness factor for gold of 2 (total surface area, 0.6 cm²). For comparison, for a close-packed monolayer of MBPA, $\Gamma \approx 0.6 \times 10^{-9}$ mol/cm². ⁱ Assuming an $\text{Al}(\text{H}_2\text{O})_3^{3+}$ unit is adsorbed. ^j Corresponding to number of moles of nucleotide phosphate per cm².

treated with hot chromic acid and then rinsed with water and EtOH until the surface was hydrophilic, as indicated by contact angle measurements. The crystal frequency was then measured in air during different stages of $\text{Al}_2\text{C}_4\text{BP}$ film formation,⁶ after DNA adsorption, and after $\text{Ru}(\text{phen})_3^{2+}$ association. The film was rinsed after each step with deionized water and dried in a stream of N_2 before measurement of the frequency. Results are shown in Table 1. Clearly, the crystal frequency decreases as the $\text{Al}_2\text{C}_4\text{BP}$ film forms and DNA and $\text{Ru}(\text{phen})_3^{2+}$ are adsorbed on the surface, showing an increase of mass on the crystal during the different stages. The mass change, Δm , can be related to the frequency change, Δf , by the Sauerbrey equation:¹¹

$$\Delta m = -[A(\rho_q \mu_q)^{1/2}/2F_0^2] \Delta f \quad (6)$$

where F_0 is the fundamental frequency of the unloaded crystal (6 MHz), A is the electrode area (0.159 cm²), ρ_q is the density of quartz (2.65 g/cm³), and μ_q is the shear modulus of quartz (2.95×10^{11} dyn/cm²). With these constants, eq 6 becomes

$$\Delta m (\text{ng}) = -1.95 \Delta f (\text{Hz}) \quad (7)$$

The mass changes calculated in this way are also given in Table 1. These can be converted to approximate surface concentrations, Γ , assuming a roughness factor of 2 (i.e., total surface area of both sides of the quartz crystal of 0.6 cm²).³ ECL could also be detected from both gold surfaces of the quartz crystal when used as a substrate for $\text{Al}_2\text{C}_4\text{BP}$ film formation, DNA adsorption, and $\text{Ru}(\text{phen})_3^{2+}$ association and then scanned in the TPrA solution.

We have demonstrated that an electrode surface can be designed which immobilizes DNA while not adsorbing a detector molecule, $\text{Ru}(\text{phen})_3^{2+}$. Moreover, ds-DNA on the surface can be detected by electrogenerated chemiluminescence of associated $\text{Ru}(\text{phen})_3^{2+}$. Characterization of the adsorbed DNA by transmission electron microscopy and atomic force microscopy is currently being investigated. We have also found that single-stranded DNA can be immobilized on the $\text{Al}_2\text{C}_4\text{BP}$ film surface and then hybridized with complementary DNA in solution with detection of the ds-DNA produced by ECL. Details of these experiments will be reported elsewhere.

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