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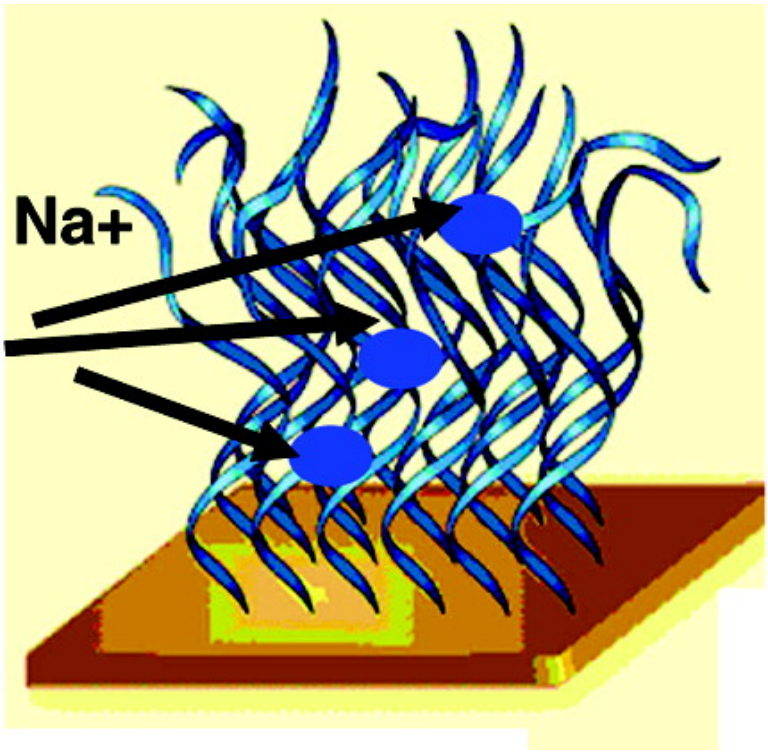

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# Where is the Sodium in Self-Assembled Monolayers of Single-Stranded DNA?

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While the primary biological function of DNA involves information storage and transfer, its unique structural properties can be exploited to build up molecular devices. Among these characteristics are the specific and reversible interaction of complementary sequences of DNA oligomers and the ease with which DNA can be synthesized and modified to comprise different lengths or contain functional groups that allow attachment to various surfaces.[1−3] Self-assembled monolayers of DNA oligomers adsorbed on gold[4−7] or silicon[8−10] substrates were studied very intensively in recent years. Films of single-stranded DNA (ssDNA) immobilized on surfaces form the basis of a number of important biotechnology applications, including DNA microarrays[11,12] and biosensors.[13,14] The organization of ssDNA as monolayers allows one to investigate various properties of the DNA in a controlled manner[15] and to use DNA for analytical applications[16,17] as well as for exploring futuristic schemes for molecular electronics.[18]

It is commonly assumed that the adsorbed DNA layer contains some structural water and the cations. Although former XPS studies were performed on monolayers of DNA, no one, to our knowledge, referred to the amount of the metal cations in the layer. Here we show, based on XPS studies, that when monolayers of ssDNA are formed from NaCl buffer and washed thoroughly, no $Na^+$ signal is detected. A finite concentration of ions is observed when the DNA is made from a solution of $Mg^{2+}$ ions, but it is still only a fifth of what it would be if all the phosphate ions were fully neutralized by the metal cations.

Self-assembled DNA monolayers on gold films were prepared according to standard procedure[19,20] by depositing 3′ thiolated 15-mers of DNA on clean gold substrates. Fifteen base single-stranded, disulfide (S−S)-protected oligonucleotides (*MWG Biotech*) were suspended in 0.4 M, pH = 7.2 sterile phosphate buffer. The clean Au slide was covered with the oligomer solution (50 mM) and kept for a given time at controlled humidity. After adsorption at room temperature, the slides were rinsed in sterile 0.4 M phosphate buffer, pH 7.5. The slides were then soaked in sterile 0.4 M sodium phosphate buffer, pH 7.2, while shaking for 15 min. This was followed by rinsing for 15 min in 0.2 M phosphate buffer, pH 7.2, and subsequently, by a through rinse in sterile deionized (Millipore) water for three consecutive times for 20 min and then kept for 12 h in sterile deionized (Millipore) water to remove any excess salt left on the surface. The next day, the slides were rinsed again with fresh sterile deionized (Millipore) water and then dried by a stream of pure $N_2$ (99.999%). We studied various single-stranded DNA.[21] Here we present data for two 15-mer long ssDNA strands: 5′-AAAAAAAAAAAAAAG-3′ (referred to as 1G) and 5′-GGAA-GAGAGAGAAGG-3′ (referred to as 8G). We studied also a 26-mer long ssDNA strand: 5′-CTA-AGA-TTT-TCT-GCA-TAG-CAT-TAA-TG- 3′.

XPS, atomic force microscopy, contact angle measurements, spectroscopic ellipsometry, and radioactive labeling were used to characterize the monolayers. The average monolayer thickness of both 15-mer ssDNA oligomers was found to be $3.2 \pm 0.2$ nm, a value considerably smaller than the length of a fully extended 15-mer (about 5.1 nm); however, the characterization clearly indicates that the molecules are standing almost perpendicular to the surface (see Supporting Information); 32P-labeled DNA oligomers were used to characterize the adsorption quantitatively. For both 15-mer ssDNA oligomers, the monolayer density was found to be $N = (1.4 \pm 0.4) \times 10^{13}$ molecules/cm$^2$ (as determined by phosphorimager analysis). This value is much smaller than the expected density of a close-packed monolayer ($12.7 \times 10^{13}$ molecules/cm$^2$ for cylindrical geometry with 1 nm diameter cross-section). Within our experimental uncertainty, this density is independent of the deposition time (see below). Since both the thickness and the density represent averages over the surface, the above results are consistent with a heterogeneous monolayer in which "solid" domains of fully stretched, densely packed ssDNA molecules coexist with a surface "gas" of ssDNA adsorbed in a mushroom-like configuration.

Figure 1 shows XPS spectra obtained from monolayers of 15 base single-stranded DNA, prepared from solutions containing sodium or magnesium ions. The deposition times for the monolayers were varied (10 s, 30 s, 15 min, and 12 h). Table 1 presents the atomic concentration ratios for the two types of monolayers, as derived from the XPS data. Each experiment was repeated several times, and the ±10% accuracy reflects the reproducibility of the results. While for all layers, the phosphate-to-carbon ratio (P/C) and the nitrogen-to-phosphate ratio (N/P) are in a reasonable agreement with the expected values, *no sodium signal is measured in the case of a layer of ssDNA deposited overnight from a sodium-containing buffer solution*. Notice that although monovalent metal ions are also never observed in crystallographic studies of DNA crystals,[22] it is sometimes argued that they are not sufficiently well localized within the crystals to be detected by X-ray diffraction.[23,24] In our case, such ions are directly observable by XPS, and their absence cannot be explained by the above argument. We further find that, for short deposition times, some low concentration of sodium is always observed, despite the extensive washing procedure (see Table 1). Even though the actual deposition time in this limit (below 30 s) is poorly defined, it seems to exhibit a maximum for the Na/P ratio at around 30 s. Since the washing procedure is identical for all samples, the observation of higher sodium concentration for short deposition periods indicates that replacement of sodium ions by protons is not a trivial consequence of the washing.

While there is no direct experimental evidence on the origin of the dependence of sodium depletion on deposition time, it is plausible that it is directly related to the kinetics of the reorganization processes that take place inside the adsorbed layer. Since, within the 15% accuracy of our XPS data, only small variations in the

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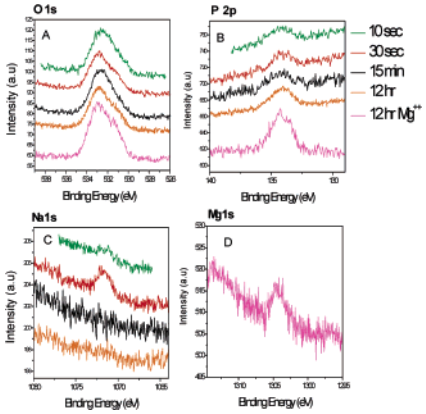


***Figure 1.*** XPS spectra taken for several samples prepared by exposing gold to sodium phosphate solution containing a single-stranded DNA oligomer containing 15 bases (14 adenine and 1 guanine) for 10 s (olive), 30 s (red), 15 min (black), and 12 h (orange), or to magnesium phosphate solution containing the same oligomer for 12 h (magenta).

***Table 1.*** XPS Results for Monolayers Made from Single-Stranded DNA Oligomers Containing 15 Bases (14 adenine and 1 guanine) and Prepared with Different Adsorption Times from Sodium Phosphate Buffer[a]

| sample prep time | N/P ±10% | C/P 10% | N/O ±10% | % of $Na^+$ (Na/P) ±10% |
|---|---|---|---|---|
| 10 s | 4.0 | 20.9 | 0.34 | 4.3 |
| 20 s | 4.9 | 21.3 | 0.51 | 6.6 |
| 30 s | 5 | 17.7 | 0.61 | 11.4 |
| 40 s | 5.8 | 24.3 | 0.61 | 9.5 |
| 5 min | 4.5 | 18.9 | 0.52 | 2.4 |
| 15 min | 4.8 | 20.6 | 0.49 | 1.8 |

[a] The data were identical for guanine located either on the 3′ (1G) or 5′ (8G) ends.

total amount of adsorbed material with deposition time are detected, the reorganization takes place at a constant amount of adsorbed DNA. Furthermore, as memory of the deposition time survives through the much longer washing time, reorganization of the layer cannot be attributed to lateral diffusion (which, if present, would continue during the washing process) and must proceed through exchange of DNA molecules in the monolayer with those in the bulk solution.

We propose that the observed decrease in the concentration of $Na^+$ ions with deposition time results from the following effects: (a) the fraction of oligomers in the stretched solid phase increases and that in the mushroom phase decreases with time, and (b) these ions are expelled from the interior of the growing "solid" domains and are replaced by protons (the absence of any significant line shifts in XPS spectra indicates that the layer is not charged). A possible reason for the depletion is that the domains are stabilized by stacking and hydrogen bonding interactions between the bases of neighboring stretched DNA molecules and that bulky solvated cations are expelled from the resulting dense structure.

Depletion of metal cations is also observed in monolayers prepared from a buffer containing magnesium ions. In these samples, however, the signal of the magnesium is slightly higher, ca. 20% of the value required to neutralize the charge on ssDNA. This is consistent with the proposed mechanism of depletion since the free energy gain from stacking and hydrogen bonding is partially offset by the entropy loss associated with replacing each divalent magnesium ion by two protons. Interestingly, deposition of longer DNA molecules (26 bases) from Na-based solutions (15 min adsorption time and 12 h washing) also gives slightly higher cation concentrations, 12−16%. The density of the monolayer in this case is found to be lower than that for the shorter oligomers (15 bases). These observations agree with the expectation that formation of ordered domains of longer (charged) oligomers is hindered by larger free energy barriers. As a result, the molar fraction of these domains is smaller than that for shorter ssDNA molecules, and higher ion concentrations are detected.

While the proposed mechanism is obviously tentative, our experimental results clearly prove that, in ssDNA adsorbed on gold substrate, the concentration of metallic cations is far below the value required to fully neutralize the DNA molecules. Since the XPS results indicate that the layer is not charged, a fact verified from the absence of any significant line shifts, DNA is believed to be neutralized mainly by protons, which are unobservable in XPS analysis.

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**Supporting Information Available:** XPS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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