Ultrasensitive Detection of CrO$_4^{2-}$ Using a Microcantilever Sensor

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Microcantilevers modified with a self-assembled monolayer respond sensitively to specific ion concentrations. Here, we report the detection of trace amounts of CrO$_4^{2-}$ using microcantilevers modified with a self-assembled monolayer of triethyl-12-mercaptopododecylammonium bromide. The self-assembled monolayer was prepared on a silicon microcantilever coated with a thin layer of gold on one side. The microcantilever undergoes bending due to sorption of CrO$_4^{2-}$ ions on the monolayer-modified side. It was found that a concentration of 10$^{-8}$ M CrO$_4^{2-}$ can be detected using this technology in a flow cell. Other anions, such as Cl$^-$, Br$^-$, CO$_3^{2-}$ (or HCO$_3^-$), and SO$_4^{2-}$, have minimal effect on the deflection of this cantilever. The mechanics of the bending and the chemistry of cantilever modification are discussed.

Chromium exists in different oxidation states in the groundwater, seawater, and soil of our environment.¹ Cr(III) is an essential trace element in the human body as a part of the glucose tolerance factor and also plays an important role in lipid and protein metabolism. But Cr(VI), in the form CrO$_4^{2-}$, on the other hand, has been found to be toxic to animals and humans.² Furthermore, CrO$_4^{2-}$ is stable in neutral and alkaline environments. Most of the methods used to determine CrO$_4^{2-}$, such as extraction,³⁻⁵ ion exchange,⁶ chromographic,⁷ and atomic absorption spectroscopy are generally time-consuming, have less accuracy, or are expensive. Therefore, developing new sensitive techniques for the in situ detection of CrO$_4^{2-}$ in the environment with high sensitivity and selectivity has immediate relevance in environmental remediation and monitoring.⁸

In recent years, microcantilevers have attracted much attention due to their potential as a platform for the development of many novel physical, chemical, and biological sensors.⁹⁻¹² Microcantilevers are the simplest microelectromechanical systems (MEMS) device that can be micromachined and mass-produced. Microcantilever sensors offer improved dynamic response, greatly reduced size, high precision, and increased reliability. In addition, microcantilever sensors also offer many advantages including fabrication of multielement sensor arrays and integration of micromechanical components with on-chip electronic circuitry. Moreover, microcantilevers can be operated in a liquid environment with ease compared to other acoustic sensors.

The principle of microcantilever operation is very simple. When molecular adsorption is confined to one surface of a microcantilever, it undergoes bending due to adsorption-induced stress. It is known from the literature that molecular adsorption changes surface forces (surface stress).¹³ If the two surfaces of the cantilevers are different (bimaterial), molecular adsorption will result in a differential stress between the top and bottom surfaces of the cantilever. Molecular adsorption-induced surface stress on a microcantilever can be observed as changes in cantilever deflections. Using this concept, we have demonstrated the feasibility of chemical detection of a number of vapor-phase analytes with high sensitivity.¹⁰⁻¹²

The radius of curvature of adsorption-induced surface stress of the microcantilever can be related using Stoney’s formula.¹³ The radius of curvature of the bending of the cantilever can be calculated using the physical parameters of the cantilever and can be written as

$$z = \frac{3(1 - \nu) L^2}{4E t^3} \sigma_s$$ (1)

where $z$ is the cantilever’s radius of curvature, $E$ is Young’s modulus for the substrate, $t$ is the thickness of the cantilever, $L$ is the length, $\nu$ is the Poisson ratio, and $\sigma_s$ is the differential stress on the cantilever. The bending of a cantilever can be measured
with subangstrom resolution using the techniques perfected for the atomic force microscope (AFM), such as optical reflection, piezoresponse, capacitive, and piezoelectric detection methods. By substituting the measured bending, z, in eq 1, it is possible to calculate the surface stress. It is expected that appropriate ion receptors could be designed and coated onto the surface of a microcantilever, which has been coated with gold on a single surface, for detecting specific ions. The binding of ions would cause a bending of the microcantilever, while other ions that do not bind to this receptor would have no effect on the cantilever. Recently, we reported that a gold-coated microcantilever modified with a self-assembled monolayers (SAMs) of calix[4]crown-6 can detect pbp amounts of Cs⁺ ions. It is known that quaternary ammonium compounds are useful as ion pair reagents for the chromatographic analysis of CrO₄²⁻, and these compounds have been used for extracting and determining CrO₄²⁻ by absorption in columns and membranes. Here, we report that microcantilevers modified by a self-assembled monolayer of triethyl-12-mercaptopododecylammonium bromide can be used to detect nanomolar concentrations of CrO₄²⁻.

EXPERIMENTAL SECTION

Reagents and Procedures. NaCl, K₂CrO₄, K₂SO₄, KCl, K₂C₃O₄, and KBr were used as received from Aldrich. Triethyl-12-mercaptopododecylammonium bromide was synthesized by reacting 12-mercaptopododecyl bromide with triethylamine in EtOH. High-purity deionized water was obtained with a Milli-Q water system (Millipore). We used commercially available silicon microcantilevers (Park Instrument, CA) in these experiments. The dimensions of the V-shaped microcantilevers were 200 μm length, 20 μm width, and 0.7 μm thickness. One side of the cantilever had a thin film of chromium (3 nm) followed by a 20-nm layer of gold deposited by e-beam evaporation.

SAM Formation on the Cantilever. The Si cantilevers (with Au on one side) were cleaned in piranha solution (7:3 H₂SO₄ (96%)/H₂O₂ (30%)) for 1 min and rinsed with H₂O (three times) and EtOH (two times) before measurement or SAM preparation. (Caution: Piranha solution reacts violently with many organic materials and should be handled with great care.) The formation of triethyl-12-mercaptopododecylammonium bromide SAM on the gold-coated cantilever was conducted by immersing the cantilever into a 3 M solution of triethyl-12-mercaptopododecylammonium bromide in EtOH for 7 days, rinsing with EtOH three times, and drying. Further details of the experiment can be found in the Results and Discussion section on the effect of treatment time on CrO₄²⁻ detection sensitivity.

RESULTS AND DISCUSSION

The predicted surface structure of the modified microcantilever used in this experiment is shown in Figure 2. We expect that when CrO₄²⁻ passes through the flow cell, it will adsorb onto the surface, displace the halide ion, and combine with the cationic ammonium group, forming a stable ionic association that will alter the surface stress, causing a cantilever deflection. The microcantilever was initially exposed to a constant flow (10 mL/h) of distilled water, and its deflection was set as the baseline (i.e., 0 nm deflection). Then, the CrO₄²⁻ solution was passed through the cell at the same 10 mL/h flow rate. After ~30 s, the cantilever started to bend up and it reached an equilibrium position within 100 s. Since it takes ~100 s to flush the cell volume once at 10 mL/h, the flow rate and not the binding kinetics currently limit the sensor response time. Significantly faster flow rates cannot be used because of an increase in background noise.

Effect of SAM Treatment Time on the Sensitivity. Initially, we conducted the experiments with SAM-modified cantilevers prepared by soaking in a 10⁻³ M solution of triethyl-12-mercaptopododecylammonium bromide overnight. It was found that the cantilever deflected only 20 nm upon exposure to a solution containing 10⁻³ M CrO₄²⁻. It was determined that immersing the cantilevers in the triethyl-12-mercaptopododecylammonium bromide solution for a longer period of time gave much better response.
toward CrO$_{4}^{2-}$. To optimize the SAM to obtain the highest sensitivity toward CrO$_{4}^{2-}$, the deflection of the cantilevers were measured for 10$^{-3}$ M solutions of CrO$_{4}^{2-}$ as a function of the SAM formation time, and the results are shown in Figure 3. The data indicate that six or seven days is needed to form the SAM for the maximum response to CrO$_{4}^{2-}$. On the basis of this observation, the cantilevers used in these experiments were all prepared by immersion into the quaternary ammonium salt solution for seven days prior to measurement of CrO$_{4}^{2-}$. It is apparent that this time effect on the sensitivity of CrO$_{4}^{2-}$ detection is due to the formation of a more compact SAM. It is expected that it would require a longer amount of time to form a compact layer of triethyl-12-mercaptododecylammonium bromide on the gold surface due to repulsion of the positive-charged headgroups in triethyl-12-mercaptododecylammonium bromide relative to other long-chain neutral compounds.

Since the formation of a SAM on the bare gold surface of a cantilever causes bending, we conducted an experiment to compare the bending resulting from the formation of a SAM from triethyl-12-mercaptododecylammonium bromide and bending due to adsorption of the neutral compound 12-mercaptododecylthiol (see Figure 4). It is observed that, after 200 s, 12-mercaptododecylthiol has almost completely covered the gold surface and formed a compact SAM. However, after 20 h, the bending of the cantilever due to the adsorption of triethyl-12-mercaptododecylammonium bromide is still going down slowly, suggesting that the formation of a compact SAM is still not complete. This observation is consistent with the contact angle experiment and appears to explain the time dependence for the formation of a SAM useful for the selective and sensitive detection of chromate.

**Cantilever Deflection Due to Anion Adsorption.** The concentration of CrO$_{4}^{2-}$ in the solution was varied from a low concentration of 10$^{-10}$ M to a high concentration of 10$^{-3}$ M. It was observed that, at different concentrations, the cantilever bent at different amplitudes, and the time dependence of the bending of the cantilever as the concentration of CrO$_{4}^{2-}$ is changed is shown in Figure 5. When the CrO$_{4}^{2-}$ concentration increased, the amplitude of cantilever deflection increased. It was also observed that when the solution passing through the cell was switched back to water, the deflection of the cantilever bending decreased. When 10$^{-3}$ M CrO$_{4}^{2-}$ is replaced with water, the cantilever deflection approaches the initial value prior to CrO$_{4}^{2-}$ addition. Since water without any added electrolyte was injected and the deflection returned to the original position, it is unclear what anion remained associated with the quaternary ammonium cations after the long period of flushing with water alone. Interestingly, we observed very similar results when using 10$^{-3}$ M NaCl with both the CrO$_{4}^{2-}$ solution and the flushing solutions. It is possible that hydroxide anions (even at concentrations as low as 10$^{-7}$ M) replaced CrO$_{4}^{2-}$...
or that an elevated CrO$_4^{2-}$ concentration on the surface has promoted dimerization to Cr$_2$O$_7^{2-}$ or some combination of chemical and physical effects resulted in a reduced surface stress. Further experiments are presently underway to elucidate the mechanism of cantilever bending. Since the microcantilever surface area-to-solution volume ratio is quite small in these experiments, surface reactions do not consume a measurable fraction of the solution species. Nevertheless, the important point is that the deflection response of the cantilever to changes in CrO$_4^{2-}$ concentration is reversible. Repeated injection of the same concentration of CrO$_4^{2-}$ causes the same deflection amplitude of the microcantilever ($\pm$10%), ensuring that the same microcantilever can be regenerated and reused for accurate CrO$_4^{2-}$ measurements. The deflection response of any surface-modified microcantilever to the concentration of CrO$_4^{2-}$ in solution will depend on the mechanical properties of the cantilever and the fraction of the surface covered by the SAM as well as the concentration of CrO$_4^{2-}$ in solution. Two closely matched cantilevers deflected 270 and 285 nm in response to $1 \times 10^{-4}$ M CrO$_4^{2-}$, indicating the measurements are reproducible between cantilevers, if the mechanical properties and surface coatings are optimized. Although the stability of a SAM-modified cantilever has not been extensively investigated, the response was stable over a period of several weeks.

Figure 6 shows the deflection of cantilever after reaching an equilibrium bending position at different concentrations of CrO$_4^{2-}$. The plot shows that the deflection of a microcantilever can be observed at a CrO$_4^{2-}$ concentration as low as $10^{-9}$ M, which indicates a higher sensitivity than ion-selective electrode (ISE) or extraction methods. The data in Figure 6 indicate the adsorption isotherm for CrO$_4^{2-}$ on the SAM-modified microcantilever is decidedly nonlinear, indicating a cooperative effect at the highest concentration investigated. Each data point was repeated three times. The experiments repeated on 10 replicate cantilevers show exactly the same behavior for CrO$_4^{2-}$ adsorption. As will be discussed in the following section on microcantilever mechanics, the increase in bending at higher CrO$_4^{2-}$ loading can be rationalized by a greater surface stress (eq 1) at high loading than at low loading. The high sensitivity is due in part to the extraordinarily large deflection response due to Cr(VI) sorption. At the highest concentration investigated ($10^{-3}$ M), the bending response of the microcantilever is 0.25% of the length of the cantilever. It should be noted that monolayer adsorption should result in a saturation that will lead to flattening of bending response at high concentrations. In Figure 6, we could not continue the experiments with higher concentrations of CrO$_4^{2-}$ ions since the bending signal reached the maximum limit for the position-sensitive detector.
Therefore, the lack of a saturation plateau in Figure 6 cannot be interpreted as due to multilayer formation. More experiments using cantilevers of different dimensions are needed to address the question of response saturation of microcantilevers.

To compare the selectivity of this sensor to CrO$_4^{2-}$ over other anions, the deflections of a modified cantilever to the same concentration (1 × 10$^{-4}$ M) of CrO$_4^{2-}$, NO$_3^-$, Cl$^-$, CO$_3^{2-}$ (and HCO$_3^-$), and SO$_4^{2-}$ were measured, and the results are shown in Figure 7. Most of the anions, such as NO$_3^-$, Cl$^-$, and CO$_3^{2-}$ (both HCO$_3^-$ and CO$_3^{2-}$ exist in the solution), showed no effect on the deflection of the cantilever. The anion SO$_4^{2-}$ causes ~20 nm deflection of the cantilever, which is much less than the effect of CrO$_4^{2-}$. Blank tests performed on an unmodified bare gold-coated silicon cantilever revealed that unmodified cantilevers do not bend in response to CrO$_4^{2-}$ even for concentrations as high as 1 × 10$^{-3}$ M. This rules out a nonspecific salt effect on the unmodified microcantilever surface. The solutions of Cr(VI) are generally under slightly alkaline conditions or close to neutral, and more than 98% of the chromium is in the form CrO$_4^{2-}$.

**Mechanics of the Bending of Microcantilevers.** Interestingly, the triethyl-12-mercaptododecylammonium bromide-modified cantilever bends up toward the gold side of cantilever upon sorption of CrO$_4^{2-}$. As illustrated in eq 2, the sorption of CrO$_4^{2-}$,

$$\text{CrO}_4^{2-}(\text{soln}) + 2\text{HS}^-\text{R}-\text{NEt}_3^+ \text{ Br}^- (\text{surface}) \rightarrow 2\text{Br}^- + 2\text{HS}^-\text{R}-\text{NEt}_3^+ \text{ CrO}_4^{2-} (\text{surface}) \quad (2)$$

dissolved in solution, at the modified surface requires two quaternary ammonium molecules, sorbed to the cantilever surface, to maintain electrical neutrality.

This ionic association changes the stress on the surface. Prior to the introduction of CrO$_4^{2-}$ into the solution in contact with the cantilever, Br$^-$ or OH$^-$ ions are associated with the quaternary ammonium cations. The cantilever surface can be described as a charged double layer in which the surface is a layer of positive charge and the aqueous solution immediately adjacent contains the adsorbed layer of negative charge. In analogy with charged electrodes, a compact layer of adsorbed anions (Helmholtz layer) will exist at the cantilever surface and a more diffuse Stern layer will be present in the adjacent solution. Furthermore, it is anticipated that the dielectric constant of the water immediately adjacent will be lower than that of bulk water.

The CrO$_4^{2-}$-selectivity of this cantilever can be explained by an electrostatic model of anion transfer from the aqueous phase to the surface of microcantilever, where the anions associated with the SAM go into the aqueous solution and the CrO$_4^{2-}$ anions becomes associated with quaternary ammonium cations. Moyer and Bonnesen discussed an electrostatic model for anion exchange in solvent extraction and resin separation in which the selectivity is determined by the hydration energy of the exchanging anions. The influence of hydration on resin anion exchange was first discussed by Diamond and Whitley. Ion pair extraction systems have also demonstrated a relationship between the logarithm of the extraction coefficient and the free energy of hydration of anions. The hydration energy of CrO$_4^{2-}$ is smaller than other anions, such as Cl$^-$, Br$^-$, SO$_4^{2-}$, HCO$_3^-$, and CO$_3^{2-}$, that are commonly found in groundwater. Low hydration energy (which generally decreases as the size of the anion increases) favors the exchange of the anion associated with the SAM to the higher dielectric medium of the aqueous solution. These anions with higher hydration energies have a weaker association with the quaternary ammonium cations of the SAM. After the CrO$_4^{2-}$ solution is introduced, the Br$^-$ or other anion is displaced by stronger ion pair formation between CrO$_4^{2-}$ and the quaternary ammonium cation. A smaller number of anions, one CrO$_4^{2-}$ per two quaternary ammonium sites, are adsorbed causing the surface charge to change. This smaller number of anions changes the surface stress, causing the microcantilever to bend upward.

**CONCLUSIONS**

Our study has shown that a quaternary ammonium self-assembled monolayer-modified cantilever can selectively respond to CrO$_4^{2-}$ at concentrations as low as 10$^{-9}$ M. The sensitivity of detection of CrO$_4^{2-}$ using a surface-modified cantilever is much higher than the ISE method. These results provide further evidence that an ion-selective SAM-coated cantilever can be applied successfully to the detection of trace amounts of ions in solution. This technique can also be applied to investigate the magnitude and direction of surface stress due to variation in pH and ionic strength as demonstrated by Fritz et al. This new concept can be applied to the detection of various ions for environment remediation and monitoring applications as well as biomedical assays. Experiments are presently underway on sensors for other ions using cantilevers modified by different molecular recognition materials. The design and fabrication of a multielement sensor array device based on cantilever elements specifically modified for the detection of specific ions is apparent.

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