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Biosensor for the Nonspecific Determination of Ionic Surfactants

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We report the analytical figures of merit for the first biosensor for ionic surfactant quantification. The biosensor consists of a silanized silica optical fiber, onto which acrylodan-labeled bovine serum albumin (BSA-Ac) is immobilized. Recent work from our laboratory on native BSA-Ac (Lundgren, J. S.; Bright, F. V. *J. Phys. Chem.* 1996, *100,* 8580−8586) has shown that ionic surfactants dehydrate the local environment surrounding the lone acrylodan residue, open up the pocket hosting the acrylodan reporter group, and dramatically increase the segmental mobility of domain I in BSA. In the current work, we use BSA-Ac as an actual biorecognition element for surfactant detection and quantification. We also compare several BSA-Ac immobilization strategies and determine the analytical figures of merit for the BSA-Ac-based biosensor to a prototypical analyte, cetyltrimethylammonium bromide (CTAB). The biosensor linear dynamic range extends from 5 to 60 μM, and the t_{90} response time (90% of the total response) is less than 30 s. Biosensor response precision (relative standard deviation) during 34 sensing cycles is 2.5%. On the down side, biosensor performance decreases 38% after 25 days of storage; however, this drift can be compensated. This work also demonstrates the utility of BSA-Ac as a model biorecognition element−reporter group system for grading the suitability of different surface immobilization strategies.

Specific and nonspecific analytical methods have been developed for the determination of surfactants both in cosmetics, paints, household soaps and detergents, and pharmaceutical formulations and in wastewater management.[1] Chromatographic methods are commonly used when the sample contains a mixture of surfactants that need to be individually quantified.[2,3] In situations where a single surfactant is present in a given sample or the total surfactant concentration is relevant, nonspecific titration methods can be used.[4,5] Titrations such as the Wickbold potentiometric procedure for nonionic surfactants[6,7] and the colorimetric methylene blue method for anionic surfactants[8] generally involve preconcentration and extraction steps to improve detectability and minimize interferences. As a result, existing schemes are not ideal, and a simple sensor for the quantification of surfactants would help to alleviate many of the aforementioned problems.

A biosensor consists of an immobilized biorecognition element (e.g., antibody, enzyme) that interacts or reacts with the target analyte to produce a measurable analytical signal.[9−12] Unfortunately, few if any antibodies or enzymes exist that "recognize" ionic surfactants. In an effort to circumvent this problem, we use bovine serum albumin labeled with the fluorescent probe acrylodan (BSA-Ac) as our biorecognition element. BSA-Ac was chosen for three reasons.[13−17] First, the fluorescence (intensity and spectra) from the acrylodan reporter group (attached at cysteine-34) in BSA-Ac changes in a predictable way, depending on changes in the physicochemical properties of the local microenvironment surrounding cysteine-34. Second, we understand the origin of the spectral shifts and intensity changes seen for native BSA-Ac as a function of surfactants, degree of hydration, and surface adsorption. Finally, recent work from our laboratory on native BSA-Ac has shown that certain ionic surfactants, like cetyltrimethylammonium bromide (CTAB), induce conformational changes in the entire BSA molecule and the microdomain surrounding cysteine-34, as well as dehydrate the cysteine-34 environment. Specifically, in the presence of ionic surfactants, there is generally an increase in the native BSA-Ac fluorescence and a concomitant blue shift in the acrylodan emission as the surfactant concentration is increased.

To exploit the inherent behavior of BSA-Ac in the presence of surfactants and develop a biosensor for surfactant quantification, one must first immobilize the BSA-Ac. Toward this end, we investigate several immobilization protocols[17−21] and determine how each influences the immobilized BSA-Ac performance relative

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to native BSA-Ac. BSA-Ac immobilization was carried out on fused silica optical fibers using the following approaches: (1) physisorption, (2) physical entrapment within a sol−gel-derived film that is coated onto the optical fiber, and (3) silanization of the optical fiber with (aminopropyl)triethoxysilane (APTES), followed by treatment with glutaraldehyde. To fully evaluate the immobilization procedure and the overall sensor performance, we used the intrinsic spectral response of the BSA-Ac system and the standard analytical figures of merit for the biosensor responding to the prototypical analyte, CTAB.

EXPERIMENTAL SECTION

Materials. The following materials were used: silica optical fibers with a core diameter of 1000 μm (Ceramoptec); acetone, methanol, glutaraldehyde, and $Na_2HPO_4 \cdot 7H_2O$ (Fisher Chemical); chloroform, hydrofluoric acid, and $NaH_2PO_4 \cdot H_2O$ (J. T. Baker); 6-acryloyl-2-(dimethylamino)naphthalene (acrylodan, Molecular Probes); essentially fatty acid-free bovine serum albumin, 12 000 MW cut-off dialysis tubing, sodium dodecyl sulfate, cetyltrimethylammonium bromide, and triethylamine (Sigma); and (aminopropyl)triethoxysilane (APTES) and tetramethoxysilane (TMOS) (United Chemical Technologies).

BSA-Ac Preparation. BSA-Ac was prepared according to published protocols.[14−16]

Biosensor Substrate Preparation. The silica fibers were mechanically stripped of the protective coating (10 mm segment) and treated with concentrated HF for 3 min to remove residual cladding. BSA-Ac was physisorbed to the fiber-optic surface by incubating the fiber in 50 μM BSA-Ac overnight. The fibers were rinsed with buffer to remove loosely adsorbed protein. Optical fibers were also dip-coated with BSA-Ac-doped, TMOS-derived sol−gel solutions. The TMOS-based sol−gel preparation has been described previously.[22] Silanization of the silica optical fibers followed from Lu et al.[21] Briefly, the optical fibers were immersed (at 20 °C for 6 h under N_2) in a solution containing 0.8 mL of APTES and 0.4 mL of triethylamine in 40 mL of toluene. Following silanization, the optical fibers were rinsed successively with chloroform, acetone, and methanol (3×) and then allowed to dry under a gentle N_2 stream. Once dry, the optical fibers were introduced into a 2.5% glutaraldehyde solution in 0.1 M, pH 7.00 phosphate buffer (the working buffer). After 90 min, the optical fibers were removed from the reaction solution and rinsed thoroughly with the working buffer. In the final step, the activated optical fibers are incubated with a 50 μM BSA-Ac solution for 12 h. Biosensors were stored in the working buffer at 4 °C when not in use.

Steady-State Measurements. All steady-state fluorescence measurements were carried out using an SLM 48000 (SLM-Aminco/Spectronic Instruments) with a 450 W Xe arc lamp as the excitation source. Excitation was at 351 ± 8 nm. Emission scans were collected at an 8 nm bandpass. Total emission measurements were carried out using a 420 nm longpass filter. All initial biosensor measurements were made in neat buffer, followed by random measurement of samples containing known surfactant concentrations. The normalized biosensor response is defined as the total fluorescence from the biosensor at a given surfactant concentration, divided by the total fluorescence from the same biosensor in neat buffer. After each measurement, the biosensor is reset by rinsing with 5 mL of distilled, deionized water.

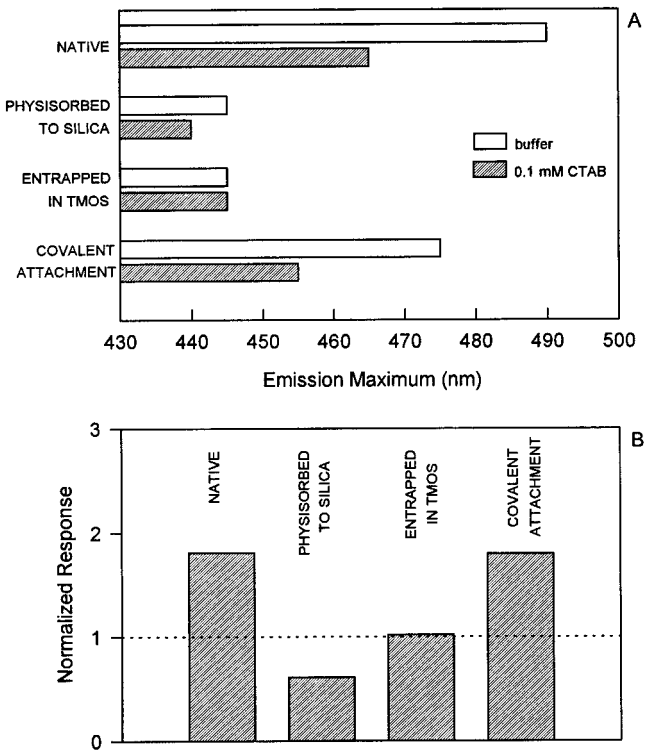

Figure 1. Emission maxima (A) and normalized emission response (B) for native and silica-immobilized BSA-Ac to buffer and 0.1 mM CTAB.

RESULTS AND DISCUSSION

Steady-State Emission of Immobilized BSA-Ac. The fluorescence response of native BSA-Ac to ionic surfactants is characterized by a blue-shifted emission spectrum and an increase in the total emission intensity. Our previous work showed these surfactant-induced changes in the BSA-Ac emission were a direct result of protein dehydration and conformational changes in BSA-Ac.[13] Given this, it seemed prudent to develop a BSA-Ac immobilization scheme in which the BSA-Ac response to surfactants was as similar as possible to the response seen for native BSA-Ac to surfactants.

Figure 1 summarizes the effects of immobilization chemistry on BSA-Ac emission maxima in the absence and in the presence of 0.1 mM CTAB (panel A) and the normalized response to 0.1 mM CTAB (panel B). In all cases, the benchmark is native BSA-Ac in buffer which exhibits an emission maximum at 490 nm in the buffer that shifts to 465 nm in the presence of 0.1 mM CTAB (panel A), with a concomitant 81% increase in fluorescence (panel B). The dashed line in panel B represents the normalized BSA-Ac response in the working buffer in the absence of CTAB.

Upon physisorption of BSA-Ac to silica, the Ac emission maximum blue-shifts to 444 nm and shifts to only 440 nm when the biosensor is emersed in 0.1 mM CTAB. These results indicate that protein physisorption to silica strongly perturbs the local environment surrounding the acrylodan and that the surfactant-

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induced blue shift is small. We also determined that the normalized emission response of the physisorption-based biosensor in the presence of 0.1 mM CTAB *decreased* to 0.61. We attribute the drop in fluorescence to BSA-Ac actually being desorbed from the silica optical fiber when the biosensor is immersed in 0.1 mM CTAB (CTAB does not quench the acrylodan emission). This hypothesis was confirmed by observing the characteristic emission from BSA-Ac in the CTAB solution once the biosensor was removed.

When BSA-Ac is entrapped within a porous TMOS-derived sol−gel glass film coated onto the silica optical fiber, the emission maximum occurs at 445 nm in buffer, and it does not shift to any detectable extent in the presence of 0.1 mM CTAB. There was also no detectable change in the response from TMOS-entrapped BSA-Ac to CTAB. Immobilization of BSA-Ac within the TMOS film either (1) precludes the binding between the protein and CTAB or (2) dehydrates the local environment surrounding the acrylodan reporter group such that CTAB binding has no net effect on the cybotactic region surrounding the Ac residue. Based on previous work from our group on BSA-Ac encapsulated within a sol−gel-derived glass, the latter scenario appears most likely.[23]

Covalent attachment of the biorecognition element to the silica substrate through the APTES/glutaraldehyde (APTES/GA) procedure resulted in fluorescence from the surface-immobilized BSA-Ac at 475 nm in buffer and a full 20 nm blue shift when the biosensor is subjected to 0.1 mM CTAB. The APTES/GA-immobilized BSA-Ac-normalized response to 0.1 mM CTAB is 1.80, which is essentially identical to the response of native BSA-Ac to CTAB (1.81). Together these results demonstrate (1) that the BSA-Ac performance and viability depend strong on the particular surface-immobilization scheme used and (2) that the APTES/GA yields a biosensor that behaves most like native BSA-Ac in buffer.

Working Curves and Linear Dynamic Range. Of the three immobilization protocols used to immobilize BSA-Ac to silica, only the covalent attachment protocol was investigated further. Figure 2 presents typical calibration curves of the BSA-Ac-based biosensor to CTAB (panel A) and SDS (panel B). Also shown is the normalized response of native BSA-Ac to the same surfactants (○). The biosensor is not specific to a single surfactant but responds to certain ionic surfactants with varying sensitivities.[13] The normalized biosensor response (●) to CTAB is somewhat similar to that of native BSA-Ac (○) with respect to sensitivity but differs in working range (5−60 μM CTAB for the biosensor). We determined the working range of the biosensor to SDS to be 75−600 μM and of nearly identical sensitivity and working range when compared to native BSA-Ac in buffer. Because the biosensor responds with equal or greater sensitivity than the native protein to the two surfactants presented in Figure 2, we propose that the local microenvironment surrounding the acrylodan reporter group is accessible to the surfactant for most of the BSA-Ac molecules immobilized at the silica optical fiber. These conclusions have been confirmed recently by a series of interfacial quenching experiments that focused on determining the accessibility of the acrylodan reporter group to O_2 as a function of several common immobilization chemistries.[24] These results do not, however, imply that we have produced a particular orientation of BSA-Ac molecules at the biosensor interface.

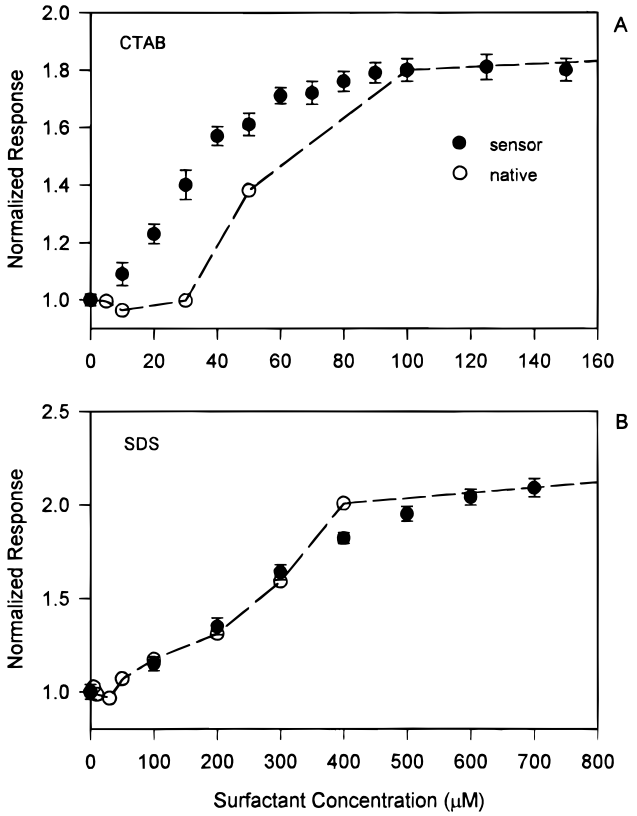

Figure 2. Calibration curves for native BSA-Ac and the APTES/GA-immobilized, BSA-Ac-based biosensor to CTAB (A) and SDS (B).

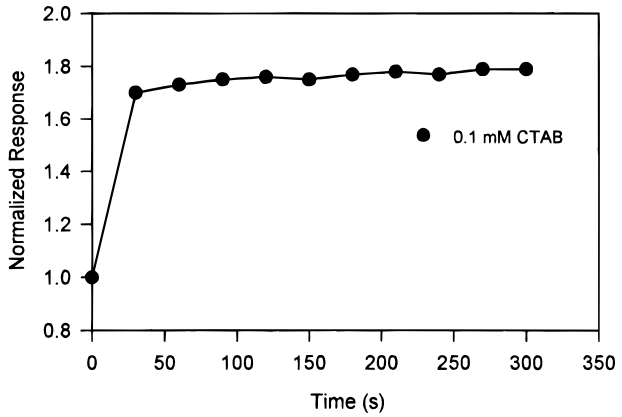

Figure 3. Normalized emission response vs time profile for the APTES/GA-immobilized, BSA-Ac-based biosensor to 0.1 mM CTAB.

Response vs Time Profile and Reversibility. Another important analytical figure of merit for any biosensor is its response time. Typically, solution phase sensors are limited by the time required for the analyte to diffuse through solution or through the entrapment matrix and associate with the recognition element. In the APTES/GA immobilization scheme, the BSA-Ac molecules (recognition elements) should form a monolayer at the surface (based on the surface loading calculations and ellipsometry data of Lu and co-workers[21]); thus, the limiting factor governing the biosensor's response time should be diffusion through the solution. Figure 3 presents the response vs time profile of our BSA-Ac-based surfactant biosensor to 0.1 mM CTAB. In our current setup, we inject CTAB into a cuvette that contains the biosensor, allow the sample to mix, and then reposition our light shielding. This entire process takes approximately 30 s. Figure 3 shows that well over 90% of the total response is achieved within

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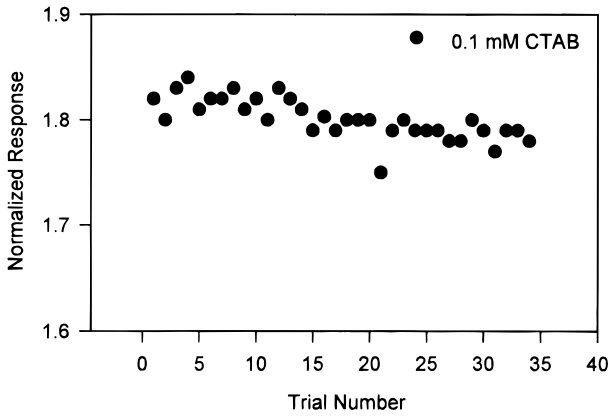

Figure 4. Reversibility profile of the APTES/GA-immobilized, BSA-Ac-based biosensor to 0.1 mM CTAB through 34 cycles.

the 30 s period; the actual response time is likely significantly faster.

The reproducibility of the biosensor response is a key measure of the sensor's analytical performance. In Figure 4, we present typical biosensor-normalized responses from a single biosensor cycled repeatedly between CTAB, buffer, and CTAB solutions. After 34 total cycles, the mean analytical response is 1.80, with a standard deviation of 0.02. These results illustrate that our new biosensor is fully reversible and exhibits excellent measurement precision (2.5% RSD).

Response as a Function of Storage Time. Biorecognition elements like proteins, antibodies, and enzymes often have limited stabilities, especially when removed from their native microdomains (e.g., membrane bound).[25] Moreover, one often adversely affects their stability and hence performance further by immobilizing them at or in a surface. Thus, it is important to determine how the biosensor performance depends on storage time. Figure 5 illustrates the effects of storage time on the emission maximum and the normalized response of our BSA-Ac-based surfactant biosensor to buffer and 0.1 mM CTAB. Inspection of these results shows (panel A) that there is a blue shift in the BSA-Ac emission in the presence and in the absence of CTAB with increasing storage time. These results indicate that there are clear changes in the cybotactic region surrounding the acrylodan residue for the surface-immobilized BSA-Ac over this time period (25 days). Based on our previous work with BSA-Ac,[13−15,23,24] the blue-shifted emission indicates that the average local microenvironment surrounding the acrylodan residue becomes less dynamical and that dipolar relaxation processes are slowed as the biosensor is kept stored. We also note (panel B) a steady decrease in the normalized biosensor response, to 0.1 mM CTAB, with storage time. After 25 days of use, the biosensor responds with an efficiency 38% lower than the initial response. These results are consistent with a slow, time-dependent changes in the BSA-Ac conformation or its reorientation at the biosensor/buffer interface.[13,14]

CONCLUSIONS

Over the past several years, we have used BSA-Ac as a model biorecognition element−reporter group system to more clearly understand how changes in the milieu influence the recognition and reporting steps in chemical sensing and the dynamics

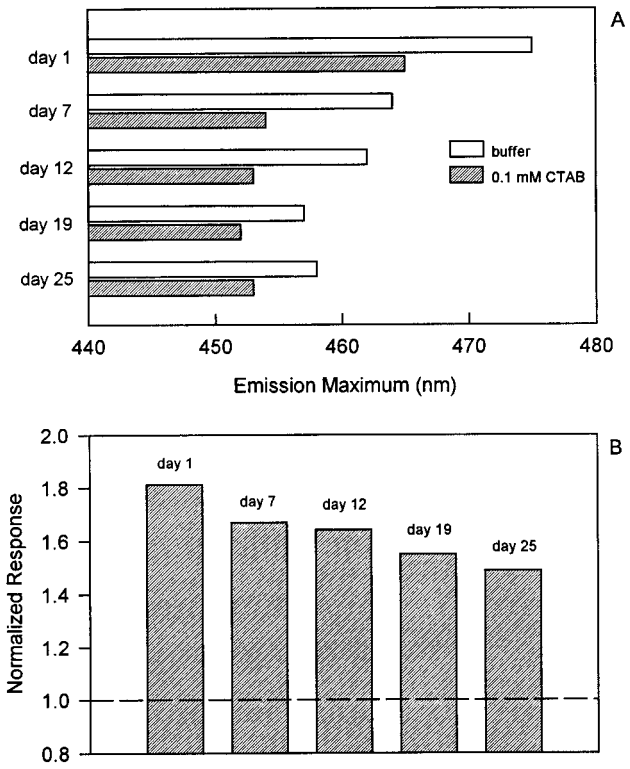

Figure 5. Emission maxima (A) and normalized response (B) from the APTES/GA-immobilized, BSA-Ac-based biosensor to buffer and 0.1 mM CTAB as a function of storage time.

surrounding the reporter group.[13−15,23,24] In the course of this program, we have determined that the steady-state fluorescence from BSA-Ac is a consequence of nanosecond and subnanosecond dipolar relaxation processes that occur between the acrylodan reporter group (attached covalently at cysteine-34) and the local microenvironment surrounding the acrylodan residue. In the current work, we have applied our understanding of the BSA-Ac photophysics when in buffer, chemically denatured, dehydrated, and surface immobilized to develop and optimize a simple surfactant biosensor using BSA-Ac as the biorecognition element. We also use BSA-Ac as the biorecognition element to detect certain ionic surfactants and as a model sensing system that reports the effects of a given surface immobilization protocol on the biosensor performance.

Immobilization of BSA-Ac to silica by physisorption or entrapment within TMOS-derived sol−gel films elicits poor biosensor responses. Based on our previous work,[13−15,23,24] we attribute the poorer response to the surface/matrix slowing the overall dipolar relaxation dynamics surrounding the acrylodan residue and/or changes in the accessibility of the acrylodan reporter group to the surfactant. Thus, surfactant molecules may, indeed, bind to the BSA-Ac when it is immobilized using these schemes, but the form of the BSA-Ac at or in the interface is such that surfactant binding cannot slow the dynamics within the cybotactic region beyond the level achieved by the actual immobilization process. As a result, there is only a small spectral shift (i.e., analytical signal) and minimal changes in the acrylodan fluorescence. Parallel experiments on the physisorbed BSA-Ac[24] on silica show that acrylodan residue accessibility is at least partially responsible for the physisorbed BSA-Ac-based biosensor's poorer performance.

Covalent immobilization of BSA-Ac to silica with APTES and glutaraldehyde yields a biorecognition element that is comparable

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to native BSA-Ac in buffer. By using this immobilization scheme, CTAB can be detected at concentrations as low as 5 μM with a working range up to 60 μM, and for SDS a working range of 75−600 μM is possible. The response time for our new biosensor is less than 30 s, and the device exhibits complete reversibility even after 34 sample/blank/sample cycles, with a measurement precision of 2.5% RSD. On the negative side, the new biosensor response drifts with time. After 25 days of storage, the sensor response decreases by 38%, indicating that there is limited stability with BSA-Ac as a recognition element. However, a simple calibration procedure can compensate for the observed drift.

Currently, we are extending the immobilized BSA-Ac scheme to quantify trace concentrations of IgG antibodies.

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