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Photoresponsive Ion-Selective Optical Sensor

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We report here on a novel concept of a photoresponsive ion-selective optical sensor. Ion-selective optical sensors (ion optodes) are based on the same compounds (ion carriers, ion-exchangers, etc.) and response mechanisms as in ion-selective electrodes (ISEs).[1] A wide range of ion optodes have been developed[2,3] over the past two decades. Typically, these sensors have been fabricated as thin polymeric films on a transparent substrate,[1] as miniature probes at the tip of an optical fiber,[4] or in the form of micron and submicron-sized polymeric beads.[5] Recently, it was demonstrated that thousands of such beads can be injected into a living cell without significant perturbation, thus allowing one to monitor intracellular activities of different ions.[6]

To date, ion optodes have been used only in a passive mode, under conditions of the thermodynamic equilibrium.[1,2] However, in the past decade, several novel analytical methods based on a nonequilibrium response mechanism have been developed for the potentiometric counterparts to ion optodes (ISEs). Ion fluxes in the ISEs can be controlled with a preset concentration gradient across a membrane[7] or by means of nonequilibrium electrochemical methods.[8] These techniques give a remarkable improvement in sensitivity,[9] allow one to drastically reduce detection limits,[7] perform multianalyte detection with a single sensor,[8] distinguish activity and total concentration of an analyte,[10,11] determine polyionic compounds such as anticoagulants,[12,13] and detect surface binding events.[14]

Due to similarities in the response mechanism, the abovementioned nonequilibrium detection methods can be applied to ion-selective optical sensors, as well. However, methods of ion flux control developed for ISEs are not applicable for bead-based assays of miniature ion probes. Rather, the most convenient way to generate and control ion fluxes in the optical probe is to make an optode photoresponsive. Light can be used both to control an ion probe with a photochemical reaction and at a different wavelength to read out the response via absorbance or fluorescence detection.

A regular cation-selective optode contains an ionophore, which selectively binds a primary ion and a second ionophore (chromoionophore) that interacts with a reference ion (usually, hydrogen) and changes the optical properties.[1−3] The competition between two ions for ion-exchange sites in the optode matrix determines the sensor response. We envision three possible methods to photochemically perturb the equilibrium in an ion optode: use photoresponsive ion carriers, photogenerate the ion-exchange sites, or photogenerate an acid or base in the optode matrix. The last of these is especially attractive since it can be easily achieved using photochemical acid generators (PAGs) widely used in chemically amplified photolithography.[15]

Of course, irreversible photolysis of a PAG does limit sensor lifetime. However, this is not a drawback for a disposable optical bead-based assay.

In this study, we employed a sodium/hydrogen ion-selective optode in "proof-of-concept" experiments.

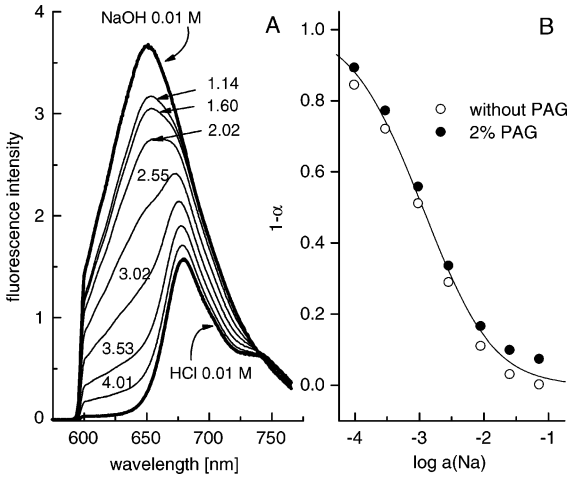

Figure 1. (A) Fluorescence spectra of the chromoionophore in the optode film containing PAG. The upper and the lower spectra were recorded in NaOH and HCl solutions and correspond to complete deprotonation and protonation of the chromoionophore. The intermediate spectra were recorded in the solution containing 0.05 M $MgCl_2$ and 0.1 M Tris (2-amino-2-(hydroxymethyl)-1,3-propanediol) at pH 8 at different activities of sodium ions (numbers on the curves correspond to pNa). (B) Calibration curves for the optode film with and without PAG. The line represents the calculated[2] theoretical response; (1-α) is a relative degree of chromoionophore protonation. Fluorescence intensity is given in arbitrary units.

The sensor was fabricated as a 5 μm plasticized poly(vinyl chloride) film deposited on a microscope cover glass. The film contained 40 mmol·kg^{-1} sodium ionophore (*tert*-butyl calix[4]arene tetraacetic acid tetraethyl ester), 20 mmol·kg^{-1} ion-exchanger (sodium tetrakis(4-chlorophenyl)borate, and 10 mmol·kg^{-1} chromoionophore I (ETH 5294). To make the optode photoresponsive, we loaded 45 mmol·kg^{-1} nonionic PAG (2,4-bis(trichloromethyl)-6-(4-methoxystyryl)-1,3,5-triazine) into the sensor matrix. This PAG produces HCl upon illumination with UV light at 350 nm.

The experimental setup consisted of an inverted fluorescence microscope and imaging spectrometer with CCD camera. UV light at 350(±25) nm was used for PAG photolysis, and fluorescence of the chromoionophore caused by excitation at 550(±25) nm was recorded from 600 to 800 nm. A xenon arc lamp with a four-filter fast wavelength switch was employed as a light source. Ratiometric fluorescence measurements were performed comparing emission peaks at 650 and 672 nm.

Figure 1A shows fluorescence spectra for the optode containing PAG at constant pH and various activities of sodium ions. As we expected, the optode response is not affected by the presence of PAG; the calibration curves for the optode film with and without PAG were very similar (Figure 1B). The parameter (1-α) is the fraction of the total chromoionophore concentration that is present in the protonated form.[2]

The initial fluorescence spectra of the optode equilibrated with aqueous solution are quite reproducible, as shown in Figure 2A. However, illumination for just 1 s with a UV light pulse caused

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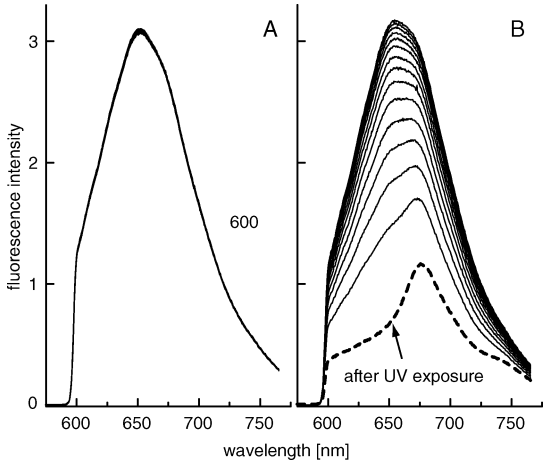

Figure 2. (A) Fifteen spectra (overlapped) recorded in sequence at 5 s intervals for an optode film containing 2% by wt. PAG in a solution containing 0.1 M NaCl, 0.05 M $MgCl_2$, and 0.1 M Tris at pH 8. (B) A sequence of 15 spectra recorded at 5 s intervals following illumination with UV light at 350 nm for 1 s. The first spectrum (dashed line) was recorded immediately after the UV exposure. Fluorescence intensity is given in arbitrary units.

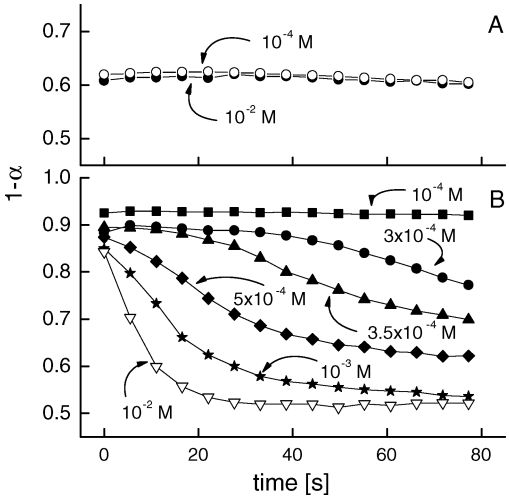

Figure 3. (A) Initial equilibrium response of the active ion optode recorded prior to UV illumination for two distinct Tris concentrations. (B) Non-equilibrium response recorded after 1 s UV exposure. The numbers on the graphs represent Tris concentrations. The solution contained 0.001 M NaCl, 0.05 M $MgCl_2$ at pH 8.

the photogeneration of hydrochloric acid, followed by instantaneous protonation of the chromoionophore and subsequent drastic change in the fluorescence spectrum.

After UV exposure, we recorded fluorescence spectra every 5 s to monitor the equilibration process, which is controlled by the diffusion of hydrochloric acid into the aqueous solution (Figure 2B). The final top spectrum in Figure 2B is almost identical to the one recorded prior to UV exposure, indicating that the photogenerated acid completely leached out of the sensor matrix.

In the next series of experiments, the concentration of sodium ions was kept constant at 10^{-3} M, and the concentration of Tris buffer was varied from 10^{-4} to 10^{-2} M. The pH was kept constant at 8.0 ($\pm$0.05 units). The optode film was replaced with a new one in each experiment.

Clearly, the equilibrium optode response (Figure 3A) did not depend on the total Tris concentration. However, the nonequilibrium response recorded after UV illumination was strongly affected by the buffer capacity of the sample (Figure 3B). Equilibration time increased as the buffer capacity of the sample decreased.

This result can be explained on the basis of a non-steady-state diffusion model. If a flux of the photogenerated hydrogen ions is sufficient to completely protonate the Tris base at the sensor/sample interface, the solution at the interface is no longer buffered, and the pH at the interface is appreciably lower than that in the bulk sample. The pH at the interface is dictated by the balance of two ion fluxes: the flux of hydrogen ions coming from the sensor matrix and the flux of Tris diffusing from the bulk of the aqueous solution sample toward the interface. A lower total concentration of buffer in the bulk sample results in a lower pH at the interface and slower diffusion of the acid.

After prolonged contact with the sample, the perturbation disappears and the sensors equilibrated to $(1\text{-}\alpha) = 0.57 \pm 0.03$, which is consistent with the recorded equilibrium response $(1\text{-}\alpha) = 0.60 \pm 0.04$ in a series of five consecutive measurements.

In conclusion, these experiments demonstrate the general applicability of *nonequilibrium* detection methods to optical ion-selective sensors. Photochemical reactions can be utilized to generate and control ion fluxes in an ion-selective sensor in the same manner as nonequilibrium electrochemical methods have been used in ISEs. In particular, the ability of the proposed photo-responsive probe to detect the buffer capacity, in addition to the activity of hydrogen ions, is a great advantage over classic ion optodes. The fact that active optical probes can be scaled down to the submicron size makes them especially attractive for intracellular applications.

Finally, we note that common optical techniques, such as fluorescence microscopy and flow cytometry, can be combined with active ion probes with only minor modification of the existing experimental setup.

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Supporting Information Available: Instrumental setup and optode fabrication. This material is available free of charge via the Internet at http://pubs.acs.org.

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