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Sensing of Carbon Dioxide by a Decrease in Photoinduced Electron Transfer Quenching

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Abstract

We described a new approach to sensing of carbon dioxide based on photoinduced electron transfer (PET) quenching. Fluorophores like naphthalene and anthracene are known to be quenched by unprotonated amines by the PET mechanism. We examined the fluorescence spectral properties of two amine-containing fluorophores, 1-naphthylmethylamine (NMA) and 9-ethanolaminomethylanthracene (EAA). When dissolved in an organic solvent, both fluorophores displayed increased intensity when equilibrated with gaseous carbon dioxide. In the case of NMA, we found that the mean lifetime increased with increasing partial pressures of CO₂. The intensity and lifetime changes of NMA are completely reversible when CO₂ is removed by purging with argon. Our results are consistent with decreased quenching by the covalently linked amino groups when CO₂ is dissolved in the solution. At present, we are not certain whether the increased intensity is due to protonation of the amino groups or to carbamate formation. In either event, these results suggest that CO₂ can be detected directly using amine-containing fluorophores without the use of bicarbonate and a pH-sensitive fluorophore.

Measurement of dissolved or gaseous carbon dioxide is important in clinical chemistry and bioprocessing (1–3). In general, sensing of CO₂ is moderately difficult because of the inertness of this molecule. In contrast to a variety of cations and anions, fluorescent probes which are directly sensitive to CO₂ are not known. CO₂ does not act as a collisional quencher of fluorescence, and chelators for CO₂ are not known.

Because of the difficulties in direct detection of CO₂, most sensors have relied on coupling the partial pressure of CO₂ to the pH of a bicarbonate solution, which is called the Severinghaus electrode. This principle has been used for optical detection of CO₂ based on the changes in absorption of indicator molecules (4) or the change in fluorescence of pH-sensitive fluorophores (5–9). A difficulty with all these sensors is the need for an aqueous compartment to contain the bicarbonate solution and a gas-permeable membrane. The need for separate compartments results in increased complexity and decreased robustness of the sensors, particularly when used with fiber optics.

To overcome the need for a separate bicarbonate solution, several groups have developed the use of pH-sensitive fluorophores in polymer films (10–14). These sensors sometimes contain

pH-sensitive chromophores which quench the fluorescence probe by resonance energy transfer (12, 15). Protonation of metal–ligand complexes has also been used for CO₂ sensing (16). Protonation of a pH-sensitive molecule appears to be the operative mechanism of most CO₂ sensors which have been reported.

It is known from early research in fluorescence that many aromatic hydrocarbons in the excited state interact with amines. In nonpolar solvents, one often observes a longer wavelength emission due to an excited state complex (exciplex) between the fluorophore and the amine (17–19). In polar solvents, this exciplex emission is usually not observed, but the fluorophore is seen to be quenched by amines. The mechanism of amine quenching appears to be photoinduced electron transfer (PET),¹ which is now thought to occur in a wide variety of fluorophores (20–22).

In the present report, we observed the use of PET to develop a direct sensing fluorophore for CO₂. We examined derivatives of naphthalene and anthracene which contained an amino group linked to the aromatic ring by an alkyl chain (Scheme I). In both fluorophores, the emission is partially quenched by the unprotonated amino group, and less quenched when this group is protonated. Since PET quenching depends on the electron pair in the amino group, we reasoned that any reaction which decreased the density of unbound electrons would result in increased intensity. One possible reaction is the formation of carbamates between the amino groups and CO₂. Carbamate formation is known to occur for amines described in organic and aqueous solvents (23, 24) and for amino groups on hemoglobin and other proteins in aqueous solution (25, 26). In the carbamate, the unbound electron density will be drawn toward the carboxyl group, as occurs in peptide bonds. Hence, the extent of PET quenching should be less in the carbamate as compared with the free amines, and the intensity should increase upon carbamate formation. This hypothesis of decreased PET quenching in the presence of CO₂ was used to develop a direct sensing method for CO₂.

MATERIALS AND METHODS

1-Naphthylmethylamine (NMA) was obtained from Fluka and used without further purification. 9-Ethanolaminomethyl anthracene (EAA) was synthesized by reacting 0.86 g of 9-chloromethylantracene with 0.5 g of 2-aminoethanol in 50 ml of acetonitrile in the presence of triethylamine (TEA). The reaction mixture was stirred for 4 h. Removal of the solvent by evaporation resulted in an off-white product that was recrystallized from benzene, yielding a product of approximately 70% purity.

Emission spectra were recorded using a SLM AB2 spectrofluorometer. Frequency–domain measurements were performed using instrumentation described previously (27). For NMA, the excitation source was a frequency-doubled rhodamine 6 G dye laser at 285 nm. The emission of NMA was measured with magic angle polarizer conditions with a M 340-nm interference filter and a 10-nm bandpass from Melles Griot. Intensity decay of EAA was measured using the excitation at 335 nm from the output of a frequency-doubled DCM dye

¹Abbreviations used: FD, frequency domain; EAA, 9-ethanolaminomethylantracene; NMA, 1-naphthylmethylamine; PET, photoinduced electron transfer; TEA, triethylamine.

laser. The emission was observed through a Corning 7–59 and 385-nm cutoff filter, again with magic angle conditions.

Intensity decays were analyzed in terms of the multiexponential model,

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \quad [1]$$

where α_i is the preexponential factor, τ_i is the decay time, and $\sum \alpha_i = 1.0$. The fractional intensity of each decay time is given by

$$f_i = \frac{\alpha_i \tau_i}{\sum_j \alpha_j \tau_j} \quad [2]$$

The values of α_i and τ_i were attained by nonlinear least squares by minimizing χ_R^2 ,

$$\chi_R^2 = \frac{1}{\nu} \sum_{\omega} \left(\frac{\varphi_{\omega} - \varphi_{c\omega}}{\delta\varphi} \right)^2 + \frac{1}{\nu} \sum_{\omega} \left(\frac{m_{\omega} - m_{c\omega}}{\delta m} \right)^2 \quad [3]$$

In this expression, φ_{ω} and m_{ω} are the measured values of the phase (σ) and modulation (m) at the light modulation frequency ω . The values of $\varphi_{c\omega}$ and $m_{c\omega}$ are the calculated values for assumed values of α_i and τ_i . The values of $\delta\varphi = 0.3$ and $\delta m = 0.008$ are the assumed experimental uncertainties in the phase and modulation, respectively. In some cases, we performed a global analysis of the frequency responses measured at more than one partial pressure of CO₂. In this case, the sum in Eq. [3] extends over the partial pressure. For the global analysis, we assumed the decay times were the same at all partial pressures of CO₂, but that the amplitudes depended on the partial pressure of CO₂.

RESULTS

Effects of CO₂ on 1-Naphthylmethylamine

Emission spectra of NMA are shown in Fig. 1. For these spectra, NMA was dissolved in dioxane which also contained 0.3 mM TEA. The TEA was used in an attempt to prevent protonation of the amino group in NMA and thereby promote carbamate formation. The intensity of NMA was found to increase by more than 50% upon equilibration of one atmosphere of CO₂ compared to equilibration with argon. The inert gas, argon, was used to avoid quenching by dissolved oxygen and thereby allow a direct comparison of the intensity seen in the absence and presence of CO₂.

For characterization of NMA as a CO₂ sensor, we wished to avoid collisional quenching by the TEA. The dependence of the intensity of NMA on the concentration of TEA is shown in Fig. 2. The extent of quenching is less than 5% of 0.3 mM TEA. Hence, we chose this concentration of TEA for the subsequent experiments.

In previous reports, we described the benefits of fluorescence lifetime as the basis for sensing (28, 29). Lifetime-based sensing requires a change in the mean decay time of the sample in response to the analyte, which in turn requires that the sensing fluorophore be

fluorescent in the presence and absence of the analyte. The data in Fig. 1 suggest fluorescence in the presence of CO₂, but the emission spectra are also consistent with complete quenching of a fraction of the NMA molecules. Hence, we measured the frequency-domain intensity decays of NMA for various partial pressures of CO₂ (Fig. 3). These measurements reveal a shift in the frequency response to lower frequencies with increasing concentration of CO₂. This shift is due to an increase in the mean decay time of NMA with increasing partial pressure of CO₂ (Fig. 4). The frequency responses at all CO₂ concentrations could be well fit globally with the global decay times and nonglobal amplitudes, with an acceptable global χ^2_R value of 1.45. Examination of the values of a_i and τ_i in Table 1 reveals that one of the decay times of NMA is 0.54 ns and this component makes only a minor contribution to the total steady-state intensity, 1% or less. Hence, the intensity decay of NMA is dominantly due to decay times of 20.3 and 49.7 ns, which were found for both the two and the three decay time fits.

We examined how the intensity decay amplitude depends on the partial pressure of CO₂ (Fig. 5). In the absence of CO₂, the intensity of NMA is due almost exclusively to the 21.1-ns component. As the partial pressure of CO₂ increases, so does the fractional amplitude of the component with a 49.5-ns decay time. We presume this component is due to either the carbamate of NMA with CO₂, or to some other species forming in response to CO₂.

For lifetime-based sensing, it is adequate to measure the phase and modulation at a single light modulation frequency (28, 29). For these measurements, we chose a light modulation frequency of 4.6 MHz (Fig. 6), at which the largest changes occur in the frequency responses. These results show that the phase angle of NMA increases by nearly 20° for 1 atm of CO₂. The modulation decreases by almost 27%. These changes are adequate for lifetime-based sensing of CO₂.

For most sensing applications, the sensors must respond reversibly to changes in the analyte concentration. Since the mechanism of interaction between NMA and CO₂ is not known with certainty, we did not know if the spectral changes would be reversible. Hence, we measured the phase and modulation values as the sample was alternatively exposed to argon or CO₂ (Fig. 7). We were pleasantly surprised by the complete reversibility of the phase and modulation changes caused by CO₂. The spectral changes appeared to occur on the same timescale as our ability to dissolve CO₂ into the dioxane sample, or pure CO₂ from the sample. If the mechanism of interaction is carbamate formation, then this reaction is reversible upon removal of dissolved CO₂.

Effects of CO₂ on 9-Ethanolaminoethyl Anthracene

In order to test the general applicability of this approach to CO₂ sensing, we examined a second aromatic hydrocarbon containing an amine (EAA, Scheme I). This structure was chosen because we expected future experiments to be performed in aqueous solution, and we reasoned the ethanol moiety would aid aqueous solubility. Additionally, we knew that carbamate formation depends on the presence of free amino groups and that hydroxyl amino groups may have a higher pK_A and would be partially unprotonated in aqueous solution at near neutral pH values.

Emission spectra of EAA are shown in Fig. 8. As found for NMA, the intensity increases in the presence of CO₂. This CO₂-dependent change in intensity was completely reversible (Fig. 9). Additionally, the intensity changes corresponded to changes in the phase and modulation (Fig. 10). Hence, EAA can also be used for lifetime-based sensing of CO₂.

In addition to the data shown in Figs. 8–10, we also measured the frequency-domain intensity decays of EAA (Fig. 11). However, it appeared that the intensity decay contained components which may have been due to impurities. We feel that the intensity, phase, and modulation changes shown in Figs. 9 and 10 are reliable, but the sample is not of adequate purity to report the multiexponential analyses.

DISCUSSION

Simple and reliable measurements of CO₂ are needed for a variety of medical and industrial purposes. These include measurement of dissolved CO₂ in blood (30, 31) and measurements of CO₂ as a predictor for shock (32, 33). Measurements of CO₂ are also used in blood septicemia assays and in monitoring of respirators (34, 35).

Despite the widespread need for CO₂ sensors, most rely on the bicarbonate–pH couple or on infrared measurements of gaseous CO₂ (36, 37). To date, few, if any, indicators or fluorophores have been found which are directly sensitive to CO₂.

The reader may question the value of a sensing fluorophore dissolved in dioxane. The purpose of the present report is not to describe the final clinical sensor, but rather to point out an approach to CO₂ sensing based on amine-containing fluorophores which are quenched by the PET mechanism. Once identified such fluorophores can be immobilized in various polymeric supports and/or sol gels to fabricate a sensor. For this same reason we have not compared the accuracy and range of our sensor with present clinical sensors.

The results presented above suggest a new approach to CO₂ sensing using amine-containing fluorophores and the PET mechanism. Based on this direct interaction, it seems probable that a number of additional CO₂-sensitive fluorophores can be developed, particularly those which display longer absorption and emission wavelengths and/or larger decay times. The use of such sensors, particularly those with larger absorption and emission wavelengths, can provide a new direct approach to direct fluorescence detection of CO₂.

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FIG. 1. Spectral characteristics of 1-naphthylmethylaniline in dioxane with 0.3 mM triethylamine. The solid and dashed lines represent NMA in the solution saturated by CO₂ and argon, respectively, at 23°C and atmospheric pressure.

FIG. 2.

Dependence of a steady-state fluorescence intensity of 1-naphthylmethylamine in dioxane on the concentration of triethylamine. Intensities were measured on the SLM AB2 spectrofluorometer, with excitation at 285 nm and emission at 340 nm. The samples were in equilibrium with air.

FIG. 3.

Frequency-domain intensity decay of 1-naphthylmethylamine in dioxane in the presence of 0.3 mM triethylamine as a function of $p\text{CO}_2/(p\text{CO}_2 + p\text{Ar})$ ratio at 23°C and atmospheric pressure. The solid lines show the best global triple decay time fit for the global three decay time fit $\chi^2_R = 1.45$.

FIG. 4.

The mean lifetime of 1-naphthylmethylamine in dioxane with 0.3 mM triethylamine as a function of $p\text{CO}_2/(p\text{CO}_2 + p\text{Ar})$ ratio. The mean lifetime was calculated using the best triple-exponential global fit of the data from Fig. 3.

FIG. 5.

Fractional amplitudes of the fluorescence decay of 1-naphthylmethylamine in dioxane with 0.3 mM triethylamine as a function of $p\text{CO}_2/(p\text{CO}_2 + p\text{Ar})$ ratio. The amplitudes are from the best triple-exponential global fit of the data from Fig. 2. The shortest component (0.54 ns) contributes to the total fluorescence intensity by less than 1% and was omitted (see also Table 1). The double exponential global fit gives essentially the same results.

FIG. 6. Calibration curve of the CO₂ sensor 1-naphthylmethylamine in dioxane at modulation frequency of 4.6 MHz. The closed and opened circles represent phase and modulation, respectively.

FIG. 7.

Phase (closed circles) and modulation (opened circles) response of 1-naphthylmethylamine in dioxane to changes of CO_2 concentration at the modulation frequency of 5.7 MHz. Arrows indicate times when solution of the sensor was exposed to CO_2 or argon atmosphere. The experiment was performed at room temperature and atmospheric pressure in the presence of 0.3 mM triethylamine in the solution. The sample was placed in the hermetically tight cuvette, stirred by a magnetic stirrer, and the specified gas was continuously bubbled through the upper part of the sample. Fluorescence was collected from the lower part of the cuvette where the signal was not perturbed by the bubbles.

FIG. 8. Spectral characteristics of 9-ethanolaminomethylantracene in dioxane with 0.3 mM triethylamine. The solid and dashed lines represent the compound in the solution saturated by the CO₂ and Argon, respectively, at 23°C and atmospheric pressure.

FIG. 9.

Response of the steady-state fluorescence intensity of 9-ethanolaminomethylanthracene in dioxane to changes of CO₂ concentration at the modulation frequency of 80 MHz. Arrows indicate time when the sensor was exposed to CO₂ or argon atmosphere. Experiment was performed at room temperature and atmospheric pressure in the presence of 0.3 mM triethylamine in the solution.

FIG. 10.

Phase (closed circles) and modulation (opened circles) response of 9-ethanolaminomethylanthracene in dioxane to changes of CO₂ concentration at the modulation frequency of 80 MHz. Arrows indicate times when the sensor was exposed to CO₂ or argon atmosphere. The experiment was performed at room temperature and atmospheric pressure in the presence of 0.3 mM triethylamine in the solution.

FIG. 11. Frequency-domain intensity decay of 1-ethanolaminomethylanthracene in dioxane in the presence of 0.3 mM triethylamine as a function of the $p\text{CO}_2/(p\text{CO}_2 + p\text{Ar})$ ratio at 23°C and atmospheric pressure. The solid lines show the best global quadrupole decay time fit.

NMA

EAA

SCHEME I.

Chemical structures of 1-naphthylmethylamine (NMA) and 9-ethanolaminomethyl anthracene (EAA).

TABLE 1

Fluorescence Decay of 1-Naphthylmethylamine in Dioxane in the Presence of 0.3 mM Triethylamine

Gas ^a	τ (nS)	α_i	f_1^d	$\langle \tau \rangle$ (ns) ^e	χ_R^2 ^c
Air	4.5 (0.2) ^b	1.0	1.0	14.5	12.7 ^c
	1.1 (0.1)	0.095 (0.005) ^b	0.008		
	14.9 (0.06)	0.905 (0.005)	0.992	14.8	1.1
Argon	19.5 (0.4)	1.0	1.0	19.5	18.8
	20.3 (0.4)	0.98 (0.01)	0.953		
	49.7 (0.9)	0.02 (0.01)	0.047	21.7	6.8 ^g
	0.54 (0.07)	0.09 (0.01)	0.003		
	21.1 (0.2)	0.90 (0.01)	0.990		
	49.5 (0.4)	0.003 (0.003)	0.007	21.3	1.45 ^h
CO ₂	41.0 (1.0)	1.0	1.0	41.0	40.0
	20.3 (0.4)	0.32 (0.02)	0.16		
	49.7 (0.9)	0.68 (0.02)	0.84	45.0	6.8 ^g
	0.54 (0.07)	0.14 (0.13)	0.002		
	21.1 (0.2)	0.25 (0.01)	0.148		
	49.5 (0.4)	0.61 (0.01)	0.850	45.2	1.45 ^h
Argon, acid ^f	30.0 (0.4)	1.0	1.0	30.0	13.2
	6.3 (1.0)	0.12 (0.01)	0.03		
	31.9 (0.4)	0.88 (0.02)	0.97	31.3	1.6

^aSolution saturated by the specified gas at room temperature and atmospheric pressure.^bStandard deviations calculated by the support plane or Monte Carlo methods.^cFor experimental uncertainties $\delta\phi = 0.3^\circ$ and $\delta m = 0.008$.^dFractional fluorescence intensity.^eMean lifetime.^fPropionic acid (1%, v/v).^gDouble-exponential global fit of all data curves from Fig. 2.^hTriple-exponential global fit of all data curves from Fig. 2.