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Reactivity of a luminescent "off–on" pyrylium dye toward various classes of amines and its use in a fluorescence sensor microtiter plate for environmental samples

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ABSTRACT

We report on a systematic study on the reactivity of the pyrylium dye Py-1 toward various classes of amines (primary aliphatic, primary aromatic, secondary, tertiary) and its use in environmental sensing of amines. While most primary aliphatic amines react almost quantitatively with Py-1 within 10 min, sterically hindered primary aliphatic amines and primary aromatic amines react more slowly. Secondary and tertiary amines just induce a decomposition of the chromophore of Py-1 but do not yield a fluorescent product. This makes Py-1 a suitable receptor and transducer in an optical sensing microplate for rapid screening of primary aliphatic amines in water and soil samples. Py-1 is embedded into a polymeric cocktail, which is deposited on the bottom of wells in microtiter plates to yield a high-throughput fluorescence sensing tool. On reaction with primary aliphatic amines, a significant fluorescence increase (λ_{exc} = 485 nm/λ_{em} = 620 nm) of the sensor spots is detected in a standard microplate reader after 10 min incubation at 25° C. The linear calibration plots of eight primary aliphatic amines are in the range of 0.350–70.0 μg mL^{-1}. The limits of detection (LODs) are from 0.119 to 0.589 μg mL^{-1} and the limits of quantitation (LOQs) are from 0.399 to 1.965 μg mL^{-1}. We further show that the sensing plate is suitable for sensing mixtures of primary amines and that the total content of amines (TAC) found is a good measure for the sum of the contents of the individual amines present. Finally, the sensing plate was successfully applied to the quantitative analysis of primary aliphatic amines in 3 samples of water and soil, respectively.

1. Introduction

The need to determine amines has increased over the last few years because of growing concentrations found in soil or in waste water [1]. Primary aliphatic amines are widely distributed in manufacturing industries and in biological bodies or food (as biogenic amines). They can be hazardous to human health due to irritation of skin, mucosa and eyes. In addition, aliphatic amines play a critical role in the formation of nitrosamines, which are potentially mutagenic and carcinogenic [2]. Therefore, new sensing tools for the determination of aliphatic amines in environmental and biological samples are desired.

Methods for quantitation of aliphatic amines in water and soil include gas chromatography (GC) [3,4], high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) [1,5]. GC is frequently combined with solid-phase microextraction [3] or headspace microextraction [4] for pre-concentration. CE methods mostly employ micellar electrokinetic chromatography (MEKC) and sometimes are combined with atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) [5]. HPLC is assisted by derivatization (pre-colum, on-column, solid-support assisted off-line derivatization on C_{18} SPE cartridges) [6], fluorescence derivatization [7] and fluorescence derivatization hyphenated with mass spectrometric detection [8]. A rapid and inexpensive screening method for the total content of aliphatic/aromatic amines, however, requires the absence of a coupled separation system. The separation methods mentioned above usually require expensive instrumentation and elevated time for separation and detection (in the order of 30 min sample). Spectrofluorimetry has the advantages of being sensitive, simple and rapid. A fluorescence readout of a 96-well microplate is finished in less than 2 min which is a huge advantage compared to chromatographic and electrophoretic methods. Those require not less than 10 min per sample for separation and reconditioning. Hence, a microplate with built-in sensor membranes can serve as a rapid screening tool to determine a sum concentration of amines before applying more advanced separation methods which, on the other hand, provide quantitative data specific for each amine present.

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Due to the absence of intrinsic emission, fluorescence derivatization of aliphatic amines has been regarded as an effective technique to achieve good sensitivity [9]. A fluorogenic reagent of choice should display good selectivity and a huge difference of the fluorescence intensity of the unreacted reagent and its derivative. We have recently reported [10–13] on the use of chromogenic and fluorogenic pyrylium dyes as protein labels, gel stains and derivatization reagents for capillary electrophoresis. For the use of such a dye in a screening sensor microplate, analyte selectivity and fast reaction kinetics and reliability toward mixtures of analytes are key factors.

Therefore, we photometrically determined the reaction of Py-1 with various classes of amines (primary aliphatic, primary aromatic, secondary, tertiary). This allows (1) the estimation of rate constants of the formation of the fluorescent reaction products and (2) determination of selectivity because yet the absorbance spectra give quantitative information on the degree of the formation of fluorescent products and the degree of decomposition of the dye. The deposition of sensor spots in the wells of a simple microplate yields a high-throughput sensor tool for rapid and parallel quantitation of the total content of primary amines (TAC) in environmental samples. We show the evaluation of the fluorescence of individual conjugates of Py-1 with amines formed in the sensor spots. Interestingly, the TAC found in mixtures of primary amines is a good measure of the sum of the contents of the individual amines. A screening of amines in real water and soil samples showed good recoveries from standard addition experiments.

2. Experimental

2.1. Materials

Hypan HN 80 polymer was obtained from Hymedix Inc. (www.hymedix.com). Py-1 was from ActiveMotif Chromeon (www.chromeon.com) [11]. The buffer N-cyclohexyl-2-amino ethanesulfonic acid (CHES) was from Roth (www.carlroth.de). All other chemicals were purchased from Sigma (www.sigmaaldrich.com). They are as follows: spermidine, putrescine, histamine, tyramine, 1-tetradecylamine, methylamine (40% (w/w) in water, Riedel-de Haen), ethylamine (70% (w/w) in water, Riedel-de Haen), dimethylamine (compressed liquid), triethylamine, diethylamine, n-propylamine, dibutylamine, isopropylamine, diethanolamine, n-butylamine, isobutylamine, tert-butylamine, aniline, N-methylaniline, N,N-dimethylaniline, p-anisidine, diphenylamine, N,N-diethylaniline, 1,4-phenylenediamine, o-phenylenediamine, benzidine, α-naphthylamine, β-naphthylamine, 9-aminophenanthrene, piperidine, pyrrole, piperazine and benzylamine. The amines were of analytical grade.

2.2. Spectrophotometric measurements

Py-1 stock solution (1.00×10^{-4} mol L^{-1}) was prepared by dissolving 4.00 mg of Py-1 in 1.00 mL of dimethylformamide (DMF) and dilution of this solution with methanol to a total volume of 100 mL. Amine stock solutions (1.00×10^{-2} mol L^{-1}) were prepared by dissolving of a certain weight or volume of amine in 10.0 mL of CHES buffer pH = 10.

The reagent solution for the photometric study was obtained by diluting 1.00 mL of a Py-1 solution (1.00×10^{-5} mol L^{-1}) and 100 μL of amine solution (2.00×10^{-5} mol L^{-1}) to a final volume of 10.0 mL with methanol. These mixtures (dye and amines) were stirred at room temperature and the absorbance spectra were measured after 10 min for primary aliphatic amines and after 10 min, 30 min, 60 min, 120 min, and 1 day for secondary and tertiary amines. UV spectra were acquired on a Shimadzu UV-1800 UV–vis spectrophotometer (www.shimadzu.de).

2.3. Fabrication of sensor microtiter plates

A 5.00% (weight) solution of Hypan HN 80 in DMSO (1.00 g of Hypan + 19.00 g of DMSO) is heated to 60 °C for 12–24 h and stirred well until Hypan is dissolved completely and a clear yellowish solution is obtained. A Py-1 stock solution of 5.00 mg of Py-1 in 1.00 mL of DMSO is made and stored at 4 °C in the dark. Subsequently, 10.0 μL of Hypan HN 80 solution and 4.00 μL of Py-1 stock solution are dispensed into each well of a microtiter plate (No. 651001, 96 well microplates, V-bottom) from Greiner bio-one (www.gbo.com). Finally, the plate is shaken in round motion in an Eppendorf Thermomixer comfort (www.eppendorf.com) for 30 min at 40 °C to obtain deeply blue colored sensing spots on the bottom of the wells of the microtiter plate. Those plates are stored at 4 °C in the dark in a desiccator over solid KOH.

2.4. Preparation of amine standard solutions for determinations in sensing plate

Specific amounts of each amine were dissolved in 5.00 mL of CHES buffer pH 10 to obtain a primary stock solution of 10.0 mM of the free base. 1.00 mL of each amine primary stock solution was placed in a 10.0 mL volumetric flask. The volume was completed to 10.0 mL with methanol to produce a secondary stock solution containing 1.00 mM of each amine. Working standard solutions were prepared by transferring 4.50, 22.5, 45.0, 90.0, 135, 180, 225, 270, 315, 360 and 450 μL of each amine secondary stock solution into a vial and completing the volume to 500 μL with methanol. All solutions were stored at 4 °C.

2.5. Preparation of soil and water samples

2.5.1. Water samples

The real samples were tap water, waste water (irrigation water), and factory waste water of a dye manufacturing plant. The water samples were filtrated from foreign particles through a whatman filter paper no. 12.5 (www.whatman.com) to obtain clear solutions. Then, triethylamine (≤10 μL) was added to 100 mL of each water sample to adjust the pH to 10 and the samples were stored at 4 °C.

2.5.2. Soil samples

10.0 g of soil sample were stirred with 100 mL of methanol for 10 min at room temperature and then filtrated through a whatman filter paper no. 12.5 to obtain clear solutions. Then, 80 μL of triethylamine were added and the sample was stored at 4 °C.

2.6. Fluorimetric quantification of amines (in real samples)

50.0 μL of the respective working standard solutions were transferred into each well of a sensor microtiter plate for quantitation of the amines. The measurement was performed at λ_{exc} = 485 nm and λ_{em} = 620 nm with a FLUOstar Optima microtiter plate reader from BMG LABTECH (www.bmglabtech.com). A 10 min incubation time was adjusted and 5 s of shaking is recommended both, before and at the end of the incubation time. The temperature was set to 25 °C. The gain of the photomultiplier of the microtiter plate reader was fixed at a value of 1550. The mean fluorescence intensity was calculated as the average of five independent measurements of each concentration of the respective amine.

A standard addition method was used for quantitation of the amines in the water and soil samples. Increasing quantities of methylamine (5.00, 10.0, 20.0, 30.0, 40.0 and 50.0 μg mL^{-1}) were