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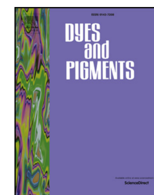

A novel water-soluble perylenetetracarboxylic diimide as a fluorescent pH probe: Chemosensing, biocompatibility and cell imaging

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ABSTRACT

Herein we pay attention to the design, synthesis and sensor activity of a novel biocompatible perylene-3,4,9,10-tetracarboxylic diimide (PDI) pH-probe in water. The synthesized compound shows selective fluorescence signalling properties as a function of pH (pK_a value of 6.35 ± 0.02) which makes the probe suitable for pH determination in the physiological range. Thus prepared water soluble fluorescent system demonstrate low cytotoxicity to L929 cell lines from 330 μM to 1.3 μM and cell permeability in the lower concentration range. That findings show the high potential of the newly prepared PDI probe for monitoring of pH variations in bio-samples.

1. Introduction

During the last few years, perylene-3,4,9,10-tetracarboxylic diimides (PDIs) have been developed to become one of the most attractive classes of fluorophores. Their attractiveness is based on the unique combination of high stability, electro optical and redox properties, which allows them to be used in broad areas such as organic compounds in photovoltaic devices [1–3], field-effect transistors [4–6], light emitting diodes [7–9], liquid crystal displays [10,11] and as dyes in photodynamic therapy [12]. The main drawback of the PDI dyes is their insolubility in common solvents, which still seriously restricted their processing, material applications and synthesis [13,14].

Recently the PDIs have become a tool for fluorescent investigations as chemosensing probes [15–18]. The fluorescent sensing molecular systems for detection and reporting of chemical species have received considerable attention due to their short response time and cheap instrumentation requiring simple manipulation [19–24]. Owning to the small dimension and fast harmless light communication allowing safe cell penetration they are excellent diagnostic tool in the medicine and biology for real-time monitoring and imaging [25–28]. That is why in the past years, more and more attention has been paid to the development of fluorescent sensing compounds for biological important ions and molecules under physiological conditions [29–33]. The use of highly water soluble probes enabling work in aqueous environments is preferred than these requiring binary water-organic solutions due to the reduced stress for the living organisms. However the water solubility is still considered a challenging objective in the molecular probe design, which makes the synthesis of novel highly water soluble organic probes of a great scientific interest [34].

The measurement of pH is very important in biological, chemical and industrial fields [35–41]. pH plays a key role in physiological and pathological process like cellular proliferation, endocytosis, modulation, apoptosis and drug resistance [42–44]. Abnormal pH values are associated with inappropriate cell function, growth, and division and are observed in some common disease types [45,46]. For example, intracellular pH in a range from 6.3 to 7.0 for cancer cells was recently reported and asthmatics lung airway pHs 5.2 have been recorded [47]. Acid responsive probes also find application for cellular imaging of acidic intracellular vesicles, such as endosomes, lysosomes, and phagosomes, where pH is in a range from 4.5 to 6.5 [48]. Hence the determination of intracellular pH has attracted increasing interests [49–52].

In previous works we have described three 1,8-naphthalimide pH-probes containing amidoamine fragments which allows their use in water [53,54]. Based on this experience it was of interest to see if the incorporation of similar amidoamine to PDI fluorophore would result in a pH sensing probe operating in water. Therefore herein we pay attention to the design, synthesis and sensor activity in water of a novel

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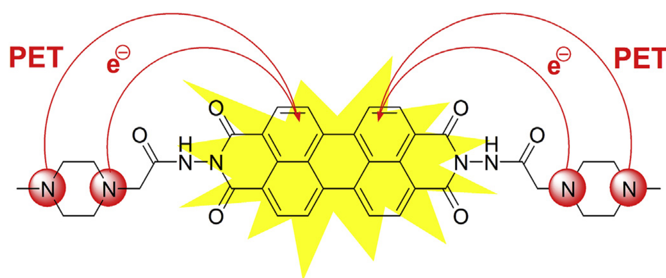

Scheme 1. Perylene-3,4,9,10-tetracarboxylic diimide based probe **4**.

PDI pH-probe containing amidoamine receptor fragments (Scheme 1). Also, it was illustrated the possibility to apply the novel fluorescent probe for intracellular imaging.

2. Experimental

2.1. Materials

Commercially available perylene-3,4,9,10-tetracarboxylic anhydride, hydrazine monohydrate, *N*-methylpiperazine and chloroacethyl chloride (Aldrich) were used without purification. All solvents (Aldrich, Fisher Chemical) were pure or of spectroscopy grade. $MgSO_4$, $CaSO_4$, $Zn(NO_3)_2$, $Cu(NO_3)_2$, $Ni(NO_3)_2$, $Co(NO_3)_2$, $Pb(NO_3)_2$, $Fe(NO_3)_3$, $Hg(NO_3)_2$, $Al(NO_3)_2$, $Cr(NO_3)_3$, $Cd(NO_3)_2$ salts were the sources for metal cations and KCl, $NaNO_3$, Na_2SO_4, $NaHSO_4$, Na_2CO_3, $NaHCO_3$, CH_3COONa, $C_7H_5NaO_3$ (Sodium salicylate), KBr, KI, $NaNO_2$, $Na_2S_2O_3$, $Na_2S_2O_4$, NaH_2PO_4, K_2HPO_4, K_3PO_4, Na_2S, NaCN and NaF were the sources for anions (all salts at p.a. grade).

2.2. Methods

FT-IR (Fourier Transform Infrared) spectra were recorded on a Varian Scimitar 1000 spectrometer. The ^{1}H NMR (Nuclear Magnetic Resonance) spectra (chemical shifts are given as δ in ppm) were recorded on a Bruker AV-600 spectrometer operating at 600.13 MHz. TLC (Thin Layer Chromatography) was performed on silica gel, Fluka F60 254, 20 × 20 cm, 0.2 mm. The UV-Vis (Ultraviolet-Visible) absorption spectra were recorded on a spectrophotometer Hewlett Packard 8452A. The fluorescence spectra were taken on a Scinco FS-2 spectrofluorimeter. The fluorescence quantum yields (Φ_F) were measured to Coumarin 6 ($\Phi_F = 0.78$ in ethanol [55]) as a standard. All the experiments were performed at room temperature (25.0 °C). A 1 × 1 cm quartz cuvette was used for all spectroscopic analysis. The spectral data were collected using FluoroMaster Plus 1.3 and further processed by OrginPro 8 software. To adjust the pH, very small volumes of hydrochloric acid and sodium hydroxide were used. The effect of the metal cations and anions upon the fluorescence intensity was examined by adding portions of the ion stock solution to a known volume of the fluorophore solution (10 mL). The addition was limited to 100 µL so that dilution remains insignificant. Dynamic light scattering (DLS) measurements were performed on a Brookhaven Instruments Corp. equipped with a He-Ne laser. The temperature was set to 22 °C and the angle of measurements was 90°.

2.3. Synthesis of probe **4**

To a suspension of 2 g (5 mmol) perylene-3,4,9,10-tetracarboxylic acid dianhydride in 25 mL of toluene, hydrazine monohydrate (6 mL, 25 mmol) was added and the resulting mixture was heated under reflux for 5 h. After cooling to room temperature the precipitate was collected by filtration, washed with toluene and dried. The crude solid was treated with 50 mL of 5% aqueous sodium hydroxide to give after filtration, washing with water and drying 1.9 g (90%) of perylene diimide **2**. Then a suspension of 1.9 g (4.5 mmol) *N*-amino-perylene diimide **2** in 30 mL of dry dimethylformamide (DMF) was heated to 80 °C and 5.6 mL (0.07 mol) chloroacethyl chloride were added dropwise. The resulting mixture was stirred under the same temperature for 5 h. After cooling to room temperature, the mixture was poured into 20 mL of water and the precipitated solid was filtered off, washed with water and dried to give 1.54 g (60%) of intermediate **3** as red crystals.

To the solution of compound **3** (1.5 g, 2.6 mmol) in 25 mL of DMF, 1.4 mL of *N*-methylpiperazine were added. The solution was heated under reflux for 10 h. After cooling to room temperature the precipitated solid was filtered off, washed with DMF and dried to give the desired probe **4** as a red solid (1.31 g, 72%). FT-IR (KBr) cm^{-1}: 3366 (ν NH); 1734 (ν N-C=O); 1707 (ν N-C=O); 1690 (ν N-C=O). ^{1}H NMR (D_2O, 600 MHz): 8.90 (s, 2H, 2 × NH); 8.50 (m, 4H, PDI); 8.36 (m, 4H, PDI); 3.78 (s, 4H, 2 × $COCH_2$); 3.63 (m, 4H, 2 × CH_2-piperazine); 3.35 (m, 8H, 4 × CH_2-piperazine); 2.98 (s, 6H, 2 × CH_3-piperazine); 2.85 (m, 4H, 2 × CH_2-piperazine). ^{13}C NMR (D2O, 150.92 MHz) ppm: 171.4, 159.6, 135.8, 131.9, 130.3, 127.1, 125.6, 62.1, 58.3, 57.2, 47.4. Elemental analysis: Calculated for $C_{38}H_{36}N_8O_6$ (MW 700.28) C 65.13, H 5.18, N 15.99%; Found C 65.34, H 5.27, N 15.68%.

2.4. Biocompatibility of PDI probe

To observe the biocompatibility of the synthesized PDI probe mouse fibroblast-like L929 cell line (ATCC®CCL-1™; American Type Culture Collection, Rockville, MD, USA) was used. The cells were grown and maintained in Eagle's minimum essential medium (MEM), (PAA, The Cell Culture Company, Cat. E15-825, Germany) containing 10% fetal bovine serum (FCS) and supplements (L-glutamine, sodium pyruvate, antibiotic, NEAA) at 37 °C and 5% CO_2. Cells were passaged at 80–90% confluence with 0.05% trypsin - 0.02% EDTA and suspended at density of 4 × 10^4 cells/mL. The biocompatibility test was performed in 96-well plates with subconfluent cell layers. The cells were incubated with PDI samples in concentration range of 0.75 mg/mL to 0.001 mg/mL for 24 h or 48 h. MTT test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Invitrogen, USA) was performed as described by Mosmann [56] with some modifications. Briefly, after the incubation period the cell medium was changed with fresh medium (200 µL/well). Then, 50 mL of MTT solution (5 mg/mL in PBS) was added. Plates were further incubated for 4 h at 37 °C and the formazan crystals formed were dissolved by addition of 250 µL solvent (5% formic acid in 2-propanol) per well and mixing. The absorbance was recorded at 570 nm with the 96-well plate reader Tecan Infinite F200 PRO (Tecan Austria GmbH, Salzburg). For each concentration six wells were used. Complete medium (200 µL) and 5% formic acid in 2-propanol (250 µL) were used as a blank solution. Biocompatibility corresponding to cell viability after incubation with the investigated probes was presented as percentage to the cell viability of the control (non-treated cells).

2.5. Fluorescence imaging in living cell

Cells were incubated as indicated above and seeded on glass slides (d = 12 mm, Superior-Marienfeld, Germany), placed in 24 well plates. After 24 h of incubation, the cells were treated with solutions of the novel PDI probe in concentration range between 1.3 µM and 26 µM and were incubated additionally for 24 h and 48 h. The fluorescent images were taken using an inverted fluorescent microscope (Leica DMI3000 B, Leica Microsystems GmbH, Germany) with the objective lens HI PLAN 20× and 40×/0.50, excitation filter BP 545/30, dichromatic mirror-565 and suppression filter- BP 610/75.

3. Results and discussion

3.1. Design and synthesis

The dye under study was designed as a highly water-soluble probe

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