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The role of the size of aza-crown recognition moiety in azaphthalocyanine fluorescence sensors for alkali and alkaline earth metal cations

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ABSTRACT

A series of fluorescence sensors bearing one 1-aza-12-crown-4, 1-aza-15-crown-5, 1-aza-18-crown-6 or 1-aza-21-crown-7 as a recognition moiety and an aza-analogue of phthalocyanine as a fluorophore was prepared. All compounds absorbed and emitted light in the red region. Sensing properties based on intramolecular charge transfer were studied *via* absorption and fluorescence titration experiments with alkali metal cations and alkaline earth metal cations. Important relationships between aza-crown size and binding affinity were observed in the group of alkali metal cations. Affinity for lithium decreased in series from the smallest crown to the largest, 1-aza-15-crown-5 bound sodium and potassium similarly, and 1-aza-18-crown-6 had the highest affinity to potassium. Alkaline earth metal cations were bound more tightly, which was obvious from more pronounced changes in the absorption spectra, and from the higher increase of fluorescence upon cation addition. A limited size preference was observed in the group of alkaline earth metal cations.

1. Introduction

Fluorescence sensors, i.e., compounds that can switch fluorescence ON and OFF upon binding a sensitive analyte, can be used in clinical, biological or environmental applications [1]. Due to their high sensitivity, ultra-fast response, and the compatibility of instrumentation with the biological environment, the fluorescence sensing devices are gaining increased attention for the analysis of metal ions over other methods. Metal cation recognition is very important for quantification of blood electrolytes [2], cations involved in ion-channel transportation [3], or for monitoring of lithium [4] and potassium [2] levels in the treatment of bipolar disorder and hypertension, respectively.

The principles by which fluorescence sensors switch between ON and OFF states are usually based on intramolecular charge transfer (ICT) or photo-induced electron transfer (PET), or less frequently on energy transfer or excimer formation. These processes have been described in detail in many excellent reviews [5–8]. The quenching of excited states (OFF state) and restoration of fluorescence (ON state) is driven by enabling and blocking ICT or PET.

Fluorescence sensors typically consist of recognition and signaling moieties. As a recognition part, the aza-crown moiety has been investigated since the discovery of crown-cation binding ability in 1967 [9] and especially after the Nobel prize was awarded to Cram, Lehn and Pedersen in 1987 [10]. Nowadays, aza-crowns are used as a recognition part in many commercially available sensors for Na^+ or K^+ recognition such as SBFI, PBFI, CD222, and Sodium Green™ [1]. The signaling moiety is represented by a fluorophore that is often recruited from the group of coumarins, indoles, imidazoles, fluoresceins, rhodamines, BODIPY dyes, and cyanine dyes [6]. The ideal sensor for metal cation recognition should possess high brightness (i.e., fluorescence quantum yield multiplied by the appropriate extinction coefficient), should absorb

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and emit light in the red region because such light is not absorbed by endogenous chromophores and is less scattered, and should be (photo)stable and insensitive to the pH of medium. A fluorophore that meets all the requirements for fluorescence sensing in the biological environment is still lacking, which leaves space for further improvement.

Dialkylamino substituted azaphthalocyanines (AzaPcs) from the group of tetrapyrazinoporphyrazines have been shown to undergo ultrafast ICT leading to efficient quenching of excited states [11,12]. Based on this finding, AzaPcs have been used recently as structurally new fluorescent sensors for monitoring pH [13], as well as for cation recognition [14] in the pilot assessments serving as a proof of concept. AzaPcs showed promising photophysical properties with light absorption in the red region above 650 nm, a high extinction coefficient (ε ~ 200 000 M^{-1} cm^{-1}) and good quantum yields of fluorescence [14,15]. Moreover, the non-basic character of the donor amine ensures the insensitivity of cation-sensitive AzaPc sensors to the pH of the medium [14].

This work is a follow-up project investigating cation-sensitive AzaPc sensors with a focus on the role of the size of aza-crown recognition moiety to gain deeper understanding into the requirements necessary for selective complexation.

2. Materials and methods

2.1. General

All of the organic solvents were of analytical grade. Anhydrous butanol was stored over magnesium and distilled prior to use. All chemicals for the syntheses were obtained from established suppliers (Aldrich, Acros, Merck, and TCI Europe) and were used as received. TLC was performed on Merck aluminium sheets with silica gel 60 F254. Merck Kieselgel 60 (0.040–0.063 mm) was used for the column chromatography. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Vx BB 300 or VNMR S500 NMR spectrometer. The reported chemical shifts are given relative to $Si(CH_3)_4$ and were locked to the signal of the solvent. Infrared spectra were measured on a Nicolet 6700 (ATR mode). The UV/Vis spectra were recorded using a Shimadzu UV-2600 spectrophotometer. The fluorescence spectra were obtained by an AMINCO Bowman Series 2 luminescence spectrometer. Atmospheric pressure chemical ionization (APCI) mass spectra were obtained using Agilent 500 Ion Trap LC/MS (Agilent Technologies, Santa Clara, California, USA) by direct infusion of the sample in methanol into the detector. HR MS spectra were measured with the use of UHPLC system Acquity UPLC I-class (Waters, Millford, USA) coupled to high resolution mass spectrometer (HRMS) Synapt G2Si (Waters, Manchester, UK) based on Q-TOF. Chromatography for this HR MS measurement was performed using Acquity UPLC BEH300 C4 (2.1 × 50 mm, 1.7 μm) column using isocratic elution with acetonitrile and 10 mM ammonium formate buffer pH 3 (90:10) at flowrate 0.4 ml/min. Electrospray ionization was operated in positive mode. The ESI spectra were recorded in the range 200–2000 *m*/*z* using glu-fibrinopeptide B as a lock mass reference and sodium iodide for calibration. Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectra were recorded in the positive reflectron mode on a 4800 MALDI-TOF/TOF mass spectrometer (AB Sciex, Framingham, MA, USA) using trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile as the matrix. The instrument was calibrated externally with a five-point calibration using Peptide Calibration Mix 1 (LaserBio Labs, Sophia-Antipolis, France). Synthesis of precursors **2b** [14], **3b** [14], **7** [16] as well as AzaPc derivatives **1bM** [14], **1eZn** [14] were prepared according to the published procedures.

2.2. Synthesis of precursors

2.2.1. 5-chloro-6-(1,4,7-trioxa-10-azacyclododecan-10-yl) pyrazine-2,3-dicarbonitrile (**2a**)

5,6-dichloropyrazine-2,3-dicarbonitrile (909 mg, 4.57 mmol) was dissolved in THF (60 mL), and the solution was cooled down in a NaCl/ice bath. Then, a cold solution of 1-aza-12-crown-4 (801 mg, 4.57 mmol) in THF (15 mL) was slowly added, and the mixture was stirred for 5 min. Finely ground anhydrous K_2CO_3 (842 mg, 6.09 mmol) was added in one portion, and the suspension was stirred for another 2 h at rt. THF was evaporated, chloroform (50 mL) and one drop of hydrochloric acid were added and the product was washed three times with brine (3 × 50 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The product was purified by column chromatography on silica with diethyl ether/acetone 5:1 as the eluent (R_f of **2a** = 0.55). The product was recrystallized from ethanol. The fine precipitate was collected by filtration. Yield: 1.44 g (94%) of yellow solid; m.p. 87.9–88.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 3.63-3.68 (m, 8 H, crown-H), 3.93 (t, J = 5 Hz, 4 H, crown-H) and 4.08 ppm (t, J = 5 Hz, 4 H, crown-H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 52.1, 69.9, 70.5, 71.4, 112.8, 113.0, 118.0, 129.0, 135.5 and 152.0 ppm; IR (ATR): 2863, 2228 (CN), 1548, 1505, 1434, 1385, 1355, 1294, 1238, 1133, 1089, 1066, 1046 and 1019 cm^{-1}; HR MS (ESI^+): calcd for $C_{14}H_{16}ClN_5O_3$ m/z = 338.1014 $[M+H]^+$; found m/z = 338.1004 $[M+H]^+$.

2.2.2. 5-chloro-6-(1,4,7,10,13-pentaoxa-16-azacyclooctadecan-16-yl)pyrazine-2,3-dicarbonitrile (**2c**)

5,6-dichloropyrazine-2,3-dicarbonitrile (600 mg, 3.02 mmol) was dissolved in THF (50 mL), and the solution was cooled down in a NaCl/ice bath. Then, a cold solution of 1-aza-18-crown-6 (790 mg, 3.00 mmol) in THF (15 mL) was slowly added, the mixture was stirred for 5 min. Finely ground anhydrous K_2CO_3 (542 mg, 3.92 mmol) was added in one portion and suspension was stirred for another 2 h at rt. THF was evaporated, chloroform (50 mL) and one drop of hydrochloric acid were added and the product was washed three times with brine (3 × 50 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The product was purified by column chromatography on silica with diethyl ether/acetone 2:1 (R_f of **2b** = 0.28) as the eluent. Yield: 1.02 g (79%) of light yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 3.61-3.67 (m, 16 H, crown-H), 3.82 (t, J = 6 Hz, 4 H, crown-H) and 4.10 ppm (t, J = 6 Hz, 4 H, crown-H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 51.9, 69.2, 70.52, 70.56, 70.65, 70.73, 112.9, 113.1, 117.9, 129.0, 135.6 and 152.0 ppm; IR (ATR): 2868, 2228 (CN), 1549, 1441, 1384, 1350, 1299, 1233, 1118, 1045, 987 and 944 cm^{-1}; HR MS (ESI^+): calcd for $C_{18}H_{24}ClN_5O_5$ m/z = 426.1539 $[M+H]^+$; found m/z = 426.1543 $[M+H]^+$.

2.2.3. 5-butoxy-6-(1,4,7-trioxa-10-azacyclododecan-10-yl) pyrazine-2,3-dicarbonitrile (**3a**)

2a (900 mg, 2.66 mmol) was sonicated in butanol (110 mL) for 10 min at rt. DBU (446 mg, 2.93 mmol) was added dropwise and the mixture was stirred at rt for another 1 h. Ethyl acetate (70 mL) and one drop of hydrochloric acid were added and the product was washed three times with brine (3 × 50 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The product was purified by column chromatography on silica with toluene/THF 10:1 (R_f of **3a** = 0.25). After several impurities were washed out, the mobile phase was changed to toluene/THF 5:1 (R_f of **3a** = 0.67) as the eluent. The purified product was recrystallized from ethanol. The fine precipitate was collected by filtration. Yield: 939 mg (94%) of yellow solid, m.p. 89.7–90.6 °C; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 0.98 (t, J = 7 Hz, 3H, CH_3), 1.45