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Azaphthalocyanines: Red Fluorescent Probes for Cations

Veronika Novakova,*^[a] Lukáš Lochman,^[b] Ivana Zajícová,^[b] Kamil Kopecky,^[b] Miroslav Miletin,^[b] Kamil Lang,^[c] Kaplan Kirakci,^[c] and Petr Zimcik^{*[b]}

Fluorescent indicators are important tools for the analysis of biological cations. For example, these probes are useful for the quantification of blood electrolytes,^[1] assaying iontransport channels,^[2] and monitoring of lithium-drug levels.^[3] While the interactions of metal cations with phthalocyanines (Pcs) have previously been studied using absorption, magnetic circular dichroism, and NMR spectroscopy,^[4,5] none of these methods is suitable for in vitro and in vivo studies, owing primarily to a lack of sensitivity, and/or to the incompatibility of the instrumentation with biological systems. In this context, we describe the development of new aza analogues of Pc, azaphthalocyanines (AzaPcs), which upon binding to cations fluoresce in the red region of the visible spectrum. The development of probes that absorb and emit red light at wavelengths greater than 630 nm is highly desirable, especially for in vivo applications, because this increases the depth that the excitation light penetrates, and reduces the effects of light scattering and the autofluorescence of endogenous chromophores that often occurs at shorter wavelengths.

Recently, we confirmed that alkylamino-functionalized AzaPcs undergo an ultrafast intramolecular charge transfer (ICT) upon excitation. The ICT relaxation pathway of the excited states results in the quenching of the AzaPc fluorescence (i.e., produces the OFF state).^[6] Their fluorescence can be switched ON by protonation of the donor center (i.e., of the amino substituent), if it is sufficiently basic.^[7] This property led us to develop a new type of AzaPc sensor

[a] Dr. V. Novakova Department of Biophysics and Physical Chemistry Faculty of Pharmacy in Hradec Kralove Charles University in Prague Heyrovskeho 1203, 50005 Hradec Kralove (Czech Republic) E-mail: veronika.novakova@faf.cuni.cz

- [b] L. Lochman, I. Zajícová, Dr. K. Kopecky, Dr. M. Miletin, Prof. P. Zimcik Department of Pharmaceutical Chemistry and Drug Control Faculty of Pharmacy in Hradec Kralove Charles University in Prague Heyrovskeho 1203, 50005 Hradec Kralove (Czech Republic) E-mail: petr.zimcik@faf.cuni.cz
- [c] Dr. K. Lang, Dr. K. Kirakci Institute of Inorganic Chemistry Academy of Sciences of the Czech Republic, v.v.i. Husinec-Řež1001, 250 68 Řež(Czech Republic)
- Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201300079.

based on the coordination of cations to the donor center. In a proof of concept study, an aza[15]crown-5 moiety, which is used as the recognition element, was attached to the periphery of the AzaPc macrocycle. This molecule was further functionalized with bulky tert-butylsulfanyl substituents to eliminate AzaPc aggregation. During the synthesis of the sensors, we observed that their fluorescence quantum yields $(\Phi_{\rm F})$ are sensitive to substituent groups in the *ortho* position to the crown ether. Therefore, a series of model compounds with a diethylamino donor center (1a-f, Scheme 1) was syn-



Scheme 1. Reaction conditions: i): 1) Mg butoxide, butanol, reflux, 6 h; 2) p-toluenesulfonic acid, THF, RT, 2 h; 3) Zn(CH₃COO)₂, pyridine, reflux, 2 h.

thesized to assess the effects of ortho substituents on quenching ability and to select the most suitable substituents for incorporation into the sensors (2a-c, Scheme 1).

Both the model compounds 1a-f and the sensors 2a-c were synthesized from two different precursors, 5,6-bis(tertbutylsulfanyl)pyrazine-2,3-dicarbonitrile and a substituted

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pyrazine-2,3-dicarbonitrile bearing a donor center by mixed cyclotetramerization using magnesium butoxide as the initiator (for details of their synthesis, see the Supporting Information).

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The absorption spectra of compounds 1 and 2 in THF were typical of monomeric species in solution and the presence of aggregates that could reduce the sensitivity of the sensor was not detected (Figure 1a, b). Typical Q- and Bbands were observed at approximately 655 and 375 nm, respectively, and another band with characteristics typical of charge-transfer (CT) bands occurred at approximately 480 nm. The CT band confirms the conjugation of the donor substituent (i.e., the lone electron pair on the peripheral nitrogen) with the macrocycle. The fluorescence emission spectra of 1 and 2 exhibited typical shapes for these compounds (λ_{em} ca. 658 nm, Figure 1c, d). However, the Φ_{F} values varied with the substituent in the ortho position to the donor center. While the $\Phi_{\rm F}$ values of **1a** and **1b** were very low and indicated that ICT was highly feasible in these cases, the $\Phi_{\rm F}$ values for **1c-f** were similar to those of com-



Figure 1. Interactions of **2a** with Na⁺ (a, c, e) and K⁺ (b, d, f): a), b) Absorption spectra in THF (1 μ M) during titration with corresponding SCN⁻. c), d) Fluorescence emission spectra (1 μ M, λ_{exc} =600 nm). Inset: Dependence of $\Phi_{\rm F}$ on the concentration of the added cation. e), f) Job's plot with highlighted stoichiometries ($c_{\rm (M}^+_{+2a)}$ =10 μ M). g) Schematic illustration of **2a**-cation complexes.

pound 3, which lacks a donor center (Table S1, in the Supporting Information). Therefore, the substituents in 1c-f induce only a limited fluorescence interval between the OFF and ON states and are not suitable as sensitive probes. The data from steady-state fluorescence measurements were corroborated by fluorescence lifetime ($\tau_{\rm F}$) results (Table 1,

Table 1. Properties of 1a-c, 2a-c, and 3 in THF.

-	$arPsi_{ m F}$	$\tau_{\rm F} [\rm ns]$	$arPhi_{\Delta}$	$\Phi_{\Delta} + \Phi_{F}$
1a	0.006	<0.5 (70%), 1.3 (30%)	0.11	0.12
1b	0.021	< 0.5 (80%), 1.7 (20%)	0.19	0.21
1c	0.135	1.54	0.62	0.76
2a	0.010	< 0.5 (73%), 1.4 (27%)	0.13	0.14
2b	0.029	< 0.5 (44%), 2.1 (56%)	0.26	0.29
2c	0.133	1.49	0.55	0.69
3 ^[a]	0.35	2.66	0.55	0.90

[a] Data from reference [8].

Table S1 in the Supporting Information). The fluorescence decay curves for 1c-f were monoexponential on a nanosecond timescale, which is typical of Zn-AzaPcs. The fluorescence lifetimes of 1a and 1b were shorter due to ultrafast ICT, and these decay curves were well characterized by a biexponential function.^[6] The singlet oxygen quantum yields (Φ_{Δ}) of **1a** and **1b**, which were employed as indicators of intersystem crossing, were also low (Table 1). The sum $\Phi_{\rm F}$ + Φ_{Λ} is less than 0.21, which is much less than the value for control compound 3 (i.e., 0.90), which does not contain a donor center. It confirms that efficient ICT occurs in compounds 1a and 1b (1a being more efficiently quenched). This result suggests that compounds similar to 1a and 1b may provide high signal-to-noise ratios in sensing applications. Therefore, three sensors, 2a-c, were prepared and investigated in cation binding studies. The $\Phi_{
m F}$ $au_{
m F}$ and Φ_{Δ} values obtained for the sensor molecules were in agreement with the results for the corresponding model compounds 1a-c (Table 1).

Both sodium and potassium cations exhibit a strong binding with aza[15]crown-5.^[9] The addition of NaSCN or KSCN to sensors **2a–c** in THF caused increases in both $\Phi_{\rm F}$ and $\tau_{\rm F}$ due to chelation of the recognition moiety (Table 2). The increase in fluorescence intensity can be attributed to the co-

Table 2. Photophysical and photochemical data of 2a-c in THF after complete binding of cation.^[11]

1	0		
	$oldsymbol{\Phi}_{ extsf{F}}^{[a]}$	$ au_{ m F} [m ns]^{[m a]}$	<i>K</i> _D [mм] ^[b]
2a+Na ⁺	0.115	0.8 (22%), 2.2 (78%)	1.03 (0.83)
2a+K+	0.105	0.8 (30%), 2.0 (70%)	
2b+Na+	0.210	1.1 (12%), 2.3 (88%)	0.38 (0.37)
2b+K+	0.205	1.1 (17%), 2.2 (83%)	
2c+Na ⁺	0.188	2.06	0.61 (0.77)
2c+K+	0.195	2.01	

[a] After the addition of NaSCN or KSCN in MeOH ($c_{(M^+)}=20 \text{ mM}$). The fluorescence lifetime for these compounds is fitted by biexponential function. The numbers in parentheses represent the fractional intensity of each decay time. [b] $c_{(AzaPc)}=1 \mu M$. K_D were determined from Φ_P values in parentheses were determined by global fitting of the absorption spectra. Std. error = $\pm 15 \%$.

ordination of the lone pair of the donor nitrogen by the cation, which blocks ICT. The fluorescence enhancement factor (FEF) reached values of 11, 7 and 1.5 for **2a–c**, respectively (Table S2 in Supporting Information). As predicted, compound **2a** had the highest signal-to-noise ratio, because it had the most efficient ICT. Compound **2c** is the least sensitive, which is in agreement with the analysis of model compound **1c**.^[10]

It has been noted that the cavity of [15]crown-5 is a good fit for the size of Na⁺, but is too small for K⁺. Therefore, K⁺ and the [15]crown-5 can form complexes with a 1:2 stoichiometry.^[5] Job's method of continuous variation revealed that this is the case for sensor molecule **2a**, which was chosen as a leading compound (Figure 1e, f). The results indicate that **2a** forms 1:1 complexes with Na⁺, but complexes with K⁺ in a 1:2 stoichiometry (Figure 1g).

Titration of sensors 2a-c (1 µM solution in THF) with NaSCN and KSCN (both in MeOH) resulted in small changes in the absorption spectra of these species (Figure 1 a, b). The notable change was the decrease in the intensity of the CT band at 480 nm, which is in agreement with the coordination of the metal cation to the donor center.^[11] The fluorescence intensity increased substantially after the addition of the cation solutions (Figure 1c, d and the Supporting Information). There was negligible change in the shape of the emission spectra for 2b and 2c; however, a small but significant blue shift was observed in both the emission and absorption spectra of 2a. This indicates that the oxygen atom in the butoxy moiety of 2a may also be involved in the coordination of the cation. Based on a nonlinear regression analysis of the fluorescence data, the dissociation constants $(K_{\rm D})$ were found to be in the range of hundreds of µM (Table 2), which is in line with previous data obtained for the complexation of the [15]crown-5 moiety to Na⁺.^[11,12] In addition, a global fitting of the absorption spectra yielded valid values for $K_{\rm D}$, which were of comparable magnitude to those obtained from the fluorescence assay (Table 2 and the Supporting Information). As mentioned above, the binding of cations may also be observed in changes to $\tau_{\rm E}$ which can be exploited in lifetime-based sensing techniques. As an example, the titration data for 2a exhibited very good response in the percentage of the slow component ($\tau_{\rm F}$ =2.1 ns) upon the addition of Na⁺ (see the Supporting Information).

Because the aza[15]crown-5 moiety is known to bind cations other than K⁺ and Na⁺,^[9,13] the change in the fluorescence intensity (expressed as FEF) of **2a** was also tested in the presence of other salts. The data from Figure 2 indicate that the inhibition of ICT occurring after binding to other cations is smaller. This is due to limited coordination to the donor center as reported, for example, for lithium.^[13] Some of the cations tested did not bind to the recognition moiety at all (e.g., Pb²⁺, Co²⁺, Hg²⁺), and the fluorescence of **2a** decreased after the addition of solutions containing their thiocyanates in methanol. A similar decrease in fluorescence intensity was observed after the addition of a corresponding volume of methanol only and is explained by a promotion



Figure 2. a) FEF of **2a** at complete binding for different cations used as SCN⁻ salts. The same for model compounds **1c** and **3** and Na⁺ ($c_{(AZBPC)} = 1 \mu M$). b) Photo of the solutions used for FEF calculations ($\lambda_{exc} = 366 \text{ nm}$).

of ICT in the more polar solvent mixture. Exclusive recognition of the cations by the aza[15]crown-5 moiety was confirmed by the limited interaction of model compound 1c or 3 with Na⁺ (Figure 2).

Azacrown-based sensors are limited by the basicity of their donor center,^[14] which precludes their use under even slightly acidic conditions because protonation of the donor switches on the fluorescence signal.^[13] However, the sensors examined in this study do not suffer any interference in an acidic environment, because of the very low basicity of their donor center.^[15] This fact and the transferability of the results obtained in THF to aqueous media were examined for sensor **2a**. In general, AzaPcs and Pcs are too hydrophobic to be used in water without losing their monomeric character and photoactivity. To overcome these limitations, compound **2a** was imbedded into porous silica nanoparticles (NPs) that were prepared using an oil-in-water microemul-



Figure 3. a) Incorporation of **2a** into silica NPs. b) Absorption spectra of **2a**@NPs in water and at pH 3 (0.5% acetic acid). c) Changes of fluorescence intensity of **2a**@NPs at 668 nm (pH 3, $c_{(AzaPc)}$ ca. 1 μ M) after the addition of NaSCN.

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Chem. Eur. J. **2013**, *00*, 0–0

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sion (Figure 3a, see Supporting Information for details).^[16] Compound **2a** remained in a monomeric form, as indicated by the absorption spectra in water (Figure 3b). The absorption spectra did not change, and the fluorescence intensity of **2a**@NPs actually decreased slightly at pH 3 (0.5% acetic acid in water) indicating that low pH has little influence on ICT in this system. The access of the analyte cations to **2a** in the NPs was preserved, and the addition of NaSCN led to a fivefold increase in the fluorescence intensity (Figure 3c and Supporting Information).

In conclusion, the concept of sensing metal cations using fluorescence changes in non-aggregating AzaPc molecules that exhibit significant ICT in the OFF state was demonstrated by binding Na⁺ and K⁺ to a aza[15]crown-5 recognition moiety. The data obtained in this study may be easily extrapolated to other ICT-based recognition moieties that are selective for different cations. It is important to note that this concept works not only in organic solvents, but also in water after the sensor molecules are incorporated into silica NPs. Aqueous sensing is typically extremely problematic for dyes based on hydrophobic AzaPc and Pc cores. The substantial advantages of AzaPcs over other types of sensors are the absorbance and emission in the red region of the visible spectrum and the presence of a very non-basic donor center that permits sensing in acidic conditions.

Acknowledgements

The work was financially supported by the Czech Science Foundation (P207/11/1200, P208/10/1678) and by Charles University in Prague (SVV 265 001). Authors thank to Antonín Cidlina for photodocumentation, Dr. Ivo Jakubec for HRTEM images of NPs, Dr. Václav Štengl for allowing us to use a DLS instrument, and Dr. Jiří Kuneš and Zdeněk Novák for NMR measurements.

Keywords: crown compounds • fluorescent probes phthalocyanines • potassium • sodium

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Received: January 9, 2013 Published online: ■ ■ , 0000

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Light up a red crown: Chelation of sodium and potassium cations by aza[15]crown-5 switches on strong red fluorescence in azaphthalocyanines. It is due to an inhibition of ultrafast intramolecular charge transfer by coordination of the cations to donor center. Sodium cations fit well into a cavity of the recognition moiety, while potassium forms supramolecular assemblies of azaphthalocyanines with 1:2 stoichiometry.



Crown Compounds

V. Novakova,* L. Lochman, I. Zajícová, K. Kopecky, M. Miletin, K. Lang, K. Kirakci,

Azaphthalocyanines: Red Fluorescent 🛛 💻 **Probes for Cations**

The concept of sensing metal cations...

... by using fluorescence changes in non-aggregating AzaPc molecules that exhibit significant intramolecular charge transfer (ICT) in the OFF state was demonstrated by binding Na⁺ and K⁺ to a aza[15]crown-5 recognition moiety. It is important to note that this concept works not only in organic solvents but also in water after the sensor molecules are incorporated into silica nanoparticles. For more details see the Communication by V. Novakova, P. Zimcik et al. on page ff.

