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Crown-annelated *p*-phenylenediamine derivatives as electrochemical and fluorescence responsive chemosensors: fluorescence studies

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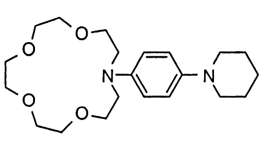
Abstract—Two *p*-phenylenediamine derivatives, having aza-crown ether substituents, are described which show high selectivity for Mg^{2+} over Na^+ in their fluorescence behavior. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Owing to its sensitivity to subtle changes in electronic structure of a molecule, and conformational change that may result from binding interactions between a fluorophore and a 'neutral' species, fluorescence spectroscopy is an important tool in the development of chemosensors.[1] The attachment of a suitable receptor, such as a crown ether, to a fluorophore has been widely used as a method for constructing organic molecules that can be used to detect a variety of analytes. Of particular interest in present day sensor research is the selective detection of metal cations. For example, detection of magnesium[2] or calcium[3] in the presence of sodium is important in biological studies because they play important roles as intracellular messengers in the regulation of cell function. Since these analytes generally have to be detected in the presence of a large (30-fold) excess of sodium in biological samples, it is important that the fluorescent chemosensor be able to bind more strongly to the metal dication, and also give a significantly different response to dication versus monocation. The accompanying papers describes the synthesis and some electrochemical studies on tetraalkyl-*p*-phenylenediamine (TAPD) systems in which one of the amine substituents is an aza-crown ether, thereby allowing complexation with metal cations. In addition to their electrochemical properties, TAPDs are also fluorophores. Consequently, we have investigated the effect of metal cation binding on the fluorescence spectra of these molecules, and we have found some interesting results that suggest these compounds may be useful as highly selective chemosensors.

2. Results and discussion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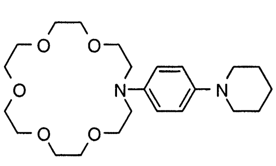
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Crown-annelated TAPD derivatives **1** and **2** were prepared as described in the preceding letter. Absorption spectra of each compound were recorded in acetonitrile over a range of concentration of added metal perchlorates (Li, Na, K, Mg, Ca, Ba), and the binding constants reported in Table 1 were obtained from this data

Table 1. Binding constants for Crown-TAPDs **1** and **2** with metal perchlorates in acetonitrile (M^{-1})[4]

	Li^+	Na^+	K^+	Mg^{2+}	Ca^{2+}	Ba^{2+}
1	1.17×10^4	9.64×10^3	1.22×10^3	9.19×10^5	2.95×10^6	1.96×10^5
2	7.70×10^3	2.60×10^4	6.55×10^4	2.11×10^6	1.51×10^7	5.29×10^7

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(blue shifts ranging from 3 to 8 nm were observed to result from complexation with the metal cations).

Fluorescence spectra for each TAPD, at a concentration of 5 μM, were recorded[5] in acetonitrile over a range of concentration of each metal perchlorate using an excitation wavelength of 265 nm, which is approximately at the isosbestic point for each series of absorption spectra. Typical spectra are presented in Fig. 1, showing the changes in fluorescence emission for TAPD **2** over a range of concentrations of $NaClO_4$ and $Mg(ClO_4)_2$. While the fluorescence quantum yield for these TAPDs is generally low (ca. 0.05), the marked difference in the effects of Na^+ versus Mg^{2+} is interesting, since it suggests a capability to selectively detect magnesium in the presence of sodium, especially given the much higher binding constant for magnesium (2.11×10^6 versus 2.60×10^4 for sodium).

Fig. 2 summarizes the fluorescence spectra at fully complexed crown ether for all the metal perchlorates recorded in this study. Alkali metal cations give very small blue shifts, with fluorescence enhancements that vary according to the metal cation, while alkaline earth metals show somewhat different effects for each TAPD. Thus, barium significantly reduces the fluorescence quantum yield of both **1** and **2**, with a large blue shift for **2**, while calcium gives a large blue shift with **1**, together with some intensity reduction, but only a quantum yield reduction with **2**. On the other hand, magnesium gives a pronounced blue shift with both **1** and **2**; the effect with **2** seems to be uniquely characteristic of magnesium. We have also tested the ability of **2** to allow detection of magnesium in the presence of sodium, by running spectra of mixtures of these two cations. Fig. 3 summarizes the results of this study (also included is the limiting spectrum for a 1:30 mixture of calcium perchlorate and sodium perchlorate).

These results are very promising in that the much greater binding constant for magnesium over sodium, coupled with the very different response for these two metal perchlorates, allows detection of magnesium in the presence of sodium. With equimolar amounts of these two metal cations, the effect for magnesium completely overrides that of sodium, whilst in the presence

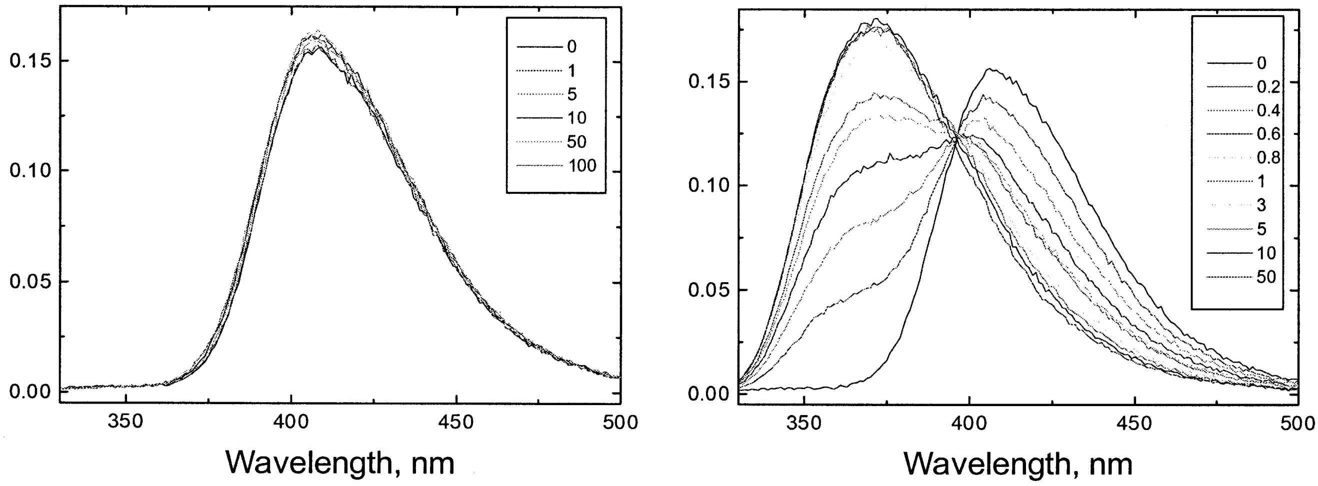

Figure 1. Fluorescence spectra for TAPD **2** over a range of concentrations of $NaClO_4$ and $Mg(ClO_4)_2$.

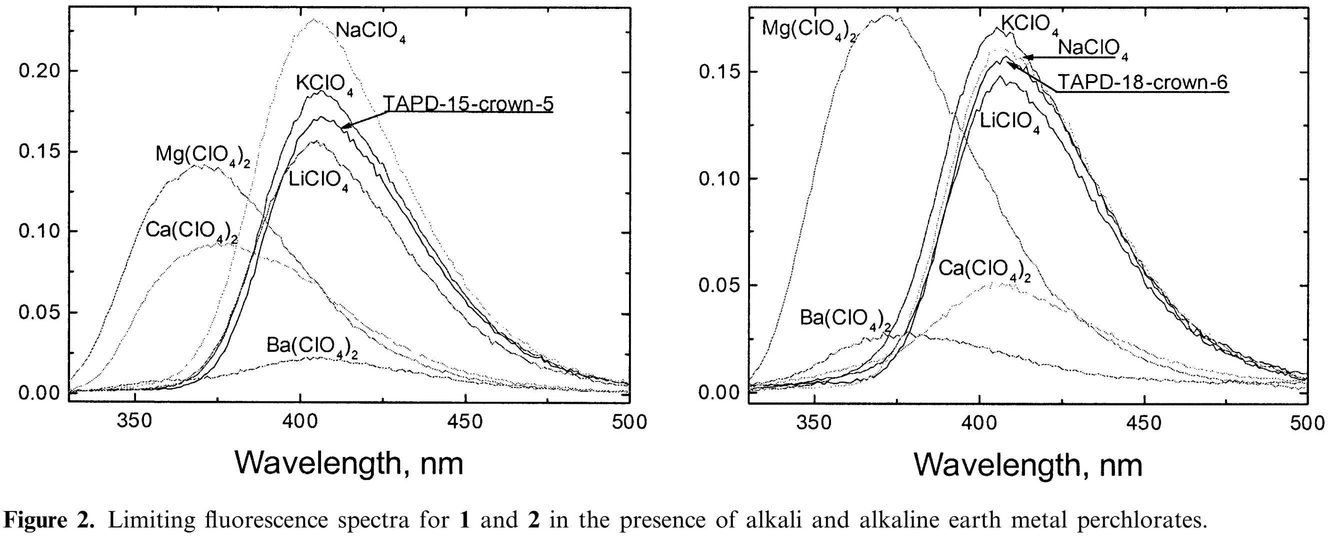

Figure 2. Limiting fluorescence spectra for **1** and **2** in the presence of alkali and alkaline earth metal perchlorates.

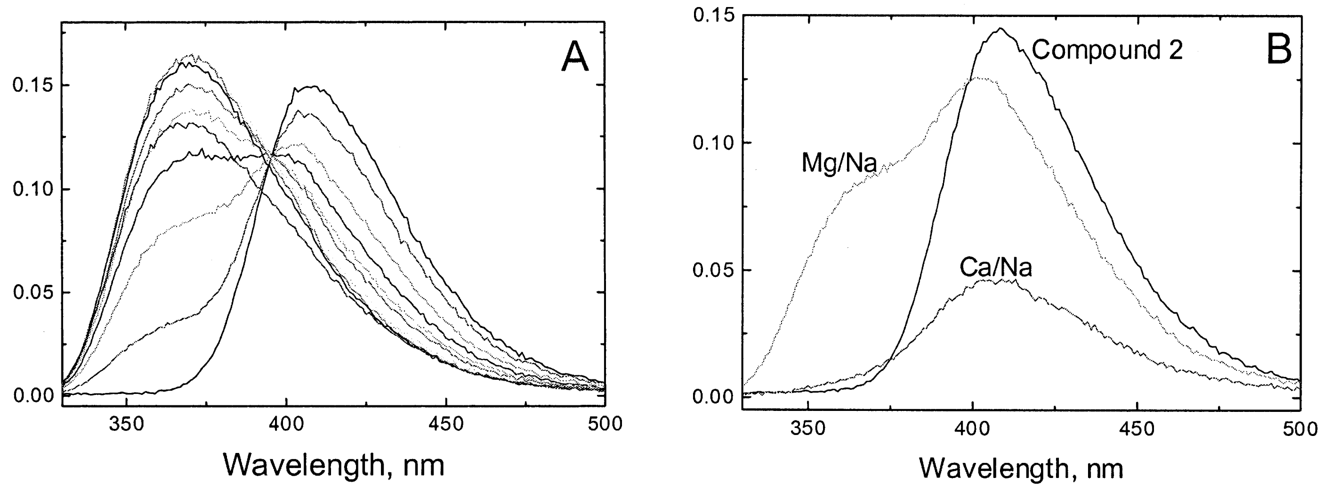

Figure 3. Fluorescence spectra: (**A**) TAPD **2** (5 μM) in the presence of mixtures of sodium perchlorate and magnesium perchlorate (1:1); concentrations (μM): 1.0, 2.0, 3.0, 4.0, 5.0, 15.0, 25.0, 50.0, 75.0. (**B**) Mixtures of sodium/magnesium perchlorates (30:1) and sodium/calcium perchlorates (30:1) at saturation (450 μM Na; 15 μM Mg and Ca).

of a 30-fold molar excess of sodium, the blue shift due to magnesium can be seen as a shoulder in the fluorescence spectrum. On the other hand, while it is apparent that calcium leads to the same level of quenching in the presence of excess sodium as it does in the absence of the monocation, the fact that there is very little shift in the emission maximum relative to free TAPD indicates that it would be much more difficult, though still possible, to use this compound as a selective chemosensor for calcium.

3. Conclusions

Crown-annelated TAPDs appear to be promising candidates as selective fluorescent chemosensors, especially for divalent cations such as magnesium. Future work in our laboratory will explore the full scope of these materials, as well as the development of chemosensors based on photoinduced electron transfer.[6] Moreover, it may be noted that this study has used acetonitrile as solvent, as is the case with numerous investigations of fluoresecent chemosensor systems reported in the literature. Our future investigations will also probe the fluorescent behavior of these TAPDs in protic solvents.

Acknowledgements

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References

1. For recent reviews, see: (a) de Silva, A. P.; Gunarante, H. Q. N.; Gunnlaugsson, T.; Huxley, A. L.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566. (b) Kaifer, A. E.; Echegoyen, L. In *Cation Binding by Macrocycles Complexation of Cation Species by Crown Ethers*; Inoue, Y.; Gokel, G. W., Eds.; Marcel Dekker: New York, 1990. (c) Valeur, B. In *Topics in Fluorescence Spectroscopy*, Vol. 4: *Probe Design and Chemical Sensing*; Lakowicz, J. R., Ed.; Plenum: New York, 1994; pp. 21–48. (d) Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3–40. For some recent examples, see: (e) Xia, W.-S.; Schmehl, R. H.; Li, C.-J. *J. Am. Chem. Soc.* **1999**, *121*, 5599–5600. (f) Xia, W.-S.; Schmehl, R. H.; Li, C.-J. *J. Am. Chem. Soc.* **1999**, *121*, 5599–5600. (g) Xia, W.-S.; Schmehl, R. H.; Li, C.-J. *Chem. Commun.* **2000**, 695–696. (h) Prodi, L.; Bargossi, C.; Monalti, M.; Zaccheroni, N.; Su, N.; Bradshaw, J. S.; Izatt, R. M.; Savage, P. B. *J. Am. Chem. Soc.* **2000**, *122*, 6769–6770. (i) Crawford, K. B.; Goldfinger, M. B.; Swager, T. M. *J. Am. Chem. Soc.* **1998**, *120*, 5187–5192. (j) Prodi, L.; Bargossi, C.; Monalti, M.; Zaccheroni, N.; Savage, P. B.; Bradshaw, J. S.; Izatt, R. M. *Tetrahedron Lett.* **1998**, *39*, 5451–5454. (k) Nakatsuji, Y.; Kita, K.; Inoue, H.; Zhang, W.; Kida, T.; Ikeda, I. *J. Am. Chem. Soc.* **2000**, *122*, 6307–6308.
2. (a) Prodi, L.; Ballardini, R.; Gandolfi, M. T.; Roversi, R. *J. Photochem. Photobiol. A* **2000**, *136*, 49–52; (b) Prodi, L.; Bolletta, F.; Montaldi, M.; Zaccheroni, N.; Savage, P. B.; Bradshaw, J. S.; Izatt, R. M. *Tetrahedron Lett.* **1998**, *39*, 5451–5454.
3. (a) Magrum, L. J.; Hickman, M. A.; Gietzen, D. W. *J. Neurophysiol.* **1999**, *81*, 1147–1149; (b) Oguz, U.; Akkaya, E. U. *Tetrahedron Lett.* **1997**, *38*, 4509–4512; (c) Akkaya, E. U.; Turkilmaz, S. *Tetrahedron Lett.* **1997**, *38*, 4513–4516.
4. Bursulaya, B. D. Protonation of coumarines in the ground and excited states. Ph.D. Dissertation, Moscow State University, 1993. 'Equili' is a global data analysis program based on several principles and assumptions: (a) activity coefficients of all chemical components of a system are equal to 1; (b) optical density is an additive function with all of the components obeying the Beer–Lambert law. The total number of components *n* is a sum of *m* basic components and their derivatives. In this case one can write an optical density of a system of *k* solutions at *l* different

wavelengths as a $k \times l$ matrix. This matrix relating to the Beer–Lambert law can be written as $\mathbf{D} = \mathbf{C} \cdot \mathbf{A} \cdot \mathbf{E}$, where $\mathbf{C}$ is a concentration $k \times n$ matrix, $\mathbf{A}$ is a diagonal amplitude $n \times n$ matrix, and $\mathbf{E}$ is a $n \times l$ matrix which consists of relative extinction coefficients. Evaluation of the thermodynamic and spectral parameters is performed by an iteration approximation of a calculated optical density matrix to the experimental one. This can be reached by minimization of a χ^2 function: $\chi^2(\Delta H, \Delta S, E, A) = \Sigma_{i=1}^{k} \Sigma_{j=1}^{l} (W_{ij}(\mathbf{D}_{ij} - \mathbf{D}_{ij}^{\mathrm{exp}}))^2$, where W_{ij} is a weight of each data point and is equal to $|W| = |W_{\mathrm{T}}||W_{\mathrm{C}}||W_{\mathrm{P}}||W_{\mathrm{L}}|$. W_{T}, W_{C}, W_{P}, W_{L} are weight of temperature, concentration, point and wavelength, respectively. Minimization of χ^2 function is achieved by a modified Newton method.

5. Experimental procedure for fluorescence measurements: Optical grade acetonitrile was purchased from Sigma and stored under argon. Absence of water in the solvent was confirmed by the lack of fluorescence signal around 320 nm while exciting at 270 nm. All metal perchlorates were dried under vacuum and stored in an evacuated desiccator. Solutions in acetonitrile were prepared by sequential dilution of a stock solution so that the absorbance of the TAPD at the maximum wavelength would be <0.1 (ca. $1–5 \times 10^{-6}$ M). Two stock solutions were made with equal concentrations of TAPD and one contained an additional 50–100 equivalents of perchlorate. Titration of the TAPD with different salt concentrations was performed in a 1 cm optical path quartz cuvette while maintaining a constant volume of 3 mL. Cuvettes were treated with conc. nitric acid in an ultrasonic bath and washed with distilled deionized water and optical grade methanol prior to use. Absorbance and fluorescence of each sample was measured. Fluorescence spectra were recorded in a ratio mode on a SLM-AMINCO model 8100 series 2 spectrofluorometer. Excitation and emission monochromators were set at 4 nm band-pass. Emission spectra were collected under magic angle conditions with excitation polarizer set at 54.7° and emission polarizer set at 0° to vertical. Background fluorescence from solvent blank was subtracted, and spectra were corrected for the instrument response using correction factors determined with a standard lamp from Optronics. Fluorescence spectra were corrected for differences in absorbencies at the excitation wavelength. A solution of quinine sulfate in 1.0N sulfuric acid was used as a fluorescence standard in order to measure quantum yield:

$$\Phi = \Phi_R \frac{I}{I_R} \frac{OD_R}{OD} \frac{n^2}{n_R^2},$$

where I is an integrated fluorescence intensity; OD is the absorbance of a sample at the excitation wavelength, and n is the refractive index, Φ of 0.542 was used for quinine sulfate.

6. Kubo, K.; Sakurai, T. *Heterocycles* **2000**, *52*, 945–976.