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Water soluble diaza crown ether derivative: Synthesis and barium complexation studies

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ABSTRACT

The combination of a mcrocyclic cavity with o-diaminobenzene derivative offers a new redox-active and water-soluble ligand for barium that incorporates 12 potential donors. The chelator was synthesized in four steps from diaza-18-crown-6-ether and fully characterized in solution by cyclic voltammetry, UV–Visible and NMR spectroscopies in the presence of barium. The corresponding complex exhibits a 1:1 stoichiometry in solution whereas it crystallizes as 3:2 (M:L) as evidenced by X-ray diffraction studies. The high affinity constant for barium estimated by NMR and electronic absorption techniques in aqueous medium, makes this ligand a promising candidate for redox-induced barium release.

1. Introduction

Calcium serves as a key signaling messenger in various biological processes such as intercellular communication [1,2]. The latter is made possible by the release of chemical messengers from an emitting cell to a target cell. For instance, the transmission in neurons is operated by neurotransmitters located in vesicles inside the emitting cell. The appropriate cell stimulation provoking a Ca^{2+} entry or increase, induces the docking of vesicles to the cell membrane followed by the fusion of the vesicle with the cell membrane and then the release and diffusion of the messenger in extracellular medium toward the neighboring cells [3]. This cascade of events known as vesicular exocytosis is biologically initiated by a significant increase in cytosolic Ca^{2+} concentration whose duration and concentration level are critical for the mechanism of vesicular fusion [4]. In vitro studies of exocytosis cascade require the stimulation of the emitting cells leading to an increase in intracellular free Ca^{2+} concentration. Among the various secretagogues *i.e.* stimulating agents that trigger exocytosis (Ca^{2+}/K^{+}, Ca^{2+}/nicotine, Ca^{2+}/digitonine, Ba^{2+}...), barium stimlation has retained our attention. Although not completely elucidated for all cells, it is firmly established that barium can efficiently substitute for Ca^{2+} and is able to induce secretion from a variety of cell types under Ca^{2+}-free conditions [5–7]. The protocol of stimulation with barium is very simple and consists in incubating the cell in a millimolar solution of Ba^{2+} in the absence of extracellular Ca^{2+}. However, Ba^{2+}-evoked release of neurotransmitters in such stimulation protocol is not spatially resolved as all regions of the cell are equally stimulated. Inspired by photo [8–10] and redox-induced [11,12] Ca^{2+} release strategies, we envisaged designing a selective redox-active barium chelator to deliver free barium as a secretagogue with high spatial and temporal resolution in extracellular medium. The oxidation or reduction of the redox reporter incorporated in the chelator provokes a charge and or/ a conformational modification that may affect sufficiently the binding ability of the ligand, thus favoring the release of a chelated ion.

The design of such selective chelator must fulfill strict requirements dictated primarily by biological constraints such as water solubility, physiological pH, and remarkable selectivity for barium over all other competing cations. Numerous exemples of selective barium ligands for sensing applications are reported in the litterature but most of them operate only in organic solvents [13,14]. The combination of macrocyclic cavities with redox-active units has been successfully exploited in organic solvents for sensing and redox-switchable applications. In particular, pioneering results with reducible subunits such as nitrobenzyl or quinone allowed the detailed description both theoretically and experimentally of the square-scheme mechansim that couples complexation and electron transfer reactions [14,15]. Oxidizable redox probes such as ferrocene, tetrathiafulvalene and phenylenediamine, were also combined to macrocycles for electrochemical cation sensing in organic solvents [16]. Few exemples of water-soluble chelating

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agents for Ba^{2+} based on crown ether motif and featuring phosphonate or carboxylate groups are known but they do not feature a redox probe [17,18]. Herein we describe the synthesis and characterization of a novel barium chelator combining diaza-18-crown-6 ether as the binding pocket, o-diaminobenzene as the redox probe and carboxylate side arms. Besides increasing the solubility in water, carboxylate groups, when ionized, provide strong electrostatic interaction with hard metals like alkali and alkaline earth cations. This new water soluble platform features 12 potential donors which corresponds to the higher coordination number encountered in barium coordination chemistry.

2. Material and methods

2.1. General considerations

All reactions were performed under argon if not stated otherwise and all chemicals were purchased from Sigma/Aldrich (France) or Alfa Aesar (France). Acetonitrile and DMF were distilled from CaH_2. ^1H and ^{13}C NMR spectra were recorded on Bruker AC-250 spectrometer in the solvents indicated at 298 K. Chemical shifts are reported using the deuterated (^{13}C NMR) or the residual monoprotonated (^1H NMR) solvent signals as reference. All coupling constants (*J* values) were measured in Hz. Elemental analysis were conducted at the Service de Microanalyses- ICSN (Gif sur Yvette, France). Thin layer chromatography (TLC) was performed on aluminium sheets precoated with 60 F_{254} silica gel. Preparative flash chromatography was performed on silica gel 60 (0.040–0.063 mm, Merck).

2.2. Electrochemistry

The electrochemical experiments were conducted in an air-tight three electrodes glass cell under argon atmosphere, controlled by a computer-controlled potentiostat (μ-Autolab, Eco-Chemie, the Netherlands). A large platinum wire and a saturated calomel electrode were used as a counter electrode and a reference electrode, respectively. A glassy carbon electrode with a disk radius of 0.5 mm, was used as working electrode and polished to a mirror finish before each experiment. Electrochemical studies in organic solvent were conducted in acetonitrile solutions of **3** (1 mM) containing either 0.1 M of $TBABF_4$ as a supporting electrolyte. The aqueous supporting electrolyte employed with the water soluble ligand **4** was prepared from ultra-pure water (<18 MΩ/cm) and contained 0.1 M $NaNO_3$, pH 7. Cyclic Voltammograms (CV) were recorded at room temperature (22 ± 2 °C) at a potential sweep rate of 0.1 V s^{-1}. Barium perchlorate was employed in organic electrolyte and barium chloride in aqueous electrolyte.

2.3. UV–Vis studies

All spectra were recorded on a computer-controlled Lambda 45-Perkin Elmer spectrophotometer. Spectroscopic measurements were performed in aqueous solutions at a constant ionic force set by 0.1 M $NaNO_3$, pH 7. UV–Vis studies for **4** (100 μM) were conducted by direct titration with sequential additions of a solution of $BaCl_2$ (or $CaCl_2$, $MgCl_2$, KCl) until full saturation is reached ([M]/[**4**] = 50 equivalents). After each concentration increment, the solution was stirred for 5 min to reach equilibrium before recording the UV–Vis spectrum. Data were analyzed at different wavelengths (263 and 239 nm) and the normalized absorbance $((A-A_0)/(A_0-A_{lim}))$ versus the ratio [M]/[**4**] plotted. Since the free cation concentration [M] could not be approximated by the total cation concentration c_M, the Benesi–Hildebrand model is not applicable. However, in the case of 1:1 complex stoichiometry, an explicit expression of the absorbance versus the total concentration c_M can be derived without approximation from the second order equation:

$$c_L.x^2 - (c_L + c_M + K_d).x + c_M = 0 \quad (1)$$

where: $x = \frac{A-A_0}{A_0-A_{lim}}$, A_0 (A_{lim}) is the absorbance at $c_M = 0$ (at full saturation respectively), A the absorbance at a given c_M, $c_M = [M]_{free} + [ML]$, $c_L = [L]_{free} + [ML]$ and K_d the dissociation constant. The explicit expression of the absorbance A versus c_M is a solution of the second order equation (Eq. 1). K_d can thus be obtained by a nonlinear least square minimization.

2.4. Determination of binding constant by ^1H NMR

For binding constant determination, ^1H NMR spectra were recorded in deuterated water at constant ionic force of 0.1 set by $NaNO_3$. Titration experiments were carried out by mixing the ligand solution in D_2O to a solution of barium chloride. Changes in the chemical shift of the methylenic protons adjacent to the carboxylate groups were exploited for the detrmination of binding constant. Data of $\Delta\delta$ (chemical shift variation) against $[Ba^{2+}]$ were fitted to a 1:1 binding model using origin 8.1 software (see Fig. S9 in the ESI).

$$\Delta\delta = \delta - \delta_0 = (\delta_{lim} - \delta_0).\frac{[ML]}{[L]_0}$$

Where $\Delta\delta$ is the measured change in chemical shift, δ_{lim} (and δ_0) is the chemical shift of the barium complex (and of the free ligand respectively). $[L]_0$ is the total concentration of ligand (free and complexed forms) and [ML] is the free barium concentration determined as a solution of the quadratic equation:

$$[ML]^2 + (-[L]_0 - [M]_0 - \frac{1}{K_a}) \cdot [ML] + [M]_0 \cdot [L]_0 = 0$$

Where $[M]_0$ is the pre-equilibrium barium concentration. Non linear curve-fitting of the experimental $\Delta\delta$ versus $[M]_0$ at a known $[L]_0$, yielded the parameters δ_{max}, K_a and [C].

2.5. Synthesis

2.5.1. Bis(2-nitrophenyl)-diazacrown ether **1**

(1,4,10,13)-Tetraoxa-7,16-diazacyclooctadecane (206 mg, 0.785 mmol), o-fluoronitrobenzene (175 μL, 1.65 mmol) and triethylamine (241 μL, 1.73 mmol) were dissolved in 2.5 mL of anhydrous DMF. The yellow solution was stirred for 6 h at 100 °C. The rsulting orange solution is concentrated under vacuum and diluted with dichloromethane and washed with aquous solution of $NaHCO_3$, water, then saturated solution of NaCl. The organic phase was dried on anhydrous Na_2SO_4, filtered then evaporated to dryness to give **1** yellow crystalline solid (696 mg, 90%).

^1H NMR (250 MHz, $CDCl_3$): δ 7.65 (dd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.2 Hz, 2H), 7.31–7.51 (m, 4H), 6.93–7.04 (m, 2H), 3.61 (t, $^3J_{HH}$ = 5.3 Hz, 8H, $OCH_2\underline{CH_2}N$), 3.55 (s, 8H, OCH_2CH_2O), 3.47 (t, $^3J_{HH}$ = 5.3 Hz, 8H, $O\underline{CH_2}CH_2N$). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 145.1 (C^{Ar}–NO_2), 136.6 (C^{Ar}–$N_{ethercrown}$), 125.4, 123.4, 117.7 and 115.2 (C^{Ar}–H), 70.5, 54.7 and 69.0 (CH_2O and CH_2N). Elemental *Anal.* Calc. for $C_{24}H_{32}N_4O_8$: C, 57.13; H, 6.39; N, 11.10. Found: C, 56.77; H, 6.54; N, 11.48%.

2.5.2. Bis(2-aminophenyl)-diazacrown ether **2**

1 (400 mg, 0.793 mmol) and Pd/C (10%, 75 mg) were placed in a nitrogen flushed schlenk flask and suspended in 7 mL of ethanol. A slow flow of H_2 was bubbled in the reaction mixture until the solu-