Oxoanion binding: a change of selectivity for porphyrin–alkaloid conjugates as a result of substitution pattern

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The selective interaction of two porphyrin–brucine quaternary salts with oxoanions in a methanol/aqueous environment is described. The influence of both *meta* and *para* substitution of the tetraphenylporphyrin core with four brucine units on anion binding selectivity is discussed. The interaction of cationic porphyrin–brucine conjugates with anions are based not only on an anion-exchange mechanism; differences between the porphyrins tested also revealed the influence of peripheral substitution on the final selectivity.

Introduction

The development of molecular sensors for detecting selectively chemically- and biologically-important anionic species has become a major research project in supramolecular chemistry. It has also been challenge to find and study receptors capable of selective anion sensing in aqueous media.[1]

The development of highly efficient systems capable of binding specific anions selectively is recognized as being a key predicate to solving a number of fundamentals, including anion sensing extraction[2] and separation;[3,4] oxoanions are of particular relevance in this regard.[5] Perhaps as a consequence of this, only a few systems capable of recognizing or extracting perrhenate or pertechnetate anions have been developed. These systems are based on the use of urea-functionalized dendrimers,[6] amino-azacryptands,[7] tetrasubstituted, lower rim-functionalized calix[4]arenes[8,9] and bimetallic cyclotriveratrylene hosts.[10] The design of modified cationic porphyrins as anion binders is based on electrostatic interactions accompanied by the formation of non-covalent hydrophobic and π–π complexes between the porphyrin core and the analyte, together with additional binding modes such as hydrogen bonding.[11]

In our group, we have devoted considerable effort to the design of porphyrin-based selectors of biologically-important species.[12,13] These functional conjugates consist of two parts, the tetraphenylporphyrin unit, which offers π–π and hydrophobic interactions, and allows them to be studied using absorption and fluorescence spectrometry because porphyrins are strong chromophores and fluorophores, and secondly brucine cation(s), which have previously shown the ability to selectively recognize anions.[14] As brucine is inherently chiral, the enantiodiscrimination of binaphthylcarboxylates has been described. Prepared porphyrin–tetrabrucine quaternary salts also have a remarkable behavior in a solution.[15,16] According to these results, they form chiral supramolecular nets and chiral polymers with various solvents.

Here, we report the interaction of two porphyrin–brucine quaternary salts with perrhenate and other anions in aqueous solutions, mainly in an anion-exchange manner. Among the radioisotopes used, technetium and rhenium complexes continue to be of great interest to the radiopharmaceutical industry.[17–19]

Suitable isotopes of these two metals are readily available. For clinical use, they are prepared from $^{99}Mo/^{99m}Tc$ and $^{188}W/^{188}Re$ generators. In both cases, the radionuclides are present in their oxoanion forms, TcO_4^- and ReO_4^-, respectively.[20] This is done in medical centers immediately prior to use.[21] Radiopharmaceutical preparation thus requires the reaction of pertechnetate or perrhenate with a reducing agent (typically $SnCl_2$) in the presence of an external ligand, or with a species capable of acting as both a reducing and chelating agent (*e.g.* heterofunctionalized phosphines).[22] The actual ligand environment is chosen to meet a range of important requirements, including those dictated by pharmokinetics and a desire to effect site-selective targeting. This, in turn, requires a suitable level of lipophilicity (important for optimizing the pharmokinetics) and the presence of a functionality that permits attachment to biomolecules for site-specific localization.[23]

The direct complexation of Tc and Re anionic forms represent an alternative approach for their transportation to a target. The ubiquity and importance of oxoanions imparts an urgency to the design of receptors that can bind such species with selectivity. Although considerable progress has been made in recent years, the generation of systems that show selectivity remains a challenge. This is particularly true for species such as perrhenate and pertechnetate anions, for which the enthalpic contributions to binding are rather small.[24]

Results and discussion

Porphyrin derivatives **1–3** were used in this study. All of them are quaternary salts based on the alkylation of different tertiary amines with 5,10,15,20-tetrakis(bromomethylphenyl)porhyrine, where the bromomethyl group is in position

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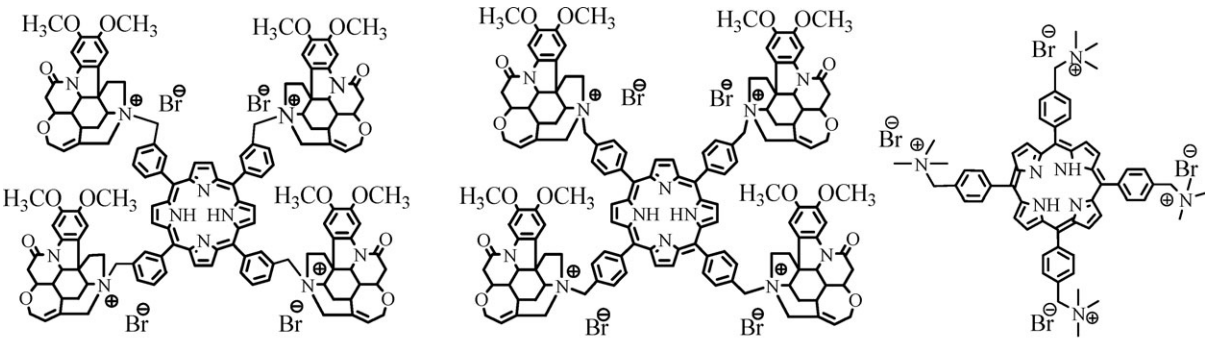

1 2 3

3′ or 4′ on the phenyl periphery. Derivatives **1** and **2** were prepared by the alkylation of alkaloid brucine;[15] derivative **3** was prepared by the alkylation of trimethylamine.[25] We used a methanol–water (1 : 1, v/v) mixture in all cases of our anion-exchange studies as gel formation in pure methanol and partial aggregation in pure water were revealed in previous studies[15,26]

Beside perrhenate, other anions (*i.e.* sulfate, perchlorate, dihydrogenphosphate and nitrite) were involved in screening interactions with **1–3** in methanol–water mixtures by monitoring the absorption of the Soret band (418 nm for **1**, 415 nm for **2** and 415 nm for **3**) of the three free porphyrins and of their mixtures with anions. Halogen anions weren't included in the study because **1–3** have been used in tetra-bromide and halide–halide anion-exchange and caused no change in the absorption spectra of **1** and **2** (data not shown). Ratios of the absorbance of mixtures of the porphyrins with anions (A_i) and the absorbance of solutions of the porphyrins (A_0) at the same porphyrin concentration are shown in Fig. 1.

Both **1** and **2** interact with perrhenate as the absorbances of the Soret band drop down to about 40% of their original value. While **1** interacts only with perrhenate, **2** also interacts with sulfate and slightly with the other anions. The ratio A_i/A_0 remains 1 : 1 after the addition of all the anions to **3**. According to this, a pure electrostatic interaction between the porphyrin tetracations and the anions studied is not enough for anion-exchange of the bromides originally present in **1–3** and to distinguish between the different anions. It also confirms the indispensability of the brucine moieties of **1** and **2** for supramolecular complexes based on anion recognition (see below), *i.e.* pure anion-exchange is not enough to describe the process observed; further donor–acceptor interactions must also be considered.

The interaction mode and stoichiometry of the complexes were studied by absorption and fluorescence spectrometry, and electronic circular dichroism (ECD) was also applied because of the inherent chirality of **1** and **2**, which are able to form chiral supramolecular polymers in solution.[16]

Stock solutions of the porphyrin derivatives were prepared in methanol–water (1 : 1, v/v) mixtures. A given stock solution was also used for the dissolution of the oxoanions so the concentration of the porphyrin derivatives remained constant during the course of titrations. Individual spectra were measured after the addition of the dissolved oxoanion.

Interaction of 1 with perrhenate

In the absorption spectra, the addition of perrhenate to **1** resulted in the Soret band decreasing (418 nm) and

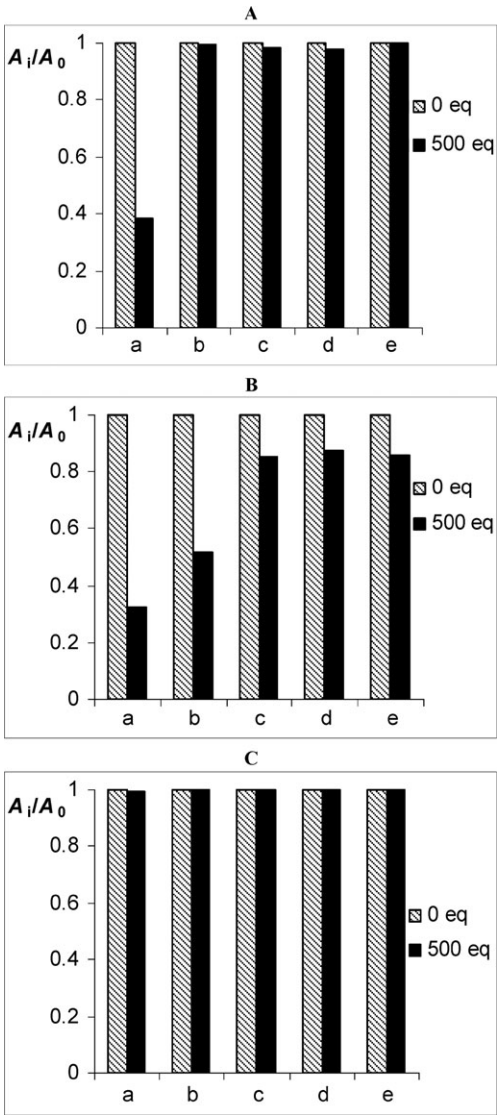

Fig. 1 Relative absorbances of the Soret band of (A) **1**, (B) **2** and (C) **3** without (0 equiv.) and with 500 (equiv.) the following anions (a) ReO_4^- (b) SO_4^{2-} (c) ClO_4^- (d) NO_2^- (e) $H_2PO_4^-$.

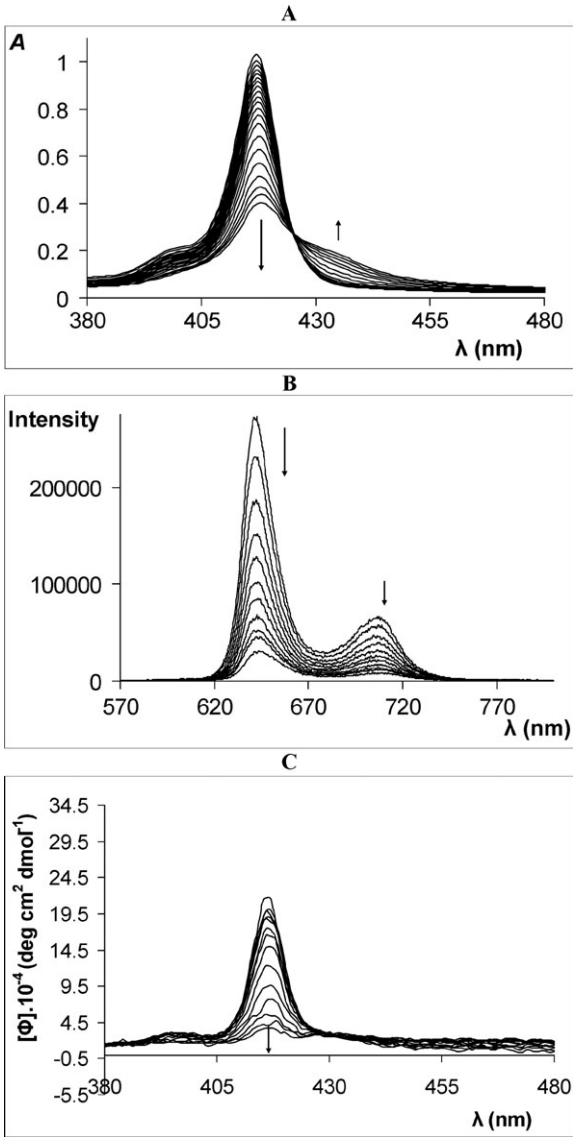

Fig. 2 (A) Absorption, (B) fluorescence emission (λ_{ex} = 418 nm) and (C) ECD spectra of **1** after the addition of 0–500 equiv. of $KReO_4$; ([**1**] = 2.4 μmol L^{-1} for the absorption and ECD spectra; [**1**] = 1.8 μmol L^{-1} for the fluorescence spectra.

simultaneously in the slight increasing of a new band at about 435 nm (Fig. 2A). According to the literature,[25,27–29] the former wavelength can be tentatively attributed to the monomeric form of H-aggregates and the latter to the monomeric aggregated form of **1**. On the contrast to high selectivity, the broad band at 435 nm indicates higher aggregates.[26] Fluorescence emission spectra of **1** after the addition of perrhenate are shown in Fig. 2B. As can be seen, the intensity of the fluorescence at 643 and 708 nm decreased with increasing amounts of added salt; radiative de-excitation was suppressed in the aggregates. Generally, quenching occurs in systems where the charge transfer state is thought to be below the fluorescence state as a result of strong local electric fields.[30] Even though the tetraphenylporphyrin core isn't chiral, the inherent chirality of **1** can be observed in the Soret band wavelength of the ECD spectra (Fig. 2C). Like in the absorption spectra, the addition of perrhenate causes a decreasing ellipticity at this wavelength. This indicates the formation of aggregates without supramolecular chirality. Had supramolecular chirality existed, it would have been demonstrated by an increasing of the ECD band intensity.[15] All of these experiments revealed complexation between **1** and perrhenate based on "monomers" of **1** surrounded by perrhenate based on an anion-exchange process.

Quantifying the interactions based on the calculation of conditional constant K_i using Letagrop Spefo software was rather complicated. Compound **1** was used as its bromide salt, so anion exchange

$$\mathbf{1}^{4+}\text{–}(Br^-)_4 + n ReO_4^- = \mathbf{1}^{4+}\text{–}(Br^-)_{4-n}\text{–}(ReO_4^-)_n + n Br^-,$$

where $\mathbf{1}^{4+}$–$(Br^-)_4$ is the expanded notation of **1**, has to be assumed. Analysis of the absorption data measured (Fig. 3A–C) indicates the exchange of three or four bromides for perrhenate anions (Fig. 3B and C), with a conditional stability constant in the 10^9–10^{12} order of magnitude. At very low concentrations of **1** (for fluorescence titrations [**1**] = 1.8 μmol L^{-1}), lower stoichiometries (*i.e.* 1 : 2; Fig. 3D) have to be considered.

Interaction of 2 with perrhenate and sulfate

The screening of interactions of porphyrins with anions showed the lower selectivity of **2** compared to **1**. The recorded data, on the other hand, were of great interest in this case.

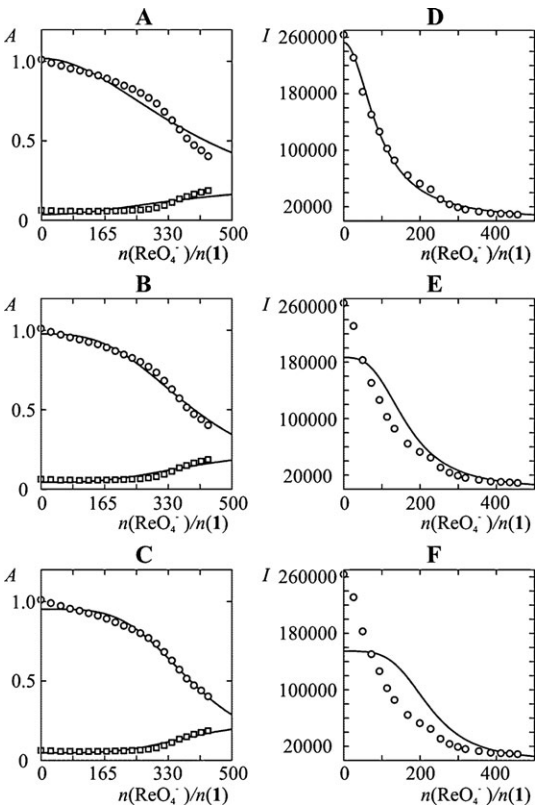

Fig. 3 Changes in absorbance (A–C) at 418 nm (○) and 435 nm (□), and fluorescence intensity (D–F) at 643 nm (○) upon the addition of ReO_4^- to **1**. Stoichiometry of porphyrin–anion fits (—): A, D—1 : 2; B, E—1 : 3; C, F—1 : 4.

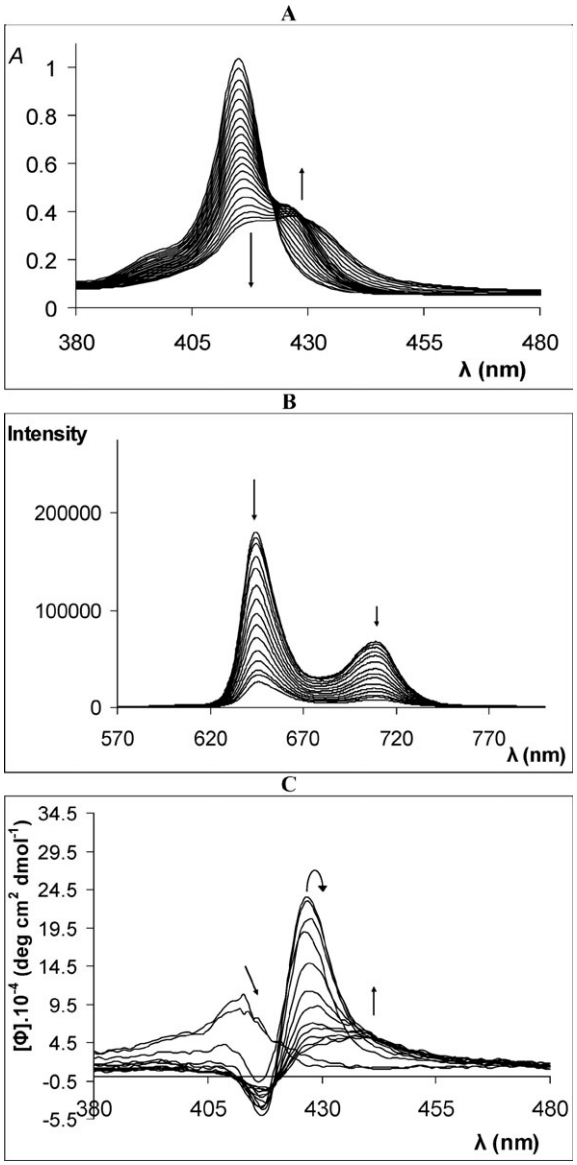

Fig. 4 (A) Absorption, (B) fluorescence emission and (C) ECD spectra of **2** after the addition of 0–500 equiv. of $KReO_4$ ([**2**] = 2.4 μmol L^{-1} for the absorption and ECD spectra; [**2**] = 2.0 μmol L^{-1} for the fluorescence spectra).

The absorption, fluorescence and ECD spectra measured after individual additions of perrhenate to **2** are shown in Fig. 4. Besides the absorbance changes at 415 and 425 nm, like after the addition of perrhenate to **1**, another new band at about 435 nm was observed after larger amounts of perrhenate had been added. Together with their bandwidths and a comparison with the band at 435 nm in the previous case of **1**, two types of aggregate (the first at 425 nm and the second at 435 nm) are gradually suggested to form following the addition of perrhenate to **2**. The intensities of fluorescence emission at 644.5 and 710 nm decrease more slowly that in the case of **1**. A rather complicated situation, completely different from that of **1**, can be observed in the ECD spectra. The addition of perrhenate to **2** causes a change of sign of the band at 413 nm and its shift to 416 nm. Simultaneously, a band at 426 nm arises, and the process of spectrum development is completed by the band at 433 nm. The reasons for the different results of the interaction of **1** and **2** with perrhenate can be explained by supramolecular complexation rather than a simple anion-exchange mechanism. The latter is only part of the entire process.

The changes in absorption (Fig. 5A), fluorescence (Fig. 5B) and ECD (Fig. 5B and C) spectra can also be observed after the addition of sulfate to **2**.

Significant changes to the ECD spectrum of **2** after the addition of perrhenate and sulfate indicate a conformational change of **2** during the ion-exchange. The existence of a supramolecular polymer based on J-aggregates[27] formed by **2** surrounded by sulfates is indicated by the strong increase in ellipticity of the new band at 425 nm in the ECD spectrum after sulfate addition; the bidentate anion can behave like an paperclip between adjacent porphyrins.

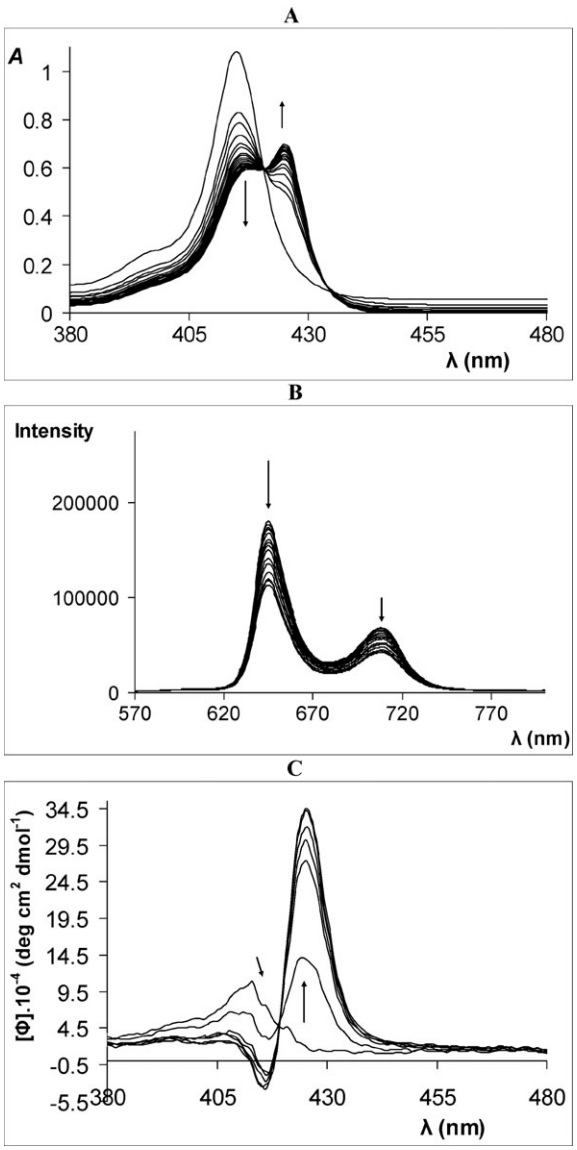

Fig. 5 (A) Absorption, (B) fluorescence emission and (C) ECD spectra of **2** after the additions of 0–500 equiv. of Na_2SO_4 [**2**] = 2.4 μmol L^{-1} for the absorption and ECD spectra; [**2**] = 2.0 μmol L^{-1} for the fluorescence spectra).

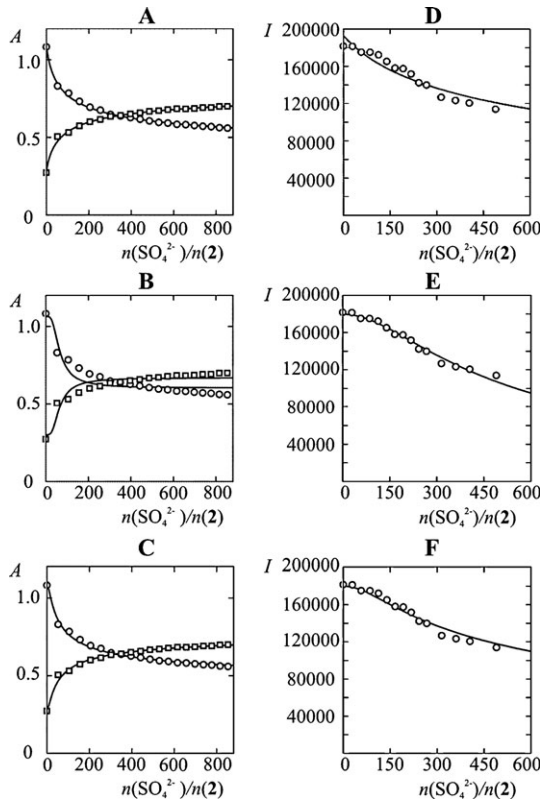

Fig. 6 Changes in absorbance at 415 nm (○) and 425 nm (□), and fluorescence intensity (D–F) at 644.5 nm (○) upon the addition of sulfate to **2**. Stoichiometry of porphyrin–anion fits (—): A, D—2 : 1; B, E—2 : 2; C, F—4 : 2.

Higher stoichiometry porphyrin–sulfate complexes are evident from fits of the experimental data in Fig. 6. Data models of monoporphyrin complexes with sulfate failed in all cases. On the contrary, supramolecular complexes between two or more porphyrins connected to two or more sulfates seem quite probable.

Conclusions

According to our screening, including two porphyrin–brucine quaternary salts and common inorganic anions, the selectivity of *meta*-substituted derivative **1** toward perrhenate was revealed. A comparison with results obtained for *para*-substituted derivative **2** showed that the lower conformational flexibility of **1** could be responsible for that selectivity. The selectivity is probably based on a complex multicomponent process leading to the formation of aggregates (J-type) based on the alternation of anions and porphyrin derivatives. As cationic porphyrins are known to be very efficient binders to proteins and DNA, our efforts to develop a selective transporter of a toxic anionic form of Re seems to be promising. Further such experiments are currently under way.

Experimental

Materials

Water (de-ionized water prepared by ionex (R = 10 MΩ)), methanol (99.5%, PENTA), sodium sulfate (99.8%), sodium perchlorate (98%, Lachema), sodium nitrite (99.8%, Lachema), sodium dihydrogen phosphate (98%, Lachema) and potassium perrhenate (99%, Aldrich) were used.

Porphyrin derivatives **1–3** used here were prepared by procedures described elsewhere.[15,25]

Titration with anions

A water–methanol (1 : 1, v/v) mixture was freshly prepared and de-gassed; it was used as the solvent for all experiments. Concentrated solutions of **1–3** ([**1**] = 39.8 mmol L^{-1}, [**2**] = 40.6 mmol L^{-1} and [**3**] = 90.0 mmol L^{-1} in DMSO) were prepared and diluted to obtain stock solutions containing 2.4 μmol L^{-1} of **1–3** for UV-vis and ECD titrations, and 1.8 μmol L^{-1} of **1** and **2** for fluorescence titrations. All titrations were made at a fixed concentration of **1–3** by dissolving the appropriate amount of anion. Titration curves were measured after the addition of an anion solution into a stock solution of **1–3** in a measuring cell (a quartz cuvette). Individual additions were realized in molar ratios ranging from (porphyrin : anion) 1 : 25 to 1 : 500.

UV-vis spectrometry

A Cary-400 UV-Vis spectrophotometer equipped with a 1 cm path length quartz cell was used to record absorbances between 300 and 800 nm.

ECD spectrometry

ECD spectra were recorded by a J-850 ECD spectrometer (Jasco, Japan) over the spectral range 300–800 nm (Soret region). The optical path length of the cuvettes used was 1 cm.

Fluorescence spectrometry

Fluorescence emission spectra were measured using a Fluoro-Max-2 fluorescence spectrophotometer equipped with a 1 cm path length quartz cell. Emission spectra were measured using a 418 nm (**1**) and 414 nm (**2**) emission wavelength at 570–800 nm.

Letagrop Spefo

We used the program LETAGROP by Sillén and Warnquist,[31] which optimizes the equilibrium constants using the experimental dependence of the estimated K.

$$m\mathrm{HBr_4} + n\mathrm{G} = m\mathrm{HBr_{4-}}_n\mathrm{G}_n + n\mathrm{Br}\ K$$
$$= [\mathrm{HBr_{4-}}_n\mathrm{G}_n]^m[\mathrm{Br}]^n/[\mathrm{HBr_4}]^m[\mathrm{G}]^n$$

where H is a host (**1** or **2**), Br is bromide and G is a guest (perrhenate of sulfate).

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