Cyclobis(paraquat-*p*-phenylene) as a Synthetic Receptor for Electron-Rich Aromatic Compounds: Electrochemical and Spectroscopic Studies of Neurotransmitter Binding

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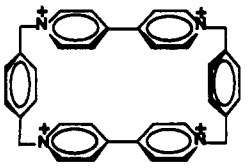
Abstract: The equilibrium binding constants of the charge-transfer complexes formed by the receptor cyclobis(paraquat-*p*-phenylene) with four neurotransmitters (dopamine, epinephrine, norepinephrine, and serotonin) and the related aromatic compounds indole and catechol were measured in aqueous media using a spectrophotometric method. The values obtained were in the range 1000–7500 M^{-1}. NMR studies confirmed that the surveyed π-donor guests are included inside the paraquat-lined cavity of the title receptor. Electrochemical studies of the receptor incorporated into thin Nafion films (~200 nm) cast on the working electrode surface suggested that charge propagation across these films is relatively slow, but the receptor is strongly retained inside the Nafion matrix. This fact was utilized to investigate, using voltammetric techniques, the binding of catechol and indole inside the Nafion polyelectrolyte film. Furthermore, thicker Nafion membranes (0.180 mm) preloaded with the receptor extracted the neurotransmitters, as well as catechol and indole, from aqueous solution, giving rise to charge-transfer absorbances linearly related to the guest concentration in the solution. Overall, the behavior of the title species qualifies it as the first redox-switchable molecular receptor.

Introduction

Cyclobis(paraquat-*p*-phenylene) (1^{4+}) is a novel synthetic receptor which has been used by one of the authors to prepare a variety of self-assembled rotaxanes and catenanes.[1] In these fascinating and complex structures, 1^{4+} acts as a π-acceptor subunit generating charge-transfer interactions with electron-rich groups that direct the self-assembly, threading, and linking of the various molecular components. Recently, we reported that 1^{4+} forms inclusion complexes with amino acids having electron-rich aromatic groups, such as tryptophan, tyrosine, and phenylalanine.[2] K_S values as high as 1000 M^{-1} were found with tryptophan in pH 7 aqueous phosphate buffer. The remarkable stability of these complexes results from the well-formed cavity of 1^{4+}, which is lined with two electron-acceptor paraquat groups separated by about 3.7 Å, a distance which perfectly matches the van der Waals thickness of an aromatic ring. Therefore, the cavity of 1^{4+} is ideally designed for the inclusion of electron-rich aromatic subunits driven by charge-transfer interactions.



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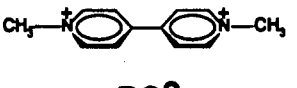


PQ2+

Receptor 1^{4+} shows reversible redox properties characteristic of the paraquat (1,1′-dimethyl-4,4′-bipyridinediium or methylviologen) subunit. These reversible redox processes are presented by the following equations:[3]

$$PQ^{2+} + e^- \rightleftharpoons PQ^+ \quad (1)$$

$$PQ^+ + e^- \rightleftharpoons PQ \quad (2)$$

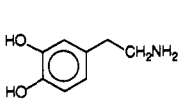
where PQ^{2+} represents the paraquat residue. Both of the two equivalent paraquat groups in 1^{4+} undergo electron transfer at similar potentials. Thus, a cyclic voltammogram of 1^{4+} in acetonitrile shows only two reversible reduction processes corresponding to the following 2-electron reactions:[1d]

$$1^{4+} + 2e^- \rightleftharpoons 1^{2+} \quad (3)$$

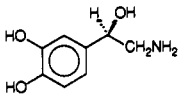
$$1^{2+} + 2e^- \rightleftharpoons 1 \quad (4)$$

Interestingly, reduction of 1^{4+} to 1^{2+} should decrease substantially the stability of the inclusion complexes formed with π-donor compounds because the electron uptake diminishes the π-acceptor character of the paraquat residues. Therefore 1^{4+} is expected to be a member of the family of receptors whose binding strength can be controlled via redox or electrochemical conversions, i.e., the so-called *redox-switchable ligands*.[4] The literature contains numerous examples of this class of ligands. However, to the best of our knowledge, all the reported examples of redox-switchable ligands are cation binders. Cyclophane 1^{4+} has the necessary structural features to become the first redox-switchable molecular receptor.

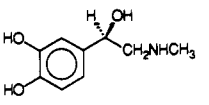
In this paper, we describe spectroscopic and electrochemical studies on the binding interactions of 1^{4+} with the following series of compounds:



Dopamine



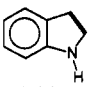
Norepinephrine



Epinephrine

Catechol

Serotonin



Indole

[†] University of Miami.
[‡] The University of Birmingham.

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The series includes three catechol-based neurotransmitters (dopamine, epinephrine, and norepinephrine) and a fourth neurotransmitter, serotonin. In addition, catechol and indole were used as model compounds since their structures resemble the electron-rich aromatic portions of the neurotransmitters responsible for the charge-transfer interactions which are at the core of the binding processes.

Experimental Section

Materials. Racemic norepinephrine was purchased from Aldrich as the hydrochloride salt. Racemic epinephrine (Aldrich) was obtained in its free-base form. Serotonin and dopamine hydrochlorides were purchased from Fluka. Catechol and indole were also commercial products (Aldrich). The cyclophane receptor $\mathbf{1}^{4+}$ was synthesized and isolated as its tetrakis(hexafluorophosphate) salt according to the reported procedure.[1a] The water-soluble tetrachloride salt was obtained by counterion exchange with tetraethylammonium chloride in nitromethane and recrystallized from methanol. Nafion (EW = 1100) was obtained from Aldrich either in solution form (5% in alcohols of low molecular weight) or as a solid membrane (thickness = 0.007 in.). All other chemicals were of analytical grade.

Aqueous solutions were always freshly prepared with distilled water further purified with a Barnstead Nanopure four-cartridge system. All solutions were thoroughly deoxygenated by sparging with nitrogen gas before use and maintained under a nitrogen atmosphere during the experiments. Neurotransmitter solutions were used immediately after preparation and protected from ambient light at all times.

Equipment. 400-MHz ^{1}H NMR spectra were recorded in a Varian VXR-400 spectrometer. Electronic absorption spectra were recorded with a Hewlett Packard 8452A spectrophotometer. Cyclic voltammograms were obtained with a Princeton Applied Research Model 173 universal programmer, a Model 175 potentiostat, and a Model 179 digital coulometer equipped with positive feedback circuitry for *IR* compensation and recorded with a Soltec VP-6423S X-Y recorder. Differential pulse voltammograms were obtained with a BAS-100 electrochemical analyzer. Glassy carbon working electrodes (projected area = 0.08 cm^2) were supplied by Bioanalytical Systems (West Lafayette, IN). The counter electrode was a Pt flag. A sodium chloride saturated calomel electrode (SSCE) built in our laboratory was used as the reference electrode.

Determination of Binding Constants. The complexation of neurotransmitters by host $\mathbf{1}^{4+}$ was monitored by measuring the absorbance of the visible charge-transfer band that developed upon mixing of the two components. In order to determine the binding constants, a 0.2–0.3 mM solution of $\mathbf{1}^{4+}$ in 0.3 M phosphate buffer (pH = 7) was spectrophotometrically titrated with aliquots from a 20–25 mM stock solution of the neurotransmitter also containing 0.2–0.3 mM $\mathbf{1}^{4+}$ in order to keep the concentration of the receptor constant during the titration. Aliquot addition was continued until a 50-fold excess of the neurotransmitter was achieved. Corrections for the neurotransmitter and receptor absorbances at the wavelength of charge-transfer absorption were made. The binding constants were obtained by computer fitting of the experimental absorbance values to the following equations:[5]

$$\frac{\Delta A}{b} = \frac{L_t K(\Delta\epsilon)[G]}{1 + K[G]} \qquad (5)$$

where ΔA is the absorbance of the charge-transfer complex measured at a receptor concentration L_t and a guest concentration [G], b is the optical path length, $\Delta\epsilon$ is the molar absorptivity of the charge-transfer complex, and K is the equilibrium constant for the formation of the complex. The experimental data points were consistent with 1:1 stoichiometry for all the complexes. A typical example of the fit to the experimental data is provided in Figure 1, showing the corrected absorbance data points during the spectrophotometric titration of $\mathbf{1}^{4+}$ with dopamine and the best line fitted to the data using K = 1006 M^{-1} and $\Delta\epsilon$ = 326 M^{-1} cm^{-1}. Similar good-quality fits were obtained with all the neurotransmitters. Reported binding constant values were calculated as the average of at least two independent determinations.

Electrochemical Experiments. The electrochemistry of $\mathbf{1}^{4+}$ was studied inside an anionic polyelectrolyte matrix, since the reduced forms $\mathbf{1}^{2+}$ and **1** precipitate in homogeneous aqueous media, giving rise to complicated voltammetric behavior.[6] The modification of glassy carbon electrodes with the anionic polyelectrolyte Nafion was performed using well-known

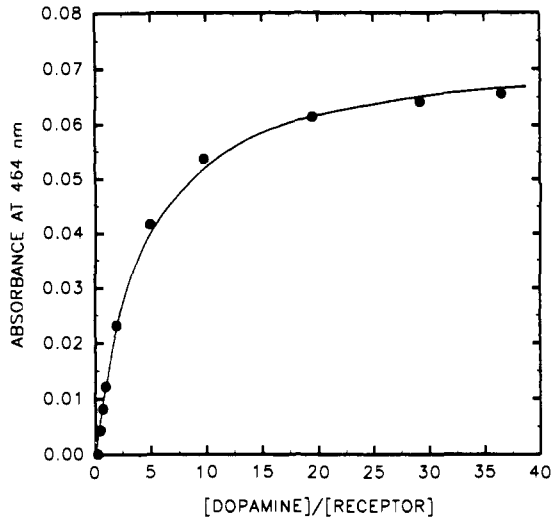

Figure 1. ΔA vs [dopamine]/[$\mathbf{1}^{4+}$]. The concentration of $\mathbf{1}^{4+}$ was kept constant at 0.23 mM. All solutions contain 0.3 M phosphate buffer (pH = 7). Optical pathway = 1.0 cm. The continuous line was calculated for K = 1006 M^{-1} and $\Delta\epsilon$ = 326 M^{-1} cm^{-1}.

casting procedures.[7] The original 5% Nafion solution—as obtained from Aldrich—was diluted 50-fold with absolute ethanol to yield a solution containing approximately 9 × 10^{-4} equiv/L. A 10-μL aliquot of this solution was syringed and carefully spread on the tip of a previously polished glassy carbon electrode. The solvent was evaporated in air at room temperature while the electrode was rotated head-up at 300 rpm. This procedure yielded reproducible Nafion coatings with an approximate wet thickness of 200 nm as estimated from the wet density of 1100 EW Na^+-form Nafion (1.58 g/cm^3).[8] Incorporation of the tetracationic cyclophane in the Nafion film was accomplished by immersing the Nafion-coated electrode in a deoxygenated 70 μM solution of **(1)**Cl$_4$ in 0.1 M phosphate buffer (pH = 7) for 45 min. The nominal surface coverage of these electrodes was ~2.9 × 10^{-8} equiv of Nafion/cm^2. After the loading of $\mathbf{1}^{4+}$ was finished (as assessed by cyclic voltammetry), the modified electrode was transferred to pure buffer solution. Integration of the cathodic wave at slow scan rates yielded an approximate surface coverage of (2.7 ± 0.5) × 10^{-9} mol/cm^2 of $\mathbf{1}^{4+}$. This is equivalent to a concentration of 0.14 ± 0.05 M of $\mathbf{1}^{4+}$ inside the Nafion film, which represents about 40% of its maximum cation-exchanging capacity.

Once the Nafion-modified electrodes were loaded with bis(paraquat) cyclophane [we will refer to these electrodes as GC/Nafion($\mathbf{1}^{4+}$) in the remainder of this work], the electrodes could be transferred to a pure supporting electrolyte solution without any detectable loss of $\mathbf{1}^{4+}$ electroactivity during periods as long as several hours. Electrochemical binding studies were performed by comparing the voltammetric behavior of GC/Nafion($\mathbf{1}^{4+}$) electrodes contacting neurotransmitter solutions with that observed for the same electrodes in pure supporting electrolyte solution.

Detection of Charge-Transfer Complexes inside Thick Nafion Membranes. The 0.007-in.-thick Nafion membrane obtained from Aldrich was first cut into small 1 × 3 cm strips. These were cleaned and conditioned by boiling in concentrated HNO_3 for 1 h and then rinsed with copious amounts of distilled water. The Nafion strips were immersed overnight in pure water and then in buffer solutions (pH = 7) for approximately 24 h. The strips were loaded with $\mathbf{1}^{4+}$ by stirring them in a 1–2 mM solution of this compound for 24 h. After this treatment, spectrophotometric analysis of the hydrated strips verified the incorporation of the bis(paraquat) derivative by the observation of the absorption band characteristic of the 4,4′-bipyridinediium moiety at 260 nm. The

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(6) Under conditions of aqueous voltammetry, the reduced forms of hydrophobic viologens are known to precipitate on the electrode surface. For example, see: Diaz, A.; Quintela, P. A.; Schuette, J. M.; Kaifer, A. E. *J. Phys. Chem.* **1988**, *92*, 3537.

(7) (a) Buttry, D. A.; Anson, F. C. *J. Am. Chem. Soc.* **1982**, *104*, 4824. (b) Buttry, D. A.; Anson, F. C. *J. Am. Chem. Soc.* **1984**, *106*, 59. (b) Buttry, D. A.; Saveant, J.-M.; Anson, F. C. *J. Phys. Chem.* **1984**, *88*, 3086. (c) White, H. S.; Leddy, J.; Bard, A. J. *J. Am. Chem. Soc.* **1982**, *104*, 4811. (d) Martin, C. R.; Rubinstein, I.; Bard, A. J. *J. Am. Chem. Soc.* **1982**, *104*, 4817. (e) Gaudiello, J. G.; Ghost, P. K.; Bard, A. J. *J. Am. Chem. Soc.* **1985**, *107*, 3027. (f) Krishnan, M.; Zhang, X.; Bard, A. J. *J. Am. Chem. Soc.* **1984**, *106*, 7371. (g) Kaifer, A. E.; Bard, A. J. *J. Phys. Chem.* **1986**, *90*, 868. (h) Szentirmay, M. N.; Martin, C. R. *Anal. Chem.* **1984**, *56*, 1898. (i) Rubinstein, I. *J. Electroanal. Chem. Interfacial Electrochem.* **1985**, *188*, 277. (j) Lieber, C. M.; Schmidt, M. H.; Lewis, N. S. *J. Phys. Chem.* **1986**, *90*, 1002. (k) Tudos, A. J.; Ozinga, W. J. J.; Poppe, H.; Kok, W. Th. *Anal. Chem.* **1990**, *62*, 367.
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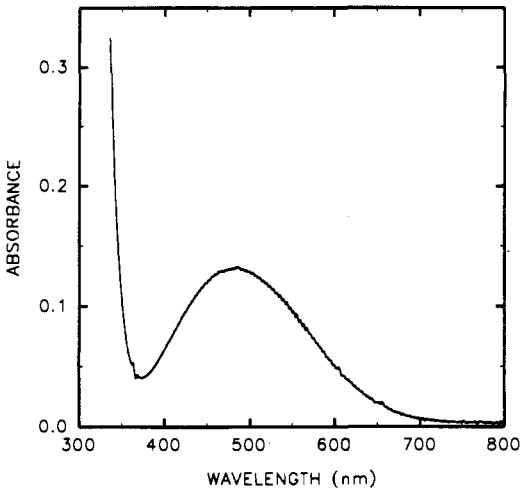


Figure 2. Electronic absorption spectrum of 0.3 mM 1^{4+} and approximately 5 mM serotonin in 0.3 M phosphate buffer, showing the charge-transfer band at λ_{max} = 486 nm.

Table I. Equilibrium Binding Constants at 25 °C, Molar Absorptivities, and Wavelengths of Maximum Absorption of the Charge-Transfer Complexes between Receptor 1^{4+} and Electron-Rich Aromatic Guests in Aqueous Phosphate Buffer (pH = 7)

guest	K (M^{-1})	$\Delta\epsilon$ (M^{-1} cm^{-1})	λ_{max} (nm)
dopamine	1070 ± 120	333 ± 11	464
norepinephrine	1480 ± 220	401 ± 19	450
epinephrine[a]	600 ± 80	475 ± 22	450
serotonin	1550 ± 60	453 ± 4	486
indole	7120 ± 860	652 ± 17	464
catechol	3850 ± 337	546 ± 10	444

[a] Due to solubility problems, these values were determined in 1 M HCl.

Nafion strips loaded with 1^{4+} were then stirred in solutions containing variable concentrations of the neurotransmitter, catechol, or indole. After this exposure, the strips were rinsed again with distilled water and analyzed spectrophotometrically under fully hydrated conditions to determine the concentration of the charge-transfer complex formed and retained inside the polyelectrolyte matrix.

Results and Discussion

Binding of Neurotransmitters, Catechol, and Indole to the Title Receptor. If an aqueous solution of 1^{4+} is mixed with a solution of any of the catechol-based neurotransmitters or serotonin, a visible absorption band develops that corresponds to the charge-transfer complex between the neurotransmitter and the tetracationic host. Similar results are obtained if catechol or indole is used in place of the neurotransmitter, clearly suggesting that the binding interaction takes place between the host's paraquat residues and the electron-rich aromatic groups of the neurotransmitters. Figure 2 shows the charge-transfer absorption of the complex formed between 1^{4+} and serotonin. The development of charge-transfer absorption bands can be advantageously used to determine equilibrium binding constants as described in the Experimental Section. The values obtained are given in Table I. The binding constants for complexation between 1^{4+} and the neurotransmitters are all higher than the values previously found with amino acids,[2] probably a reflection of the stronger π-donor character of the aromatic groups in the former compounds. For comparison purposes, we also determined the binding constants for complexation between 1^{4+} and catechol and indole. The value for catechol is higher than those for the related neurotransmitters dopamine and norepinephrine, while the value for indole is also higher than the value for serotonin. The lower affinity of the neurotransmitters for the receptor (compared to their aromatic parent compounds) must be related to the positive charge on the neurotransmitter side arms that develops by protonation of the amine group at neutral or acidic pH values. Electrostatic repulsions between the protonated side arm and the tetracationic receptor are thus responsible for a certain loss of stability in the

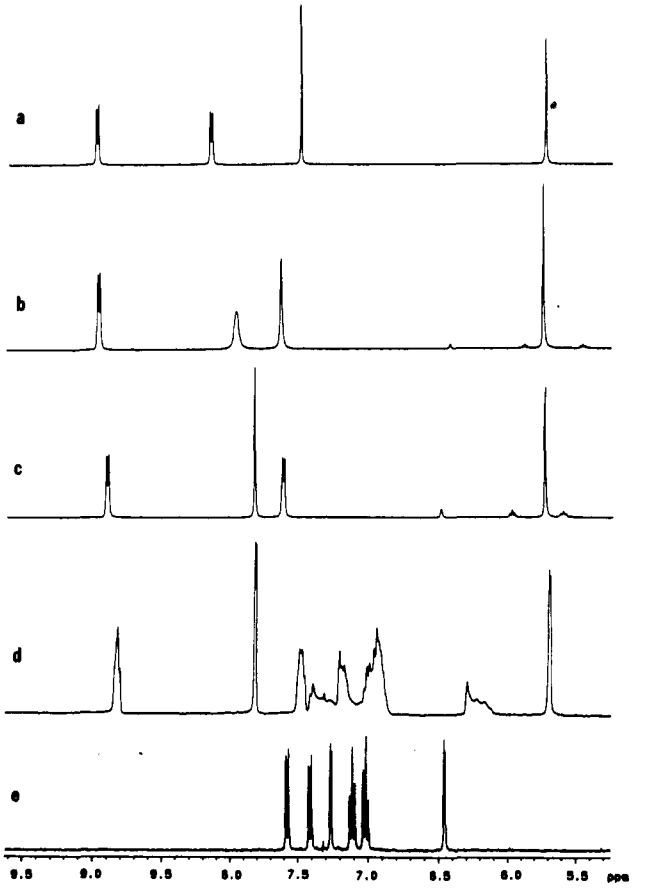


Figure 3. Low-field region of the 400-MHz ^1H NMR spectra of (a) 1.9 mM 1^{4+}, (b) 1.86 mM 1^{4+} and 0.97 mM indole, (c) 1.83 mM 1^{4+} and 1.9 mM indole, (d) 0.91 mM 1^{4+} and 25.7 mM indole, and (e) 10 mM indole. All spectra were recorded in D_2O. The resonance peak of residual acetone was used as the reference for chemical shifts.

neurotransmitter–1^{4+} complexes. The binding constant of the indole–1^{4+} complex is larger than that for complexation between catechol and 1^{4+}. This is probably due to the excellent electronic donor ability of the former compound and its greater hydrophobic character, which should indeed favor complexation in aqueous media. The binding constant quoted for epinephrine in Table I was obtained in acidic medium (1 M HCl), and thus, it is not directly comparable to the remaining values.

NMR Binding Studies. Although the interactions between the surveyed guests and 1^{4+} were clearly detected and quantitated using visible spectrophotometric techniques, we also studied the binding phenomena with NMR spectroscopy in order to gain a better understanding of the interactions involved. These studies proved to be very useful and confirmed that the primary interactions responsible for binding take place between aromatic π-systems. Figure 3 shows the changes in the ^1H NMR spectrum of indole as the relative concentration of 1^{4+} increases. Note that all the protons of indole are strongly affected by the presence of the receptor, undergoing substantial shifting and broadening. Under conditions of stoichiometric excess of the receptor, the indole resonances are so broadened that they become difficult to observe. Similar changes in the NMR spectrum of catechol and the neurotransmitters were detected upon addition of the tetracationic receptor. Interestingly, the aliphatic protons of the neurotransmitters are not significantly affected by the presence of 1^{4+} whereas the aromatic protons undergo changes similar to those shown for indole in Figure 3. Again, this observation confirms that the cyclophane receptor interacts with the neutrotransmitters by including their electron-rich aromatic systems inside the π-acceptor cavity delimited by the two paraquat subunits.

Although NMR spectroscopy could also be utilized to determine binding constants for the neurotransmitters and 1^{4+}, the values were already available from simpler spectrophotometric mea-

Table II. Chemical Shift Displacements ($\Delta\delta$ Data)[a] in the 400-MHz ^{1}H NMR Resonances of Receptor 1^{4+} upon Addition of 1 equiv of π-Donor Guests

guest	α-CH (bipy)	β-CH (bipy)	C_6H_4	CH_2
dopamine	0.003	−0.016	0.018	0.000
norepinephrine	0.003	−0.018	0.024	0.013
epinephrine[b]	0.013	−0.100	0.119	0.002
serotonin	−0.006	−0.175	0.117	0.009
indole	−0.087	−0.526	0.438	0.009
catechol	−0.033	−0.212	0.171	−0.012

[a] $\Delta\delta$ values were calculated as $\Delta\delta = \delta_{complex} - \delta_{free}$. [b] Spectra obtained in 1 M DCl in D_2O. All other spectra obtained in unbuffered D_2O.

surements. However, it was convenient to verify that the NMR data were at least in qualitative agreement with the binding constants of Table I. In order to do this, we measured the changes in the chemical shifts ($\Delta\delta$) of all the proton resonances of receptor 1^{4+} upon addition of 1.0 equiv of guest. The corresponding data are collected in Table II.

The $\Delta\delta$ values in the table generally display the same trends observed with the binding constant values (see Table I). For instance, the largest displacement of the β-protons on the bipyridinediium rings is caused by the addition of the uncharged model compounds, indole and catechol, in this order. This is precisely what is expected from the binding constant values. In apparent disagreement with the order predicted from the values of Table I, epinephrine causes larger $\Delta\delta$ values than norepinephrine or dopamine. However, it is difficult to extract meaningful conclusions in this case because the insolubility of epinephrine in neutral media forces the use of a different, highly acidic medium for this neurotransmitter in all types of experiments. However, the NMR data clearly confirm that the neutral π-donors, indole and catechol, are more strongly bound by 1^{4+} than any of the neurotransmitters. In the receptor, the β-protons of the bipyridinediium rings and the *p*-phenylene protons experience the most significant changes of chemical shift upon complexation of all the surveyed guests. Furthermore, the small $\Delta\delta$ values observed for the peripheral methylene protons of the receptor (see Table II) confirm that the binding interactions essentially involve the aromatic units of the host and the guest.

Electrochemical Studies. As noted above, the voltammetric behavior of 1^{4+} in acetonitrile is characterized by two consecutive 2-electron processes, corresponding to the reversible reductions given in eqs 3 and 4.[1c] The situation is more complicated in aqueous media because the reduced forms of the cyclophane host (1^{2+} and **1**) are not sufficiently soluble and precipitate on the working electrode surface (see Figure 4). The resulting voltammetric behavior is so strongly affected by the precipitation of 1^{2+} and **1** that it becomes practically impossible to extract thermodynamic or kinetic data concerning binding interactions from these experiments. However, a good fraction of the interest in this tetracationic cyclophane receptor results from its ability to bind biologically relevant compounds, such as certain amino acids and neurotransmitters. In order to maintain the biological significance of this work, it is imperative to preserve the aqueous nature of the reaction medium. Therefore, we concluded that a reasonable compromise would be to study the electrochemistry of receptor 1^{4+} inside a water-compatible polymeric matrix that could prevent the precipitation of the reduced forms of the receptor on the working electrode surface. We selected the perfluorinated anionic polyelectrolyte Nafion for this purpose because of the large body of research available on the modification of electrode surfaces with cast films of this polyelectrolyte.[7] In these easy-to-prepare electrodes, the cast Nafion film provides an ionically conducting matrix into which hydrophobic electroactive cations, such as 1^{4+}, can be readily incorporated and retained, sometimes for long periods of time. Furthermore, the positive charge on the neurotransmitters at neutral and low pH is known to favor their partitioning into the anionic polyelectrolyte matrix.[7k] Therefore, both components of the neurotransmitter–1^{4+} complexes can be readily incorporated into Nafion, which appears to be a promising medium for the electrochemical investigation of these complexation reactions. The experimental situation is pictorially depicted in Scheme I.

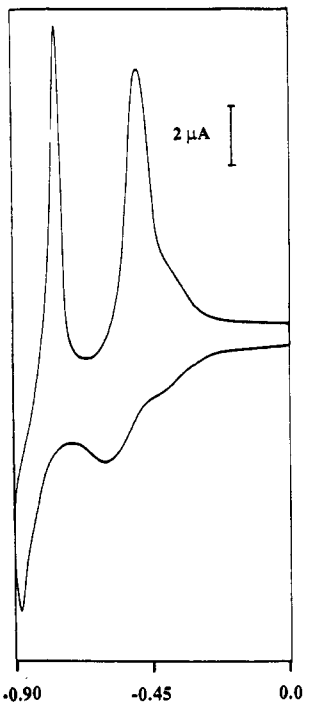
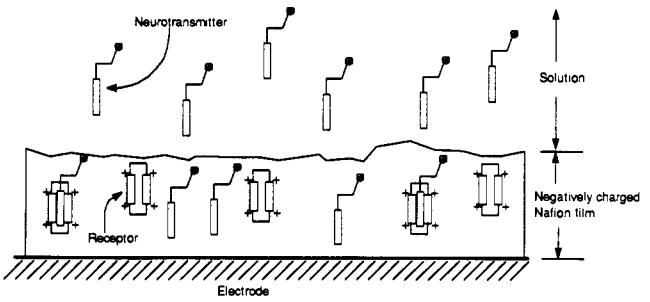


Figure 4. Cyclic voltammogram on a bare GC electrode (0.08 cm^2) of a solution containing 0.1 mM 1^{4+} and 0.1 M phosphate buffer (pH = 7). Scan rate = 100 mV/s.

Scheme I. Neurotransmitter Binding by the Tetracationic Cyclophane Host in a Nafion-Modified Electrode



When a GC electrode modified with a cast Nafion film (estimated thickness ~200 nm) is immersed in a 0.07 mM aqueous solution of 1^{4+}, a set of voltammetric waves develops corresponding to the reversible reduction process of eq 3. The voltammetric currents increase as a function of time revealing the gradual incorporation of 1^{4+} into the Nafion film. Once a steady-state voltammogram was obtained, the GC/Nafion(1^{4+}) electrode was transferred to a pure phosphate buffer solution. No detectable loss of the electroactivity due to the cyclophane receptor was observed for periods of time as long as 24 h. Thus, we conclude that the incorporation of 1^{4+} in the negatively charged polyelectrolyte matrix is quite irreversible. This is in agreement with the well-known Nafion selectivity for hydrophobic and highly charged cations.[7] The voltammetric behavior of 1^{4+} inside the Nafion film is shown in Figure 5A. The large peak-to-peak splitting (ΔE_p) and the linearity of the cathodic peak current vs the square root of the scan rate clearly indicate that the voltammetric response is controlled by the diffusion of the receptor in the Nafion film. This is interesting because the thickness of the Nafion film is rather small and suggests that the propagation of the electrochemical conversions (between 1^{4+} and 1^{2+}) is slow. For comparison purposes, we performed similar experiments with Nafion-modified electrodes loaded with PQ^{2+}. A comparison of

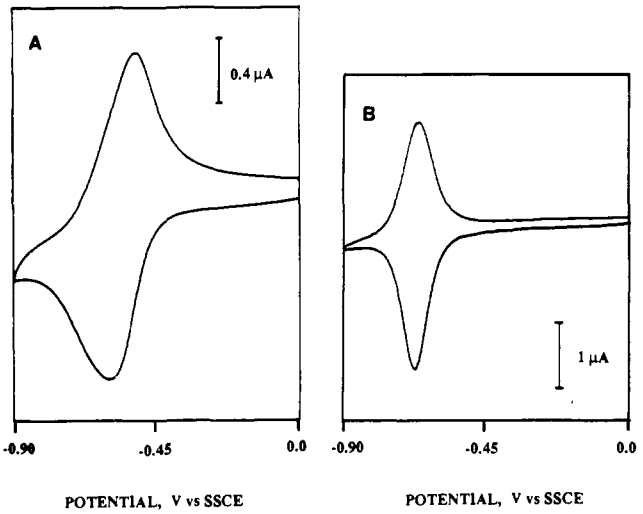


Figure 5. (A) Cyclic voltammogram of a GC/Nafion(1^{4+}) electrode in 0.1 M phosphate buffer (pH = 7). The modified electrode had surface coverages of 2.7×10^{-9} mol of 1^{4+}/cm^2 and 2.9×10^{-8} equiv of Nafion/cm^2. (B) Cyclic voltammogram of a GC/Nafion(PQ^{2+}) electrode in 0.1 M phosphate buffer (pH = 7). The modified electrode had surface coverages of 5.3×10^{-9} mol of PQ^{2+}/cm^2 and 2.9×10^{-8} equiv of Nafion/cm^2. Scan rate = 5 mV/s.

Table III. Electrochemical Data for Nafion-Confined 1^{4+} and PQ^{2+}

parameter	1^{4+}	PQ^{2+}
ΔE_p (mV, measd at 5 mV/s)	80 ± 8	15 ± 5
$E_{1/2}$ (V vs SSCE)	−0.564 ± 0.005	−0.675 ± 0.007
Γ (mol/cm^2)[a]	$(2.7 \pm 0.5) \times 10^{-9}$	$(5.3 \pm 0.3) \times 10^{-9}$

[a] Measured by integration of the first cathodic current wave recorded at scan rates ≤5 mV/s.

electrochemical data for Nafion-confined 1^{4+} and PQ^{2+} is provided in Table III. Note that this comparison was appropriately performed by loading the Nafion film to similar surface coverage (Γ) values of total paraquat.

In spite of this intended similarity, there are remarkable differences between the voltammetric behavior of both compounds in Nafion. The half-wave potential for the tetracationic receptor is about 130 mV more positive than the value for PQ^{2+}. This difference has also been observed in acetonitrile solution[1c] and seems to be related to the relief of electrostatic repulsions between the two paraquat subunits of the receptor that takes place upon reduction. For GC/Nafion(PQ^{2+}) electrodes, the current response can be characterized as corresponding to a surface-confined electroactive species[9] (see Figure 5B); ΔE_p is small, the width at half-height of the cathodic peak is ~95 mV, and the cathodic peak current is directly proportional to the scan rate [more precisely, the slope of log (peak current) vs log (scan rate) plots is about 0.9 while the corresponding value for 1^{4+} is about 0.7]. The differences in the voltammetric behavior between GC/Nafion(1^{4+}) and GC/Nafion(PQ^{2+}) electrodes having similar coating thicknesses indicate that the propagation of the electrochemical conversions across the Nafion film is substantially faster in the case of PQ^{2+}. Furthermore, the leaving rate of this dication from the polyelectrolyte film is also faster than that observed with 1^{4+}, as only ~50% of the initial electroactivity due to PQ^{2+} was detected after 30 min of exposure of a GC/Nafion(PQ^{2+}) electrode to pure buffer solution. All these observations suggest that the diffusional movements of 1^{4+} inside the polyelectrolyte matrix are substantially slower than those of the monomeric analog PQ^{2+}. This can be easily rationalized considering the higher charge on the cyclophane receptor as well as its larger molecular size compared to that of paraquat.

Other groups have investigated the electrochemistry of paraquat in Nafion films.[7e,7i,10] Our results are essentially in agreement with previous findings. For instance, Gaudiello et al. concluded that charge propagation across PQ^{2+}-loaded Nafion films is essentially due to actual diffusion of the dication owing to the relatively small electron-transfer self-exchange rate constant ($k_{ex} \leq 8 \times 10^5$ M^{-1} s^{-1}) that was measured in their experiments.[7e] The charge propagation differences that we observed between PQ^{2+} and 1^{4+} in our Nafion films are perfectly consistent with this finding. Our results could not be easily rationalized if charge propagation is assumed to take place via electron self-exchange among neighboring paraquat groups.[11] As compared to those of other literature reports, the most distinctive feature of our results is the fast leaving rate of PQ^{2+} from the Nafion films. However, this is probably due to the thin nature (~200 nm) of our Nafion coatings, since the retention of any cationic species is favored by thicker films.

When a GC/Nafion(1^{4+}) electrode was immersed in a 0.1 M phosphate buffer solution containing submillimolar concentrations of neurotransmitters, we observed that the voltammetric waves corresponding to the $1^{4+/2+}$ couple broadened and shifted in potential. Both effects increased with the concentration of the neurotransmitter. At least a fraction of the observed potential shift can be attributed to neurotransmitter complexation by the tetracationic receptor, but the substantial broadening of the peaks made it difficult to measure the complexation-induced shifts. The origin of peak broadening is the incorporation of the neurotransmitter into the Nafion film. Before exposure to the neurotransmitter, about 40% of the sulfonic sites in the polyelectrolyte matrix are compensated by the tetracationic receptor and the remaining 60% are compensated by K^+ ions from the buffer system. Since removal of 1^{4+} from the film is a very slow process, the positively charged neurotransmitters exchange into the film by replacing the hydrophilic K^+ ions. An undesirable outcome of this ion-exchange process is a substantial decrease in the film's water content since the hydration of the protonated neurotransmitters is much poorer than that of the hydrophilic potassium cations. This increases dramatically the resistance across the polyelectrolyte matrix. Using Nafion-modified electrodes loaded with cationic ferrocene derivatives (at the 20% level), we recently demonstrated that the exchange of tetrabutylammonium cations into the remaining sulfonic sites results in a complete loss of the electrode's faradaic response due to the substantial increase in the film's resistance.[12] A similar phenomenon seems to result from the incorporation of the protonated form of the neurotransmitters into the Nafion films. Thus, our initial experiments with Nafion-modified electrodes indicated that the simultaneous presence of 1^{4+} and protonated neurotransmitter inside the film creates an ionically resistive matrix which is not suitable for quantitative voltammetric investigations.

To overcome this difficulty, we performed similar voltammetric measurements with the aromatic model compounds. Catechol and indole, lacking a positive charge, were expected to be substantially less efficient at decreasing the water content of the Nafion film since they cannot replace the hydrophilic K^+ ions from the sulfonic sites. Thus, we thought that the bindng of catechol and indole by 1^{4+} would be more amenable to voltammetric studies inside Nafion films. This expectation was indeed confirmed by the experimental results.

Differential pulse voltammetry (DPV) was chosen over cyclic voltammetry for these binding studies because of the rather small magnitude of the potential shifts caused by catechol and indole. Furthermore, DPV allowed easier and earlier detection of peak broadening from film-resistive effects. Figure 6A shows a series of differential pulse voltammograms obtained with a GC/Nafion(1^{4+}) electrode immersed in buffer solutions containing pro-

(9) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*; John Wiley & Sons: New York, 1980; Chapter 10.

(10) (a) Hodges, A. M.; Johansen, O.; Loder, J. W.; Mau, A. W.-H.; Rabani, J.; Sasse, W. H. F. *J. Phys. Chem.* **1991**, *95*, 5966. (b) Porat, Z.; Tricot, Y. M.; Rubinstein, I.; Zinger, B. *J. Electroanal. Chem. Interfacial Electrochem.* **1991**, *315*, 217.

(11) (a) Dahms, H. *J. Phys. Chem.* **1968**, *72*, 362. (b) Ruff, I.; Friedrich, *J. Phys. Chem.* **1971**, *75*, 3297.

(12) Garcia, O.; Kaifer, A. E. *J. Electroanal. Chem. Interfacial Electrochem.* **1990**, *279*, 79.

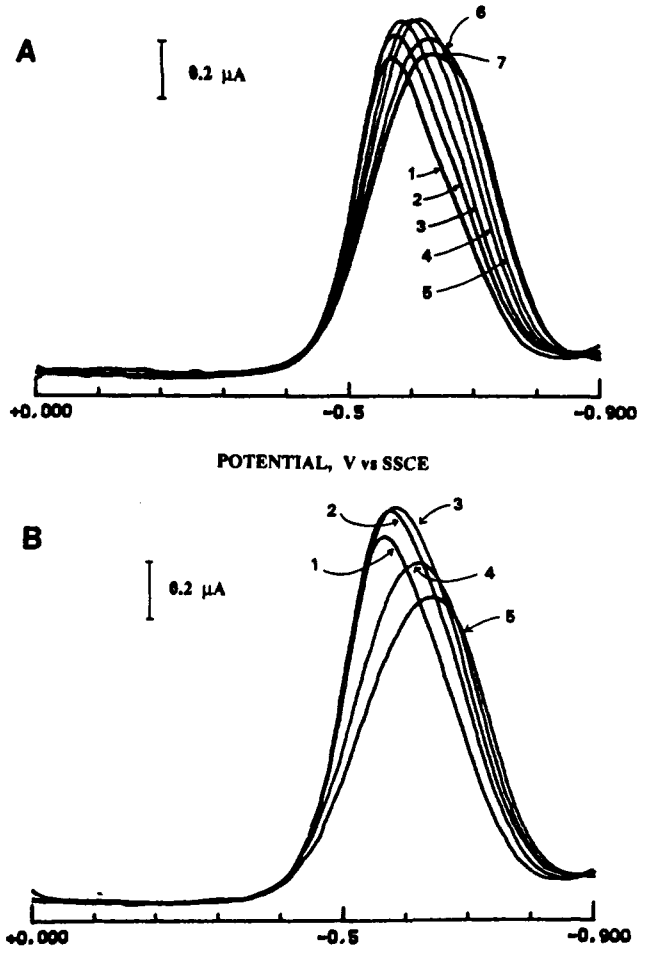


Figure 6. DPV response of the electrode of Figure 5A in contact with solutions containing the following concentrations of aromatic compounds. (A) Catechol: (1) 0 mM, (2) 0.030 mM, (3) 0.081 mM, (4) 0.3 mM, (5) 0.6 mM, (6) 1.9 mM, (7) 2.7 mM. (B) Indole: (1) 0 mM, (2) 5.5 μM, (3) 19.7 μM, (4) 54.6 μM, (5) 162 μM. Scan rate = 4 mV/s. Pulse amplitude = −10 mV.

gressively larger concentrations of catechol. In the absence of catechol, the DPV peak exhibits a half-height width of 193 ± 7 mV. This value remains essentially constant at submillimolar concentrations of catechol whereas the peak potential (and, thus, the half-wave potential) shifts to more negative values as the concentration of catechol increases within this range. For [catechol] > 1.0 mM the half-height width starts to increase quickly, indicating the development of substantial resistance through the film. Even though the catechol-induced peak potential shifts continue to be observed at concentrations over 1.0 mM, the increasing film resistance casts doubts on the use of these data for quantitative purposes. Nonetheless, these experiments demonstrate that complexation-induced shifts can be obtained with GC/Nafion($\mathbf{1}^{4+}$) electrodes contacting solutions of the guest catechol, provided that its concentration is kept below 1.0 mM. Above this level, the permeation of catechol through the Nafion film seems to be extensive enough to increase the resistance to a point that interferes with the voltammetric experiments. A plot of $\Delta E_{1/2}$ vs concentration of catechol in the solution is shown in Figure 7A. Interestingly, it shows the typical shape expected for a complexation process.[13] Figure 6B shows the DPV's obtained with a GC/Nafion($\mathbf{1}^{4+}$) electrode immersed in buffer solutions containing gradually increasing concentrations of indole. With this guest, the onset of resistive peak broadening takes place in the concentration range 20–50 μM, a value substantially lower than

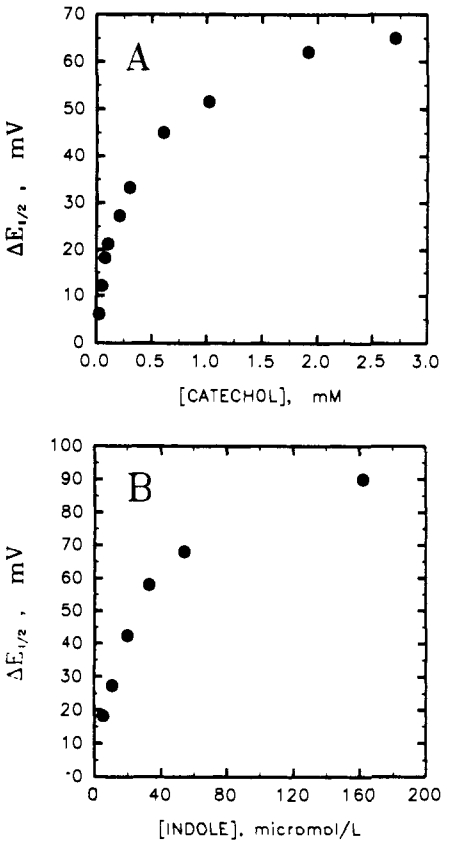


Figure 7. Change in the $\mathbf{1}^{4+/2+}$ half-wave potential measured in GC/Nafion($\mathbf{1}^{4+}$) electrodes immersed in 0.1 M phosphate buffer solutions (pH = 7) also containing variable concentrations of (A) catechol and (B) indole.

that found for catechol. This finding agrees well with the greater hydrophobic nature of indole compared to catechol. The corresponding half-wave potential data are plotted as a function of indole concentration in Figure 7B. Note that indole concentrations as low as 5–10 μM produce perfectly measurable potential shifts. Again the shape of the graph is similar to that predicted for a complexation process. The $PQ^{2+/+}$ potential recorded with GC/Nafion(PQ^{2+}) electrodes exposed to increasing concentrations of catechol or indole does not shift at all if the concentration of guest is maintained below the level at which substantial film resistance starts to develop. Above this concentration, the peaks broaden and shift due to the increasing resistance across the Nafion matrix. Therefore, complexation-induced potential shifts are observed only with $\mathbf{1}^{4+}$ and not with PQ^{2+}.

From the DPV's in Figure 6, it is clear that there is a concentration range in which the half-wave potential of the $\mathbf{1}^{4+/2+}$ redox couple reflects the complexation of the guest without significant resistive effects. The half-wave potential shifts can then be expressed by the following equation:[14]

$$\Delta E_{1/2} = E_{1/2} - E_{1/2}^{\text{free}} = \frac{RT}{nF} \ln\left(\frac{1 + K_r[G]}{1 + K[G]}\right) \qquad (6)$$

where $E_{1/2}$ is the half-wave potential in the presence of a guest concentration [G], $E_{1/2}^{\text{free}}$ is the half-wave potential in the absence of guest, K and K_r are defined in Scheme II, and the rest of the symbols have their usual meanings.

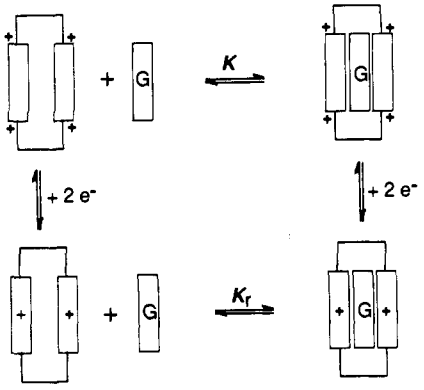
Since 2-electron reduction $\mathbf{1}^{4+}$ decreases tremendously the π-acceptor character of the receptor, K_r can be safely assumed to be zero, yielding

$$\exp\left(-\frac{nF(\Delta E_{1/2})}{RT}\right) = 1 + K[G] \qquad (7)$$

(13) Galus, Z. *Fundamentals of Electrochemical Analysis*; Ellis Horwood: London, 1976; Chapter 14.

(14) Gagne, R. R.; Allison, J. L.; Ingle, D. M. *Inorg. Chem.* **1979**, *18*, 2767.

Scheme II. Electrochemical and Chemical Equilibria Relevant to Guest-Binding by the Tetracationic Receptor



Therefore, if one assumes that the binding constants in the aqueous Nafion matrix are the same as those measured in homogeneous aqueous solution, the experimental $\Delta E_{1/2}$ values can then be used (with the help of eq 7) to calculate guest concentrations inside the Nafion film. Figure 8 shows the calculated $[G]_{Nafion}$ values as a function of guest concentration in the adjacent solution. The plot for catechol (Figure 8A) is linear (correlation coefficient = 0.9976) and has a slope of ~14. Therefore, according to this analysis, catechol partitions favorably inside the Nafion films. The situation for indole is more complicated since the corresponding plot (Figure 8B) clearly departs from linearity (correlation coefficient = 0.9630). The graph seems to indicate that indole partitioning in the anionic polyelectrolyte matrix becomes progressively more favorable as the concentration of indole in the contacting solution increases; that is, its partition coefficient depends on concentration. The reasons for this departure from linearity could be related again to the greater hydrophobicity of indole which may prefer hydrophobic microenvironments inside the Nafion(1^{4+}) matrix at high concentration levels. The interactions between 1^{4+} and indole molecules in Nafion could then be very poorly represented by the binding constant value obtained in water. However, this approximation seems to be more appropriate in the case of catechol, probably because of its greater hydrophilic character.

These electrochemical studies reveal that it is possible to modify an electrode with a thin film (~200 nm) of an ionically conducting matrix containing electroactive hosts whose electrochemical properties are used to detect the presence of the guests indole and catechol in the contacting solution. In fact, quite low concentrations of catechol and, especially, indole produce measurable potential shifts. *These experiments demonstrate the principle of application of redox-active receptors to the design and construction of voltammetric sensors for biologically relevant molecules.* Furthermore, the complexation of indole or catechol by 1^{4+} shifts the reduction potential of the $1^{4+/2+}$ redox couple to more negative values, reflecting the stabilization of 1^{4+} caused by its charge-transfer interactions with the π-donor guest. Scheme II provides a cartoon representation of the relevant electrochemical and chemical equilibria involving 1^{4+}, 1^{2+}, and the guest molecule. It is noteworthy to point out that reduction of 1^{4+} to 1^{2+} removes the π-acceptor character of the cyclophane receptor. Therefore, electrochemical or chemical 2-electron reduction of this host constitutes an excellent mechanism to release the guest from the complex. *Cyclophane 1^{4+} is thus the first reported example of a redox-switchable molecular receptor.*

Spectrophotometric Studies of Complexation in Nafion. The results of our electrochemical studies for complexation of indole and catechol by 1^{4+} inside very thin (~200 nm) Nafion films reveal that Nafion is a convenient complexation medium because the guest partitions readily into it. Unfortunately, these studies could not be expanded to the neurotransmitters because their protonated forms exchange readily into the Nafion matrix, causing a substantial increase in the film resistance that hampered the voltammetric experiments. Therefore, in order to verify the

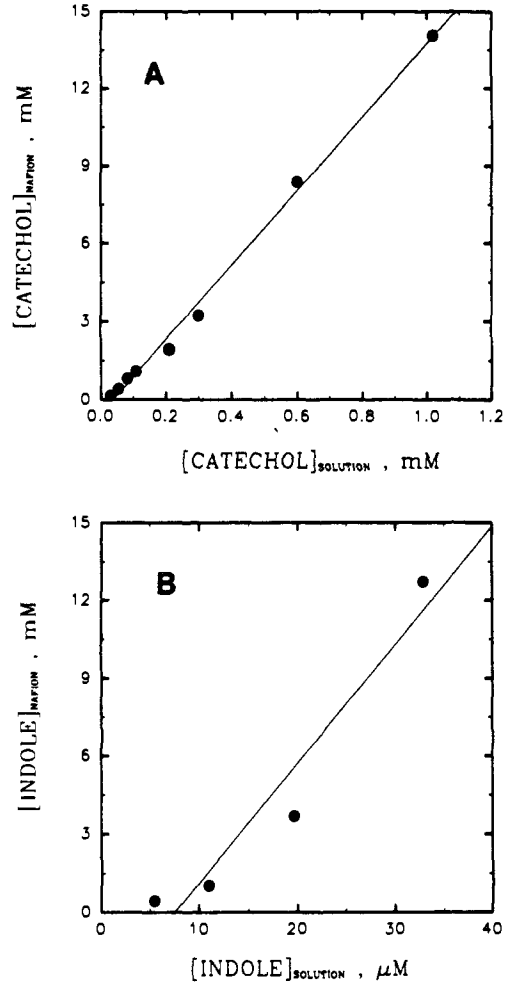


Figure 8. Plots of (A) catechol and (B) indole concentrations inside Nafion as a function of the corresponding solution concentrations. The concentrations inside Nafion were calculated from the $\Delta E_{1/2}$ values using eq 7 and K values given in Table I.

complexation of the surveyed neurotransmitters inside Nafion, we performed spectrophotometric studies using thick (~0.18 mm) commercial Nafion membranes as the complexation medium. All the spectra were recorded with fully hydrated Nafion membranes, i.e., membranes immersed in phosphate buffer (pH = 7) solutions. Exposure of these membranes to aqueous solutions of 1^{4+} (see Experimental Section) resulted in the stable incorporation of the tetracationic receptor into the membrane structure. The characteristic absorption band at 260 nm of the 1,1′-dimethyl-4,4′-bipyridinediium group was clearly detected, giving large absorbance values ($A \geq 2.0$). This finding can be used to estimate a minimum value of 2.7 mM for [1^{4+}] in the Nafion membrane, taking b = 0.18 mm and assuming ϵ = 21 000 M^{-1} cm^{-1} (the literature value for the paraquat dication in water).[15] However, the cyclophane concentration is probably much higher.

The Nafion membranes loaded with 1^{4+} offer a very attractive environment for indole, catechol, and the neurotransmitters. These guests are extracted readily into Nafion(1^{4+}) membranes from their buffered solutions (pH = 7, guest concentration = 0.1–1.0 mM). The corresponding charge-transfer complexes form easily in the thick polyelectrolyte membranes as judged from their characteristic visible absorption bands (data not shown) which exhibit λ_{max} values similar to those measured in homogeneous aqueous solution. More interesting from the analytical point of view is the observed linearity between the visible absorbance of the charge-transfer complex and the guest concentration in the solution. For instance, Figure 9 shows the charge-transfer absorbance of Nafion(1^{4+}) membranes after immersion for 15 min

(15) Watanabe, T.; Honda, K. *J. Phys. Chem.* **1982**, *86*, 2617.

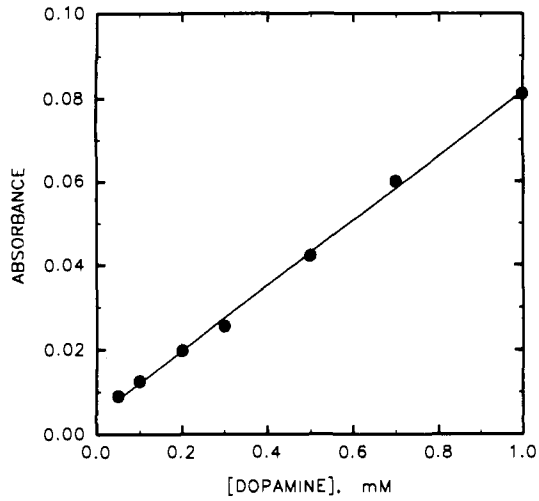


Figure 9. Charge-transfer absorbance (at 464 nm) of Nafion membranes (0.180 mm) preloaded with 1^{4+} after 15 min of exposure to phosphate buffer solutions (pH = 7) containing variable concentrations of dopamine.

in solutions of varying dopamine concentrations. The measured absorbance is linearly related to the dopamine concentration. Linear absorbance vs concentration plots were also obtained with indole, catechol, serotonin, and norepinephrine. The slopes of these straight lines were all similar; 1.0 mM solutions of any of the surveyed guests produce membrane absorbances in the range 0.08–0.14. However, in the cases of serotonin and norepinephrine, longer immersion (loading) times were needed to reach this absorbance range. Specifically, loading periods of 25 min and 3 h were needed in our experiments with serotonin and norepinephrine, respectively. In contrast, dopamine, indole, and catechol gave rise to absorbance values in the indicated range with shorter loading times of 15 min in all three cases. Although the origin of these loading time differences is still unclear, these experiments clearly offer additional evidence for the formation of charge-transfer complexes inside Nafion between the surveyed guests and 1^{4+}. Furthermore, the linearity of the absorbance values as a function of the guest concentration in the loading solution can be utilized for analytical purposes as a method to determine these neurotransmitters, as well as catechol, indole, and probably other neutral or cationic compounds possessing electron-rich aromatic rings. We are currently exploring in detail the potential of this simple analytical methodology for the determination of compounds with biological significance.

Conclusions

This work has shown that receptor 1^{4+} has a substantial affinity for the surveyed neurotransmitters, indole, and catechol, forming inclusion complexes with all of them in aqueous media. The origin of these binding properties dwells in the rigid π-acceptor cavity (defined by the two paraquat groups) of the title cyclophane. We have also shown that it is possible to manipulate the oxidation state of the receptor's paraquat groups to alter its affinity for π-donor guests. Thus, this species provides the first reported example of a redox-switchable molecular receptor. The tetracationic nature of the receptor makes possible its stable incorporation in a polyelectrolyte Nafion matrix where it also binds the same guests as concluded from our electrochemical and spectrophotometric studies. However, improved methods for the immobilization of this receptor—or structurally related ones—on electrode surfaces are required for the design and construction of practical sensor devices for biochemically relevant species.

Acknowledgment. The support of this research by the NSF (Grant CHE-9000531) is gratefully acknowledged. We thank Timothy T. Goodnow for performing some preliminary experiments and the Eastman Kodak Co. for the donation of the BAS-100 electroanalyzer.

Long-Range Spin Density Propagation in Saturated Hydrocarbons: 3-[*n*]Staffyl Radicals

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Abstract: The bridgehead radicals derived from the first three [*n*]staffanes (n = 1–3), oligomers of [1.1.1]propellane, have been generated from the corresponding bromides, and their solution EPR spectra have been recorded. Remarkably long-range hyperfine coupling has been found to ϵ, ζ, and even ι hydrogens, in qualitative agreement with ab initio UHF calculations. The coupling to the bridgehead hydrogen is attenuated by a factor of about 25 per added bicyclo[1.1.1]pentane cage. The long-range propagation of spin density can be attributed to strong interaction between the orbitals used to make the exocyclic bonds in the 1 and 3 positions of each bicyclo[1.1.1]pentane cage. The situation can be understood simply in terms of a linear σ-hyperconjugated chain of orbitals interacting through resonance integrals whose effective magnitude alternates in an about 1:5 ratio. A more detailed analysis is provided by considering the effect on the spin density of the various types of off-diagonal elements in the UHF Hartree–Fock matrix expressed in terms of maximally spin-paired natural bond orbitals (MSP-NBO). This permits a clean separation of through-space and through-bond interactions as well as further separation of each of these into contributions due to bond delocalization and those due to bond spin polarization.

Introduction

Inert and relatively rigid linear molecules terminated with axial substituents and available in a selection of lengths with small increments, such as the functionalized oligomers **1** of [1.1.1]-propellane (**2**), called [*n*]staffanes for short, have been proposed as building elements of a molecular engineering construction set of the "Tinkertoy" type.[2,3] [*n*]Staffanes[4] and their terminally

(1) This project was initiated at the University of Texas, Austin, Texas.

(2) Kaszynski, P.; Michl, J. *J. Am. Chem. Soc.* **1988**, *110*, 5225. Michl, J.; Kaszynski, P.; Friedli, A. C.; Murthy, G. S.; Yang, H.-C.; Robinson, R. E.; McMurdie, N. D.; Kim, T. In *Strain and Its Implications in Organic Chemistry*; de Meijere, A., Blechert, S., Eds.; NATO ASI Series, Kluwer Academic Publishers: Dordrecht, The Netherlands, 1989; p 463, Vol. 273.

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