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Supramolecular Association of Dopamine with Immobilized Fluorescent Probes

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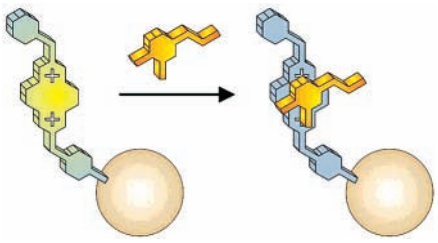
ABSTRACT



We have designed a two-step procedure to coat silica particles with fluorescent 2,7-diazapyrenium dications. The electron-deficient character of the dications encourages the association of dopamine at the particle/water interface. The supramolecular event produces a significant decrease in the fluorescence intensity. Model binding studies with catechol and propylamine revealed that the interfacial complexation of dopamine is dominated by the interaction of its electron-rich dioxyarene fragment with the electron-deficient fluorophore in neutral aqueous environments.

Neurotransmitters ensure the propagation of information in the central nervous system.[1] They travel across synaptic clefts establishing chemical communication between presynaptic and postsynaptic neurons. This general mechanism for information transfer appears to involve the participation of stereoelectronically diverse organic compounds. The understanding of their fundamental role in neurotransmission and of their implication in disease states requires, first of all, the identification of efficient analytical procedures for their reliable determination in vivo and in vitro. The need to elucidate the biosynthesis, metabolism, and functions of catecholamine neurotransmitters,[2] for example, continues to stimulate the development of experimental methods for their detection and for the analysis of their precursors and metabolites. Perfusion and microdialysis sampling coupled to chromatographic analyses have evolved over the years as sensitive and selective methods for determining catecholamines.[2] These procedures, however, involve a significant perturbation of the local brain environment, have limited spatial resolution, and are extremely slow. Electrochemical sensors, instead, offer millisecond response with micrometer resolution.[2] Unfortunately, they suffer the interference of compounds that are oxidized at potentials very similar to those of catecholamines. Despite their intrinsic limitations, these analytical methods have provided already invaluable information on the functions of catecholamine neurotransmitters.[2] For example, it is known that dopamine plays a fundamental role in motor functions and its activity has been associated with various diseases, including Parkinson's and schizophrenia.[3] Nonetheless, much remains to be unraveled

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on the mechanism of action of this particular molecule in the central nervous system.

The emergence of fluorescent probes[4] for the fast and reliable determination of organic analytes is encouraging the exploration of optical methods for dopamine detection.[5] These strategies require (1) the identification of molecular building blocks that can recognize the target analyte producing a detectable fluorescence response and (2) the immobilization of the molecular probes on solid supports that can be incorporated into reliable sensor architectures. A simple analysis of the structure of dopamine reveals electron-rich catechol and ethylamine fragments. These functional groups are expected to associate with electron-deficient 2,7-diazapyrenium dications. Indeed, these particular compounds are known to form stable complexes with dioxyarenes and aliphatic amines as a result of electrostatic forces and charge-transfer interactions.[6] The synergism of these noncovalent bonds has been exploited extensively to assemble interlocked molecules and supramolecular arrays.[6,7] In addition, the fluorescence intensity of the 2,7-diazapyrenium core decreases dramatically upon complexation. Therefore, these electron-deficient compounds appear to be promising building blocks for the assembly of dopamine-responsive surface coatings.

The syntheses of 2,7-diazapyrenium dications rely on the N-alkylation of a preformed 2,7-diazapyrene core.[6] These procedures can be adapted to assemble 2,7-diazapyrenium dications on solid supports in two steps (Figure 1). Initially,

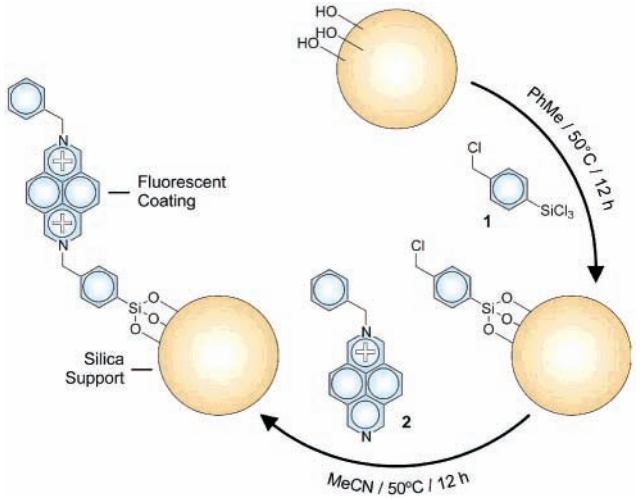

Figure 1. Two-step assembly of 2,7-diazapyrenium dications on the surface of silica particles.

the hydroxylated surfaces of silica particles (surface density = 200 m^2/g) are functionalized with the trichlorosilane **1**. After a continuous solid/liquid extraction of physisorbed materials, the surface-confined benzyl chloride groups are coupled to the preassembled monocationic compound **2**. The final result is the formation of fluorescent coatings on the silica supports. Once again, a continuous solid/liquid extraction is necessary at this point to ensure the removal of physisorbed reagents. The combustion analysis of the resulting materials indicates a carbon content of ca. 13%, confirming the presence of an organic layer on the inorganic support.

The absorption (***a*** in Figure 2) and excitation (***b***) spectra of the 2,7-diazapyrenium dication **3** are remarkably similar

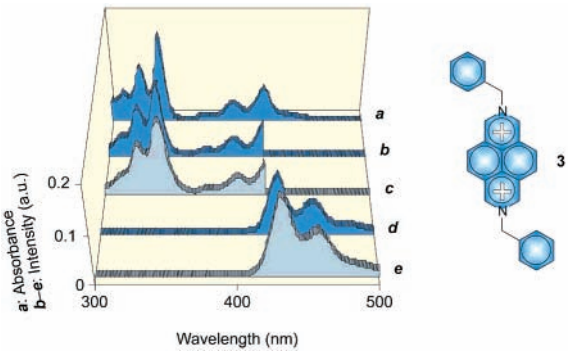

Figure 2. Absorption (***a***), excitation (***b***), and emission (***d***) spectra of a solution of the hexafluorophosphate salt of **3** (1.2×10^{-5} M, MeCN, 25 °C, $\lambda_{em} = 432$ nm, $\lambda_{exc} = 342$ nm). Excitation (***c***) and emission (***e***) spectra of a suspension of the fluoroscent particles (0.5 mg/mL, MeCN, 25 °C, $\lambda_{em} = 432$ nm, $\lambda_{exc} = 342$ nm).

to the excitation spectrum of the modified silica particles (***c***). The emission spectrum of **3** (***d***) and that of the coated particles (***e***) show the characteristic bands of the 2,7-diazapyrenium dication (quantum yield = 0.28). These observations confirm the attachment of the fluorescent compounds to the silica supports and indicate that the surface-confinement has a negligible effect on their absorption and emission properties. A loading of ca. 2×10^{-6} mmol

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(5) Lin, V. S. Y.; Lai, C. Y.; Huang, J. G.; Song, S. A.; Xu, S. *J. Am. Chem. Soc.* **2001**, *123*, 11510−11511.

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of 2,7-diazapyrenium dications per mg of coated particles can be estimated from the emission intensity of **3** and that of the fluorescent particles. Comparison of the loading with the carbon content determined by combustion analysis indicates that the fluorescent particles contain several unreacted surface-confined benzyl chlorides.

The response of the fluorescent particles to dopamine was tested in aqueous environment at neutral pH.[8] Emission spectra revealed a dramatic fluorescence decrease after the addition of the analyte. A plot of the relative intensity of the emission band at 432 nm against the concentration of dopamine shows a rapid decay to a saturation value of ca. 40% (Figure 3). The surface-confined 2,7-diazapyrenium

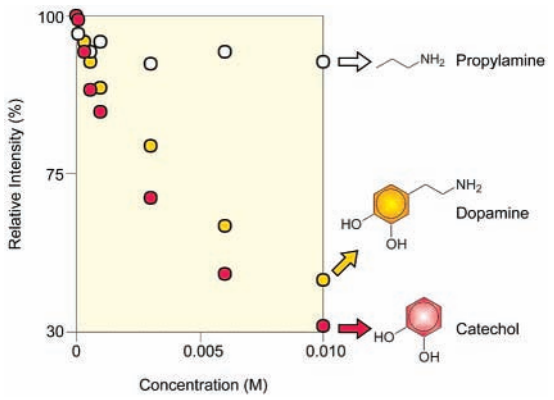

Figure 3. Relative emission intensity of the fluorescent particles (0.3 mg/mL, sodium phosphate buffer, pH = 7.0, 32 °C, λ_{em} = 432 nm, λ_{exc} = 342 nm) in the presence of various concentrations of catechol, dopamine, or propylamine.

units interact with dopamine at the particle/water interface producing a detectable fluorescence response.

Under the experimental conditions employed, dopamine is preferentially in a protonated form.[9] The lack of electron-rich character in the resulting ammonium group suggests that the interaction with the surface-confined electron-deficient dications involves the aromatic portion of dopamine. To confirm this hypothesis, the response of the fluorescent particles to propylamine and catechol was assessed. No significant changes in the emission intensity (Figure 3) could be observed even at a relatively large propylamine concentration (ca. 0.01 M).[10] Instead, a pronounced fluorescence decay was observed for catechol (Figure 3).[11] The remarkable similarity between the dopamine and catechol trends demonstrates that the dioxyarene fragment dominates the supramolecular association of dopamine with the immobilized 2,7-diazapyrenium dications.

The analysis of the fluorescence decay (Figure 3) with a multiple-equilibria binding model[12] revealed that the electron-deficient dications and the electron-rich analytes form 1:1 and 1:2 complexes at the particle/water interface. The interfacial association constants of the 1:1 complexes are 180 ± 7 and 260 ± 10 M^{-1} for dopamine and catechol, respectively. The small difference between them is, presumably, a result of disfavoring electrostatic repulsion between the cationic ammonium group of the protonated form of dopamine and the 2,7-diazapyrenium dication. Instead, the 1:2 complexes are extremely weak and their association constants are ca. 1 M^{-2}.

The influence of the surface-confinement of the electron-deficient component on the binding event was estimated by comparing the response of **3** and that of the fluorescent particles to catechol in MeCN.[13] The association constants for the corresponding 1:1 complexes are 77 ± 3 and 70 ± 7 M^{-1}, respectively, and those for the 1:2 complexes are, again, extremely small (ca. 1 M^{-2}). The negligible difference between the association constants of the 1:1 complexes indicates that the supramolecular association of the complementary electron-rich and electron-deficient components is not affected by the inorganic support. This observation suggests that the dications are positioned away from each other on the particle surfaces and cannot cooperate in the binding event. It is interesting to note, however, that the transition from an aqueous environment to an organic solvent decreases the interfacial association constant by approximately 1 order of magnitude. Thus, the hydrophobic contribution to the binding energy appears to be significant in these complexation processes.

In summary, fluorescent recognition sites can be attached to silica supports following a simple two-step procedure. The electron-deficient character of the surface-confined building blocks encourages the supramolecular association of dopamine at the solid/water interface. The interfacial complexation is transduced into a detectable optical signal. These sensitive surface coatings can be integrated, at least in principle, with fiber optical probes and lead to the development of a new generation of fast sensors for the real-time detection of catecholamine neurotransmitters.

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Supporting Information Available: Experimental procedures for the preparation of the fluorescent particles and their precursors and determination of the association constants. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Surface-confined 2,7-diazapyrenium dications are not stable under basic conditions. As a result, we have not explored the response of the fluorescent particles to dopamine at high pH values.

(9) At a pH of 7.0, the ratio between the ammonium and amine forms is ca. 80:1 and the concentration of the phenolate anion is negligible. The pK_a values for the dissociation of the ammonium cation and the first hydroxy group of dopamine are 8.9 and 10.6, respectively; see: Martin, R. B. *J. Phys. Chem.* **1971**, *75*, 2657−2661.

(10) At a pH of 7.0, the ratio between propylammonium and propylamine is ca. 5000:1. This value was estimated using the literature pK_a of 10.7 for propylamine; see: Frenna, V.; Vivona, N.; Consiglio, G.; Spinelli, D. *J. Chem. Soc., Perkin Trans. 2* **1985**, 1865−1868.

(11) The concentration of the phenolate anion of catechol is negligible at a pH of 7.0. Its pK_a value is 9.3; see: Boggess, R. K.; Martin, R. B. *J. Am. Chem. Soc.* **1975**, *97*, 3076−3081.

(12) Connors, K. A. *Binding Constants*; Wiley: New York, 1987.

(13) Binding properties were compared in an organic solvent because of the low solubility of **3** in aqueous solutions.