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(*R*)-Lipo-Diaza-18-Crown-6 Self-Assembled Monolayer as a Selective Serotonin Receptor

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A highly selective receptor for serotonin was designed using cages formed by the (*R*)-lipo-diaza-18-crown-6 self-assembled monolayer (SAM) on gold and experimentally verified by a variety of electrochemical experiments in solutions containing large amounts of dopamine and ascorbic acid, as well as other interferents. The molecular modeling study showed that parameters such as the H−π interaction provided important driving forces for the cage to form a strong inclusion complex with serotonin. The charge-transfer resistance (R_{CT}'s) to/from redox probe ions, $Fe(CN)_6^{3-/4-}$, was greatly enhanced because of their electrostatic attractions to ammonium ions of serotonin molecules captured by cages. The changes in R_{CT}-values were shown to be remarkably selective for serotonin in the presence of many interferents.

Serotonin (5-hydroxytryptamine), a monoamine neurotransmitter synthesized in the serotonergic neuron in the central nervous system, is mainly associated with modulation of depression, migraine, bipolar disorder, and anger.[1] A number of investigators have attempted to analyze the serotonin level in the brain slices while other neurotransmitters such as dopamine, norepinephrine, epinephrine, and histamine are present in large excess of ascorbate (0.2−0.5 mM).[2−7] These compounds are easily oxidized or reduced at the electrode during voltammetric experiments. Neurotransmitters thus oxidized electrocatalytically oxidize the ascorbic acid because of its lower oxidation potential than the neurotransmitters[8] causing a large current to flow, at the potential where they are oxidized.[9] To overcome the matrix effects of ascorbic acid during analysis of neurotransmitters and to improve their selectivity and sensitivity, techniques such as high-speed chronoamperometry,[8] fast-scan cyclic voltammetry,[10] and voltammetry at modified electrodes by incorporating enzymes,[11,12] ion-exchange membrane,[13] and inorganic catalysts[14] have been used. However, the amperometric methods based on analyte oxidation have suffered from oxidation peak overlaps and large background currents. Recently, Broderick et al. reported an electrochemical sensor for low level serotonin at a high sensitivity using "semiderivative" voltammetric experiments in the ascorbate-dopamine matrix using an electrode made of proprietary materials.[15] Further, the authors have not described the circuit for conducting the semiderivative functions of the current signals.

In the process of looking for a selective receptor for serotonin, we realized that a molecular cage created by a (*R*)-lipo-diaza-18-crown-6 (hereafter "*lipo-diazacrown*")[16] self-assembled monolayer (SAM) formed on a gold electrode could be the best candidate because of its *unique molecular configuration* as well as its *size* in a solution matrix containing other commonly encountered neurotransmitters such as dopamine, ascorbic acid, and other neurotransmitters. This is because the lipo-diazacrown should capture a serotonin molecule inside its cage via host−guest interactions between its alkyl hydrogens and π-electrons of the serotonin molecule via so-called the H−π interaction. The H−π interaction has been known to play an important role in protein structures, as well as in the protein−ligand recognition.[17−19] In addition, the lipo-diazacrown SAM would be free from the adverse effect of ascorbic acid as it lacks an amine group. If the serotonin molecule

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is indeed captured by the lipo-diazacrown cage and forms a host−guest inclusion complex by donating electron pairs to the ammonium nitrogen, it would enhance the rate of charge-transfer to a negatively charged redox probe, for example, $Fe(CN)_6^{3-/4-}$, in solution via the SAM as the positive charge of the complexed ammonium ion would electrostatically attract the negatively charged redox probe and also because the oxidized serotonin inside the cavity would mediate electron transfer to the redox probe.[20] Depending on charges of the SAM, an enhancement or inhibition of electron transfer to the charged probe ions has been observed.[16,20−23] In this work, we describe the lipo-diazacrown SAM as a novel selective receptor of serotonin with a variety of sensing experiments presented as proof.

EXPERIMENTAL SECTION

Materials. Serotonin hydrochloride (Sigma Aldrich) and chloroform (J. T. Baker) were used as received. All other reagents were obtained from Aldrich. (*R*)-Lipo-diaza-18-crown-6 (lipo-diazacrown) synthesized according to the procedure published elsewhere[16] was used as a molecule to form the SAM. The lysate was obtained by first putting 150 mg of the rat brain hippocampus into a test tube, followed by injection of 1.00 mL of the phosphate buffer saline (PBS) solution and sonication for 2 min. The supernatant solution was immediately transferred to the clean tube and stored at −20 °C.

Theoretical Calculations. The structure and stability of the complexes formed between lipo-diazacrown adsorbed on gold atoms and serotonin, as well as with dopamine, were studied using a density function based tight binding (DFTB) method[24] and an n-layered integrated molecular orbital (ONIOM) method implemented in the Gaussian 03 programs.[25] We first optimized the structures of lipo-diazacrown, its SAM, serotonin, and dopamine, as well as their complexes, that is, lipo-diazacrown/serotonin and lipo-diazacrown/dopamine complexes, using the DFTB method, followed by the ONIOM method. In the ONIOM calculation, the model is divided into a high layer treated at the MP2/6-31+G** level of theory and a low layer treated at the B3LYP/6-31G* level of theory, where the CRENBEL ECP basis set was used for the Au atom.[25]

Electrochemical and Impedance Measurements. A Bioanalytical Systems (West Lafayette, IN) model MF-2014 gold disk electrode (0.02 cm^2) was used as a working electrode with a homemade Ag/AgCl electrode (in saturated KCl) and a platinum spiral wire used as reference and counter electrodes. The gold disk electrode was first polished sequentially with alumina powder of 14.5, 5, 1, 0.3, and 0.05 μm sizes. The condition of the surface was then checked by recording cyclic voltammograms in 1.0 M H_2SO_4. The cleaned gold electrode was dipped in a 1 mM lipo-diazacrown solution in chloroform for 15 min. Impedance data were then obtained at the lipo-diazacrown SAM covered electrode before and after the electrode was exposed to various concentrations of serotonin in a 10 mM bis-tris buffer (pH 7.0) solution containing a redox probe, that is, 2.5 mM $K_3Fe(CN)_6$/2.5 mM $K_4Fe(CN)_6$, and 0.25 M $LiClO_4$.

The impedance measurements were made with a Solartron model SI 1255 HF frequency response analyzer connected to an EG&G 273 potentiostat/galvanostat. The open circuit potential (0.245 V vs Ag/AgCl electrode) established by the equimolar mixture of the probe ions was used as a DC bias potential, on which an ac wave of 5 mV (peak-to-peak) was overlaid in a frequency range of 100 kHz down to 100 mHz. The measurements were stopped when the impedances began to be affected by mass transfer as the mass transfer observed at the low frequency offered no significance in our measurements.

We also made impedance measurements using the Fourier transform electrochemical impedance spectroscopic (FT-EIS) technique.[26−30] A homemade fast-rise potentiostat, which has a risetime of shorter than 50 ns/V, was utilized for the FT-EIS measurements. The programmed function shown in Figure 1a was generated by an Agilent 33120 Arbitrary Waveform Generator and applied to the working electrode via the fast potentiostat every 5 s. The potential step and chronoamperometric current signals obtained from the potential step of 10 mV for the first 2.5 s were sampled at a rate of 100 kHz and used for acquisition of impedance data.[26,29,30] For the next 2.5 s, a cyclic voltammogram (CV) was recorded from 0 to 0.50 V at the scan rate of 400 mV/s. A National Instrument NI-5922 high-speed data acquisition system having 24-bit resolution was interfaced to a Pentium-4 PC through its PCI slot. All the data were recorded at a rate of 100 kHz upon application of the step and sweep functions as shown in Figure 1a. Impedances were computed by the Matlab program of MathWorks by first transforming the first derivative signals of the stepped voltage and the resulting current into the ac voltages and currents in frequency domain in a frequency range of 0.4 Hz−10 kHz and then by dividing the ac voltages by ac currents at desired frequencies.[26,20,30] Impedance data thus obtained were

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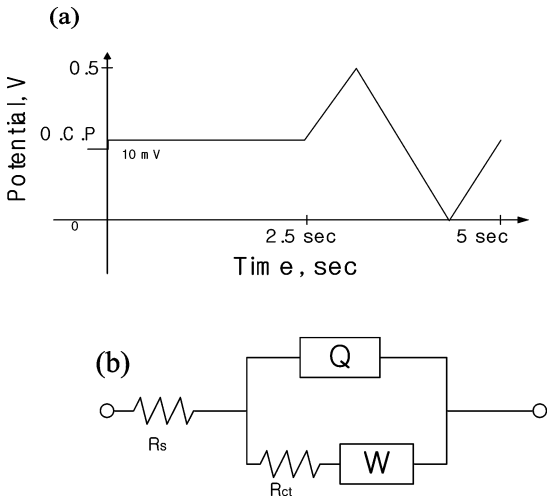

Figure 1. (a) Potential program to sequentially acquire both FT-EIS and CV data. A potential step of 10 mV is first applied on the DC bias of 0.245 V for 2.5 s, followed by a ramp signal with its upper and lower vertex voltages of 0 to 0.50 V with a scan rate of 400 mV/s. (b) Equivalent circuit used for analysis of impedance data: R_s, R_{CT}, and W represent the solution resistance, charge-transfer resistance, and Warburg impedance, respectively. A constant phase element (CPE), Q, was used instead of a capacitor to represent capacitive dispersions at the SAM-covered surface.

then fitted to a circuit model shown in Figure 1b using the ZsimpWin program (Princeton Applied Research).

RESULTS AND DISCUSSIONS

Molecular Modeling Study and Experimental Verification. To visualize how serotonin would be effectively captured by an (*R*)-lipo-diazacrown cage, we carried out a molecular modeling study on the modes of interactions. It is readily expected that ascorbic acid would be effectively discriminated against as it has no amine group to form an ammonium ion, for which the crown ring of the (*R*)-lipo-diazacrown molecule acts as a receptor by donating its nonbonding electron pairs on oxygen and nitrogen atoms. With an -NH_2 group separated from the aromatic nucleus by an ethylene group, both the dopamine and the serotonin molecules are expected to be effectively captured within the cage.

Figure 2 shows the energy optimized structures of the complexes formed between serotonin and dopamine with (*R*)-lipo-diazacrown, which forms a cage above the gold surface by sitting on its surface with four S−Au covalent bonds footed on the surface. The optimized structures of the complexes in vacuum were calculated, and the actual structures in electrolyte solutions could be different from those shown here because of their strong interactions with solvent molecules, that is, water. Both serotonin and dopamine have binding energies of −70.14 and −65.61 kcal/mol, respectively, in vacuum with the lipo-diazacrown molecule sitting on the gold surface with a difference of 4.53 kcal/mol. This indicates that lipo-diazacrown forms a strong complex with dopamine as well. The complex with serotonin, for which the formation constant (K_f) was calculated to be 2.77×10^{51} from the free energy of complex formation, is stronger than that with dopamine ($K_f = 1.32 \times 10^{48}$) by about 2100 times in vacuum. While the K_f values were obtained by optimizing the free energy of formation of the inclusion complexes in vacuum, their relative strengths should be maintained in solvated states as well.

To confirm the formation of complexes, we ran experiments in which the SAM covered electrode was dipped in a solution containing varied amounts of serotonin for 10 min to capture the serotonin in solution. The electrode was then taken out of the solution, washed thoroughly with water, and the amounts of dopamine and serotonin captured were determined by the differential pulse voltammetric experiments. Typical differential pulse voltammograms (DPVs) obtained for dopamine and serotonin at two different concentrations are shown in Figure 3a. These DPVs were obtained separately from solutions containing designated concentrations of dopamine *or* serotonin. This was because serotonin molecules were found to completely expel dopamine molecules out of the cages even if the dopamine molecules were precaptured in a solution containing dopamine alone. No dopamine peak was detected in solutions containing both dopamine and serotonin together. This qualitatively indicates that the inclusion complex of the diazacrown cage with serotonin is much stronger than that with dopamine. It should also be pointed out that one DPV cycle stripped these compounds off from the cage and the electrode was completely restored.

The DPV currents obtained at different serotonin concentrations in solution are plotted in Figure 3b. The formation constant, K_f, for the serotonin-diazacrown cage complex was obtained from the plot employing the equations

$$\frac{[S]}{I_p} = \frac{1}{K_f \cdot C} + \frac{[S]}{C} \quad (1)$$

which has been derived based on the Langmuir isotherm.[23,31] Here [S] is the serotonin or dopamine concentration in solution, I_p is the DPV peak current, and C is a constant. The formation constants obtained from the plot were 1.18×10^4 and 1.81×10^3 M^{-1}, respectively, for serotonin and dopamine (data for dopamine not shown). Although the K_f value for the serotonin-cage complex is greater than that for the complex with dopamine by only 6.5 times, which is much smaller than theoretically expected, this difference is large enough to prevent dopamine from forming a complex when present together with serotonin. The large discrepancies shown by the complexes formed in vacuum obtained by the molecular modeling calculation and those determined by experiments in solution are attributed to the strong solvation energies for these compounds; both molecules have strongly hydrophilic groups. The pK_a values of serotonin[32a] and dopamine[32b] are 9.8 and 8.87, respectively, indicating that the association constants of these compounds with protons are in the range of $\sim 10^{10}$ M^{-1} in their basic forms, and strong competitions between the complex formation with the cage and the association reaction with water would weaken the complex considerably, not to mention fewer cages available only on the surface in comparison to the number of water molecules in solution. Thus, what

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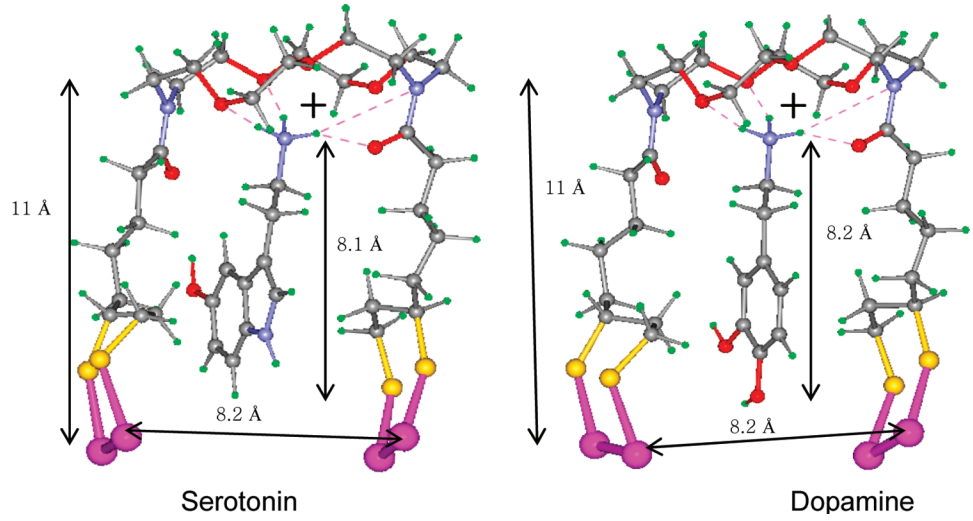

Figure 2. Energy optimized structures for the complex formed between diazacrown and serotonin (left) and dopamine (right) on a gold surface. The gold, sulfur, carbon, hydrogen, oxygen, and nitrogen atoms are marked in pink, yellow, gray, green, red, and blue colors, respectively.

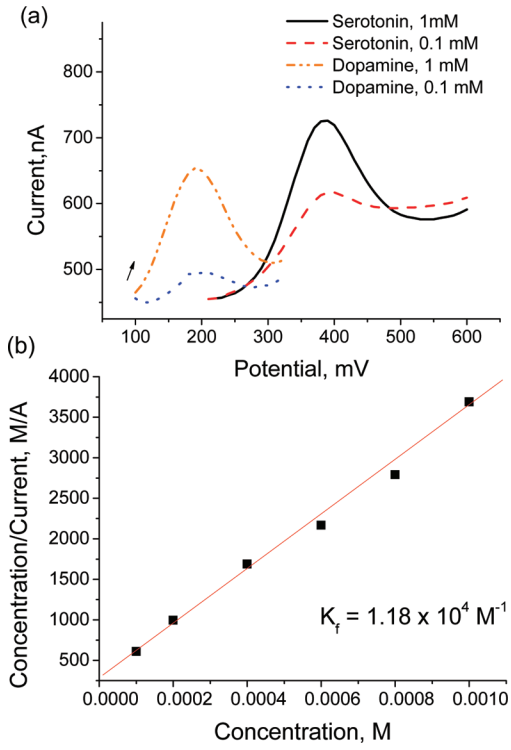

Figure 3. (a) DPVs recorded for dopamine (left) and serotonin (right) captured by dipping the SAM-covered electrode in a solution containing 0.10 (blue dotted line) and 1.0 (red dashed line) mM dopamine, and 0.10 (red dashed-dotted line) and 1.0 (black solid line) mM serotonin for 10 min; (b) the DPV peak currents plotted as a function of the serotonin concentration.

is important here is the relative strength of the two complexes, not the absolute magnitudes of the formation constants; the relative strength of the formation constant of the cages with serotonin is large enough to expel the dopamine molecules precaptured in cages.

This explains why the R_{CT} value decreases upon exposure to a solution containing dopamine, which further decreases upon addition of serotonin, as will be described below. The serotonin molecules get dopamine molecules out of the cages when added later to the dopamine-lipo-diazacrown complexes already formed. A similar observation has been reported for a complex formed between α-cyclodextrin and ferrocene, in which ferrocene was replaced by β-(D+)-glucose, with only the difference of 3.4 times of formation constants for complexes formed for ferrocene and glucose with α-cyclodextrin complexes,[23] which is a much smaller difference of 6.5 times shown by the two complexes in this work.

Two important modes of interactions account for the formation of a stronger complex with serotonin than with dopamine. The first is the H−π interaction, which has been known to play an important role in determining protein structures.[17−19] As can be seen from the optimized structures, serotonin has a little more extensive H−π interaction due to the π-conjugated system because of the indole ring attached to the benzene ring. The second is perhaps the hydrophobic interaction between serotonin molecules and gold atoms on the floor, which is lacking for the dopamine molecule. The serotonin molecule has two hydrophobic =C−H ends interfacing with gold atoms while the dopamine molecule touches gold atoms with a hydrophilic hydroxide group inside the cage. The metal atoms, which are hydrophobic, tend to strongly adsorb many organic compounds onto metal electrodes and would thus reject the hydrophilic compounds. Also, there is a slight difference in the length of serotonin and dopamine molecules. The overall length of dopamine, which is about 8.5 Å, is a bit longer than that of serotonin (8.1 Å), making it uncomfortable to be confined within the (*R*)-lipo-diazacrown cage. Notwithstanding the actual difference in the free energy of formation, the two reasons we provided above give a good justification for (*R*)-lipo-diazacrown's capability of selectively recognizing serotonin from dopamine. For this reason, the serotonin molecule containing an indole ring is different from many catecholamines such as dopamine, epinephrine, and norepinephrine on the lipo-diazacrown SAM. Thus, the cage formed on the gold surface allows this unique interaction to be possible, and serotonin is selectively recognized by the (*R*)-lipo-diazacrown SAM by forming a strong inclusion complex. For proof of the selective binding of the cage with serotonin, we present results of a few other experiments.

Impedance Measurements. The recognition of serotonin was studied first by making EIS measurements with and without

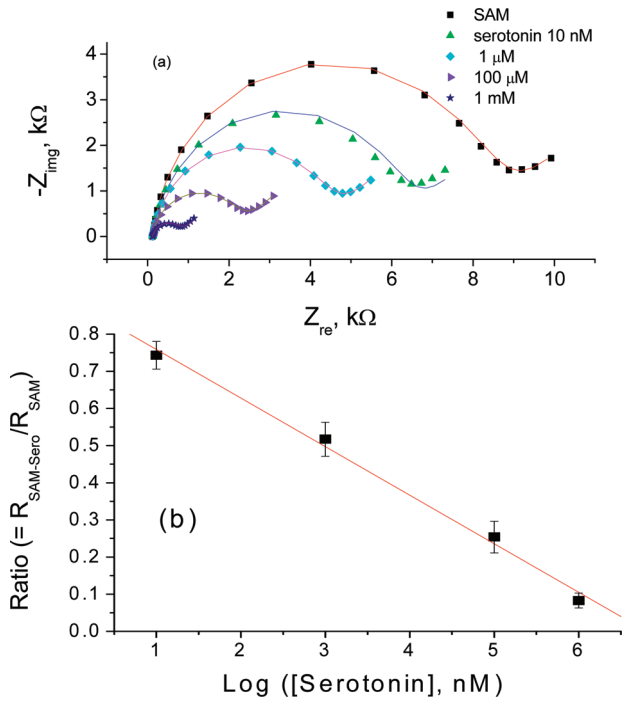

Figure 4. (a) Nyquist plots obtained at the SAM covered electrode for a charge-transfer reaction to/from the redox probe ions, 2.5 mM each $K_3Fe(CN)_6^{3-/4-}$, in 0.25 M $LiClO_4$ at pH 7.0 (10 mM bis-tris buffer) before (solid black squares) and after (other symbols) the SAM-covered electrode captured serotonin; and (b) the ratio of R_{CT} plotted vs log (serotonin concentration, nM). The detection limit was 0.59 nM. The frequency response analyzer was used for these impedance measurements.

serotonin. After the electrode had been modified by the lipo-diazacrown SAM, it was dipped into solutions containing various concentrations of serotonin for 1 h. The EIS measurements were then made at the electrode, which had picked up serotonin molecules from the solution *without* the electrolytes, in a solution containing redox probe ions (a mixture of 2.5 mM each of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$) and 0.25 M $LiClO_4$ as a supporting electrolyte in a 10 mM bis-tris buffer solution (pH 7.0)), first without and then with serotonin captured. Note in this series of experiments that the complexation reaction was first allowed to occur without any competing ions such as K^+ or Li^+ toward the lipo-diazacrown receptor. When the SAM modified electrode had been exposed to the solution containing serotonin for 1 h, the charge-transfer resistance underwent large decreases as shown in Figure 4. This indicates that the rate of electron transfer increased upon formation of the complex between the lipo-diazacrown and serotonin, which is explained only if the complex renders the access of highly negatively charged redox probe ions easier. This occurs as the surface is positively charged because of the capture of the protonated amines such as serotonin; similar observations have been made in the case of polyamines,[20] in which the positive charges on the electrode surface made the approach of the probe ions easy. When other adducts are formed blocking the approach of probe ions, however, the opposite behavior, that is, an increase in charge transfer resistance (R_{CT}), is observed instead because of the poorer accessibility of the probe ions to the electrode for electron transfer.[16,21,33] The R_{CT} value decreases linearly with respect to the log(serotonin concentration) as shown in Figure 4b for concentrations ranging from 10 nM to 1 μM. This result also indicates that the serotonin molecules captured do not dissociate and go back into the solution due perhaps to such a large formation constant (vide supra).

To monitor the reaction in real time, we first added serotonin to the electrolyte solution and monitored the changes in impedances followed by recording CVs employing the programmed function shown in Figure 1a. Thus, a potential step of 10 mV is applied for impedance measurements for the first 2.5 s followed by a cyclic ramp signal for another 2.5 s at a sweep rate of 400 mV/s, during which a CV was recorded between 0 and 0.50 V. Both impedance and CV data were sequentially obtained as soon as a 400 μL aliquot of 1 mM serotonin was injected into a 4 mL electrolyte solution. The solution was then sufficiently stirred to make the final serotonin concentration to 90.9 μM. The impedance data were then obtained; the R_{CT}-value was shown to decrease rapidly as seen in the Figure 5a. A series of CVs shown in Figure 5b demonstrate that the peak currents are hardly affected but the peak separation (ΔE_p) decreases as the complexation reaction progresses. These FT-EIS and CV results indicate that the electrochemical reversibility for the $Fe(CN)_6^{3-/4-}$ pair is significantly improved upon capturing the serotonin molecules by the cages. The ratio of charge-transfer resistances ($R_{Sero-SAM}/R_{SAM}$) before and after injection of serotonin is decreased because of the binding of serotonin to the receptor, the lipo-diazacrown molecule. When the concentration of serotonin is low at 1 μM, the degree of the decrease in the charge-transfer resistance is rather small, and it takes relatively long to reach a steady R_{CT}-value when compared with higher concentrations as seen in Figure 6a. The $R_{Sero-SAM}/R_{SAM}$ ratios taken at 500 s are shown to linearly decrease for an increase in the log(concentration of serotonin) plot (Figure 6b).

To study the matrix effects of physiological concentrations of dopamine and ascorbic acid on the serotonin recognition, experiments were conducted with the electrode exposed to a high concentration of 200 μM in both dopamine and ascorbic acid under otherwise the same conditions as used before. Because of the similarity of the molecular structure of dopamine to serotonin, R_{CT} decreased from its initial value by about 30% even before the addition of serotonin (data not shown). Upon injection of serotonin to this solution, the R_{CT}-values were further decreased for the next 10 min (data not shown). The R_{CT} ratios taken at 10 min after injection of serotonin show similar log[serotonin] dependency as those without dopamine and ascorbic acid as shown in Figure 7 (solid black squares). This indicates that the interaction of lipo-diazacrown is much stronger with serotonin than with dopamine, which was also shown by the difference in their formation constants (Figure 3). When only ascorbic acid was present, no effect on R_{CT} was detected, indicating that ascorbic acid does not form a complex with the cage.

Figure 7 shows responses of the lipo-diazacrown SAM to serotonin in the presence of large amounts of dopamine and ascorbic acid (solid black squares) and in their absence (solid red circles). Note here that the slopes of the two plots, −0.288 and −0.284 in the absence and presence of ascorbic acid and

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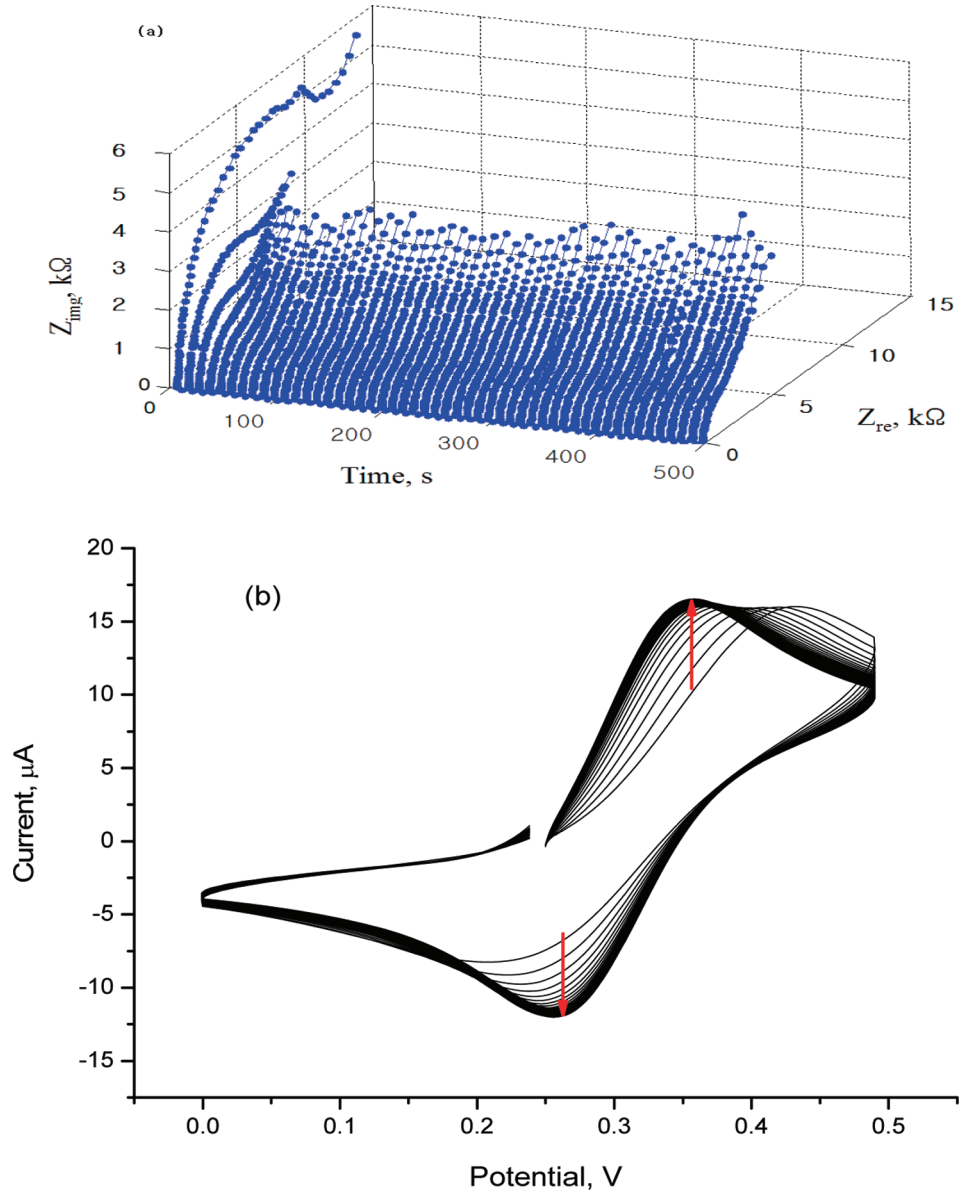

Figure 5. Series of (a) FT-EIS data and (b) CVs obtained as a function of time upon injection of 400 μL of 1 mM serotonin to 4 mL of the probe solution. The experimental conditions were the same as those used for Figure 3. The FT-EIS method has been used in all experiments unless otherwise stated.

dopamine, respectively, are practically the same with R_{CT}-values slightly lifted in the presence of interferents. The detection limit is lower at 150 nM without dopamine and ascorbic acid than that with them, 370 nM. This indicates that serotonin can be determined in a biological fluid with the same sensitivity (or slope) regardless of whether the interferents interacting with the SAM are present or not, although the interferents suppress the responses to some extent.

Effects of Other Interferents. To evaluate the effects of various other interferents in brain fluids, we ran the experiments in a PBS buffer solution containing 10 μM each tryptophan, 5-hydroxytryptophan, epinephrine, norepinephrine, acetylcholine, glutamate, aspartate, glycine, and adenosine. However, the presence of these interferents caused no changes in R_{CT} values (data not shown) except for histamine, which affected R_{CT} in the same way as serotonin does as can be seen from Figure 8a. The only difference shown by these two neurotransmitters was that the reaction kinetics was much faster for histamine than for serotonin. The response obtained when both (100 nM histamine and 1 μM serotonin) were present is shown in Figure 8b; we see clearly that the electrode responds to both histamine and serotonin with different enough decay times that the two can be time-resolved. The decrease in R_{CT} because of the presence of 1 μM serotonin with 100 nM histamine present is exactly the same as that without histamine as can be seen from the data point overlapped with the data point without histamine marked by a circle in Figure 6b. This indicates that both histamine and serotonin can be completely time-resolved and determined simultaneously in the same solution.

We finally ran experiments in a lysate solution prepared from rat brains. When the lysate solution alone was tested, the R_{CT} value initially stayed unchanged for about 300 s indicating that there was practically no serotonin and then began to increase slowly to about 2.1 times of the initial value in about 600 s (data

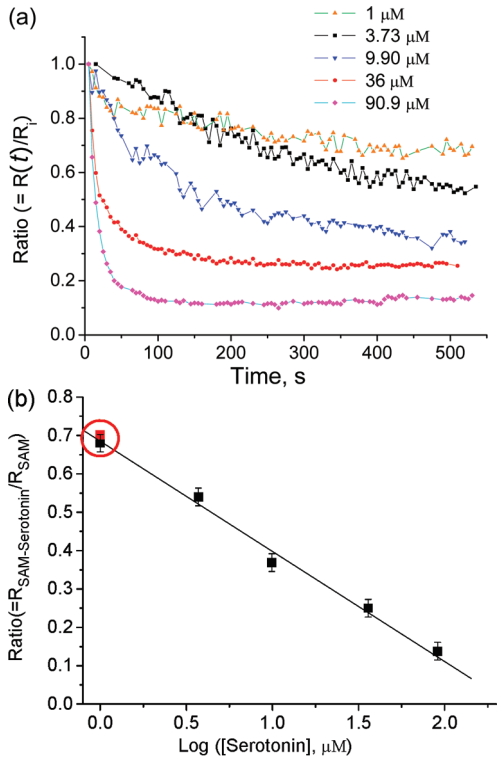

Figure 6. (a) Variation of R_{CT} (= $R(t)$) values normalized with respect to their initial value (= R_i) for 500 s upon exposure of the SAM covered electrode to various concentrations of serotonin, and (b) the R_{CT} ratio taken at 500 s vs log[serotonin, μM]. The detection limit is 0.15 μg/mL. Two data points, one without (black) and the other (red) with histamine present (Figure 8b), are marked by a red circle.

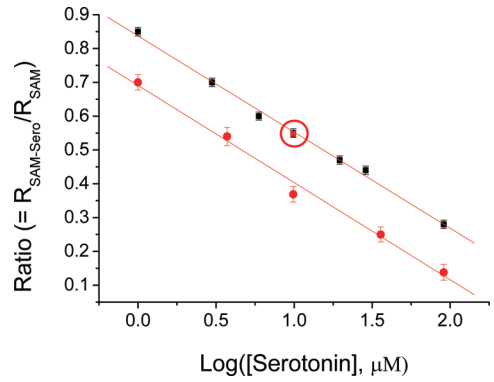

Figure 7. Comparison of calibration lines with and without the matrix effect: serotonin detection in the presence of 200 μM in both ascorbic acid and dopamine (solid black squares), and in their absence (solid red circles).

not shown). The increase in R_{CT} at later times must be due to the slow adsorption of large protein molecules in the lysate onto the SAM, blocking the electrode surface and raising the R_{CT} value. We have seen similar effects for other protein molecules.[16,33] In the second experiment, a solution containing both 10 μM serotonin and the lysate was used. The solution was prepared by adding a 40 μL aliquot containing 1 mM serotonin and another 40 μL aliquot of the lysate into 4.00 mL of the PBS to make the serotonin concentration of 10 (actually 9.98) μM (data not shown). The R_{CT} value decays rapidly to below 0.60 of its initial value and increases to about 0.67 followed by a slow

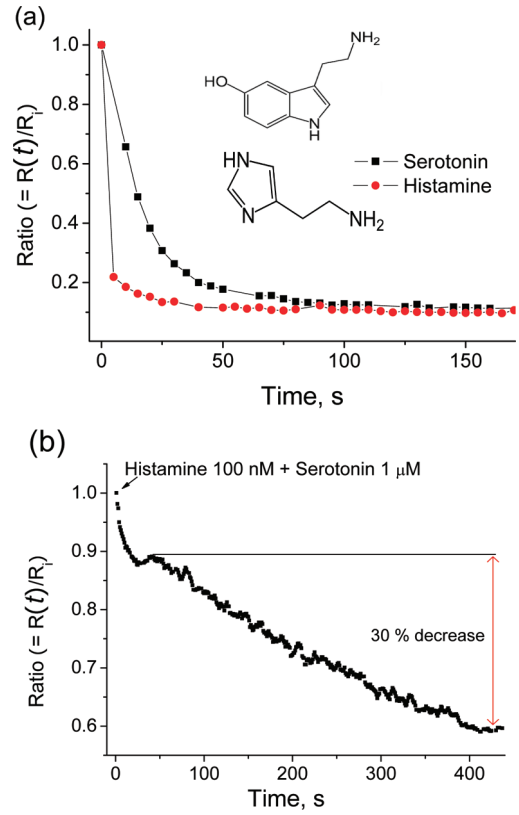

Figure 8. EIS responses to: (a) 90.9 μM serotonin (solid black squares) and 90.9 μM histamine (solid red circles) in separate PBS solutions; and (b) 100 nM histamine + 1 μM serotonin in the same PBS solution.

decay. The ratio (R_{CT} to $R_{CT,SAM}$) read from this falls right on the calibration line as marked by a circled red data point in Figure 7 on the line obtained with large amounts of ascorbic acid and dopamine present (solid black squares). This indicates that the detection of serotonin can be made in the solution containing the lysate as well. Thus, serotonin can be determined selectively, or concurrently with histamine, under a variety of experimental conditions, provided that real time FTEIS measurements are used for its detection.

CONCLUSIONS

We have demonstrated that the cage formed by lipo-diazacrown SAM on the gold electrode acts as a novel serotonin receptor because of its unique interaction with serotonin. The result of molecular modeling showed that the serotonin molecule fits snuggly into the lipo-diazacrown cages and is thus selectively recognized by the cage thanks to their H$-\pi$ interactions, the hydrophobic interaction between the aromatic =C$-$H group and gold atoms, and its correct molecular length. The selective recognition of serotonin has been demonstrated in the presence of large amounts dopamine and ascorbic acid along with other interferents present, as well as in the lysate containing solutions.

Voltammetric methods for analysis of serotonin in biological fluids suffered from the selectivity problem because of the presence of various electroactive interferents including ascorbic acid of high concentrations.[2−8,34,35] For this reason, techniques

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such as electrophoresis,[36] chromatography,[37,38] and chemiluminescence[39] have been used for its analysis. The receptor we describe in this work offers an alternative to these methods without prior separation or changing scan rates as in voltammetric methods at a microelectrode. Another important point is that these determinations can be possible only if the FTEIS experiment is used as a detection tool thanks to its time-resolving capability. As were shown above, the responses due to many macromolecules in the lysate solution began to dominate at later times, and it would be impossible to use slow responding traditional impedance measurement techniques such as frequency response analysis, as they would sum up all the responses making it impossible to resolve the relevant signal from the irrelevant ones. Finally, it should also be pointed out that serotonin may be collected from a biological fluid first and then analyzed by "stripping" differential pulse voltammetric experiments as shown by DPVs in Figure 3. Work is in progress along this line in our laboratory.

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