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RNA Aptamer-Based Electrochemical Biosensor for Selective and Label-Free Analysis of Dopamine

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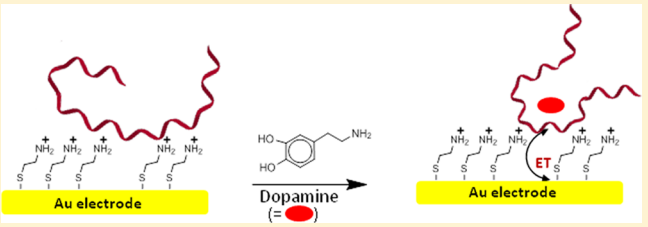
Supporting Information

ABSTRACT: The inherent redox activity of dopamine enables its direct electrochemical in vivo analysis (Venton, B. J.; Wightman, M. R. *Anal. Chem.* **2003**, *75*, 414A). However, dopamine analysis is complicated by the interference from other electrochemically active endogenous compounds present in the brain, including dopamine precursors and metabolites and other neurotransmitters (NT). Here we report an electrochemical RNA aptamer-based biosensor for analysis of dopamine in the presence of other NT. The biosensor exploits a specific binding of dopamine by the RNA aptamer, immobilized at a cysteamine-modified Au electrode, and further electrochemical oxidation of dopamine. Specific recognition of dopamine by the aptamer allowed a selective amperometric detection of dopamine within the physiologically relevant 100 nM to 5 μM range in the presence of competitive concentrations of catechol, epinephrine, norepinephrine, 3,4-dihydroxy-phenylalanine (L-DOPA), 3,4-dihydroxyphenylacetic acid (DOPAC), methyldopamine, and tyramine, which gave negligible signals under conditions of experiments (electroanalysis at 0.185 V vs Ag/AgCl). The interference from ascorbic and uric acids was eliminated by application of a Nafion-coated membrane. The aptasensor response time was <1 s, and the sensitivity of analysis was 62 nA μM^{-1} cm^{-2}. The proposed design of the aptasensor, based on electrostatic interactions between the positively charged cysteamine-modified electrode and the negatively charged aptamer, may be used as a general strategy not to restrict the conformational freedom and binding properties of surface-bound aptamers and, thus, be applicable for the development of other aptasensors.



A number of psychiatric and neurodegenerative diseases are directly connected with abnormalities in neurotransmitter (NT) metabolism in the brain.[1] Such neurological disorders as Parkinson's and Huntington's diseases, Alzheimer's disease, Tourette syndrome, and schizophrenia, psychosis, and drug addiction are related to abnormal levels of dopamine, an NT that belongs to the monoaminergic neuroreceptor family.[2] The action of dopamine, similarly to the action of other NTs, depends on its overall levels as well as short bursts of its activity in the extracellular space of the brain. Thus, real-time, sensitive, and selective in vivo detection of dopamine and monitoring its concentration dynamics within neural tissue represent one of the most challenging tasks in neuroscience. The most widely used approach to monitor dopamine levels in the brain is microdialysis of a perfusion fluid through the brain-implanted dialysis membrane and subsequent external high-performance liquid chromatography (HPLC)/electrochemical analysis of the dialysate (EC HPLC).[3] In vivo measurements of dopamine are also performed by positron emission tomography (PET) of the radiolabeled receptor ligand uptake and binding to dopaminergic receptors.[4] Neither of these techniques can be performed in behaving animals or humans, and both are slow compared to the rapid dynamics of neurotransmission and dopamine metabolism: >20 min for either microdialysis or PET scanning.

On the other hand, the inherent electrochemical activity of dopamine promoted application of direct electrochemical schemes for real-time in vivo monitoring.[5−10] Both fast and sensitive in situ electrochemical detection of dopamine is currently performed with carbon fiber microelectrodes, shown to be practically useful in biomedical and neurophysiological studies,[11−19] and the methodology was further optimized by using fast-scan cyclic voltammetry (FSCV).[20−22] These methods have enabled in vivo analysis of dopamine release and metabolism in dopamine-rich animal brain areas[6,13,18,23] and in simpler dopamine-dependent living systems.[9−11] Therewith, the interference from such electrochemically active species as ascorbic acid (AA), present in a large excess over dopamine in biological samples,[24] can be overcome by oxidative pretreatment of carbon microelectrodes[25] or exploitation of

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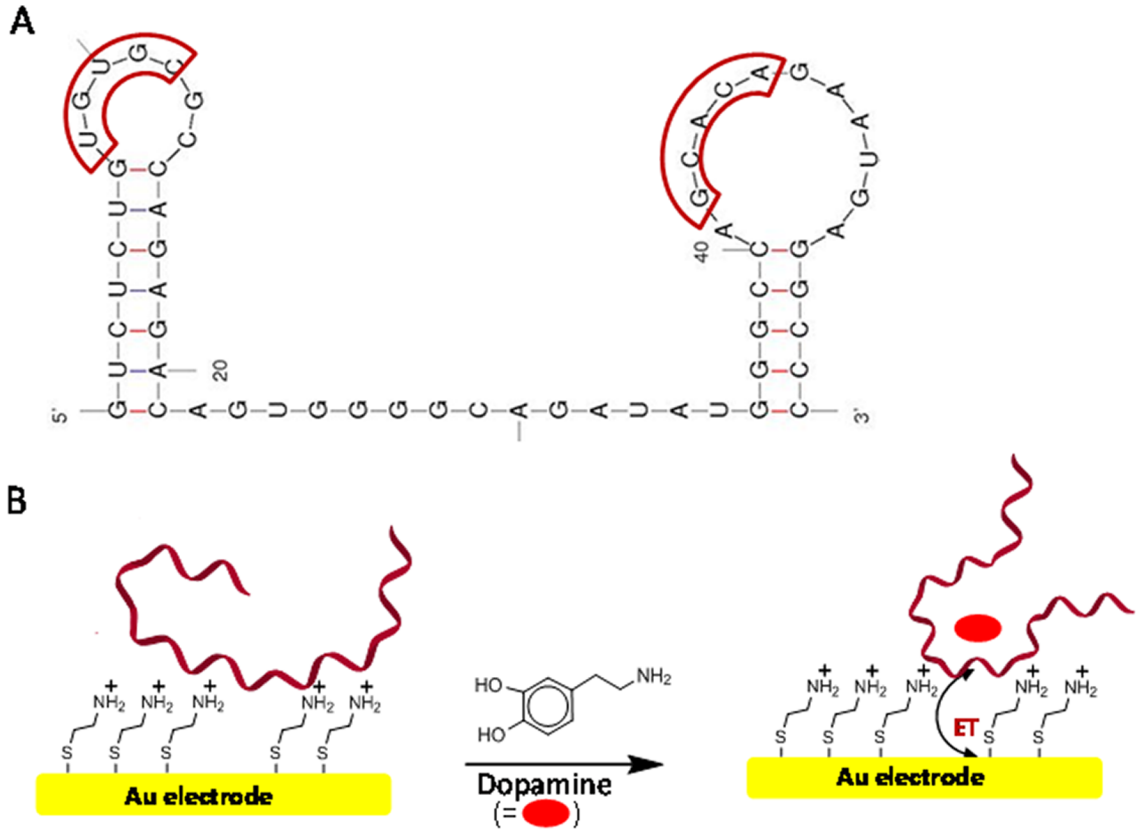

Figure 1. (A) Dopamine-specific RNA aptamer sequence (dopamine binding regions are marked with red lines) and (B) schematic representation of the electrochemical aptamer-based assay for dopamine detection.

carbon nanotubes (CNT) fiber microelectrodes[26] and especially pretreated single-wall CNT-modified electrodes,[27] providing discrimination between dopamine and AA redox signals. Negatively charged membranes were also shown to be useful for preconcentration of positively charged dopamine and cutoff of such anionic interfering compounds as 3,4-dihydroxyphenylacetic acid (DOPAC) and AA.[28,29]

However, electroanalysis of dopamine in the presence of other NT is still considered as relatively unspecific, which results from the overlapping electrochemical signals.[23] Dopamine redox potentials are similar to those of other NTs present, and that complicates direct electroanalysis of neuronal activity and disturbances in dopamine release and metabolism during neurodegenerative diseases. EC HPLC of brain microdialysates allows selective analysis of dopamine; however, this extremely useful technique is not applicable for direct in vivo measurements of the dopamine release and uptake that occur within the subsecond scale.[30,31] Thus, selective analysis of dopamine in the presence of other NTs is still considered as a challenge.

Specificity of dopamine analysis might be achieved at enzyme-functionalized electrodes[21,32] or by using aptamers as synthetic alternatives to antibodies.[33] Aptamers are single-stranded nucleic acids selected by an in vitro SELEX (systematic evolution of ligands by exponential enrichment) procedure and possess a unique binding affinity for their ligands.[34−36] Aptamer−ligand binding coupled with electrochemical detection schemes was shown to be a sensitive and versatile way of designing fast, simple, and cost-effective assays for a broad range of analytes, ranging from small molecules to proteins and cells.[34,37,38] In general, the catalytic signal amplification of enzyme-coupled electrodes makes them the best candidates for sensitive analysis of their substrates if the necessary selectivity, such as in theophylline analysis,[39−41] can be achieved. However, enzymatic assays do not provide the selectivity sufficient for dopamine discrimination over other catecholamines, due to too broad enzyme specificity and general reactivity toward phenolic groups.[42−45] On the other hand, the high specificity of the aptamer−ligand biorecognition might facilitate the design of a biosensor for selective analysis of dopamine, similarly to the already developed aptasensors for such small molecules as cocaine, ATP, and theophylline.[46−49]

In the present work the ability of a 57 nts RNA aptamer sequence to selectively bind dopamine[50] was used to develop an electrochemical aptasensor for dopamine (Figure 1). Two conserved loop regions (Figure 1A) were shown to be responsible for dopamine binding with the affinity and selectivity that should allow specific analysis of dopamine in the presence of other NTs.[50] Using the theophylline RNA aptamer as a model, we have previously demonstrated that, along with DNA aptamers, RNA aptamers can be successfully used in electrochemical biosensors operating both in pure buffer[49] and in serum-containing solutions.[51,52] RNA can be chemically modified to ensure its stability toward RNase digestion[53] for in vivo applications.

Dopamine binding to the aptamer involves two conserved loop regions (Figure 1A),[50] and certain conformational changes of the aptamer upon dopamine binding are expected. Previously, the general property of aptamers to undergo a significant structural rearrangement upon ligand binding has been actively exploited in construction of biosensors based on the electronic beacon approach.[46,49,54−57] In these works,

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binding of the analyte to the aptamer induced conformational changes of the redox-labeled aptamer, which, in turn, resulted in changes in the electron transfer (ET) between the redox label and the electrode. The latter was electrochemically read out and calibrated versus the concentration of the analyte. In our preliminary experiments, we tried to use the electronic beacon approach in the dopamine biosensor design, with redox labels placed in terminal and several internal positions of the dopamine aptamer sequence. We have determined that the conformational reorganization of the aptamer upon dopamine binding was insufficient for the development of a biosensor exploiting the conformational beacon approach, and therefore, we have focused on the alternative ways of the aptamer immobilization and studies. Since the conformational rigidity of the aptamer was no longer important, we here elaborated the protocol for the aptamer immobilization that involves electrostatic binding between the positively charged cysteamine self-assembled monolayer (SAM) on gold and the negatively charged aptamer sequence (Figure 1B). This approach was earlier shown to be highly efficient in genosensor design, even though it affected the conformational state of the immobilized DNA.[58] Since dopamine is an electrochemically active ligand, binding of it to the RNA aptamer was simply monitored by direct electrochemical oxidation of the bound dopamine.

We aimed at the aptamer-based electrochemical assay that may be in perspective applied for in vivo analysis of dopamine. Several issues were addressed: (1) Does the immobilization protocol lead to conformational changes that interfere with dopamine binding? (2) Can dopamine be selectively detected at its physiologically relevant levels in the presence of other catecholamines? (3) What is the time scale of dopamine binding/sensing with the aptamer-modified electrodes?

EXPERIMENTAL AND METHODS SECTION

Materials. Cysteamine, 6-amino-1-hexanethiol hydrochloride (C6−NH_2), 11-amino-1-undecanthiol (C11−NH_2) hydrochloride, Nafion (5 wt %), norepinephrine, epinephrine, 3,4-dihydroxy-phenylalanine (L-DOPA), 3,4-dihydroxyphenylacetic acid (DOPAC), catechol, 3-hydroxy-4-methoxyphenethylamine hydrochloride (HMP or methyldopamine), tyramine, dopamine, cysteamine, ascorbic acid (AA), and uric acid (UA), as well as primers, were from Sigma-Aldrich, Germany. *N*-Hydroxysuccinimide ester (NHS) activated methylene blue (MB-NHS) was from empBiotech, Berlin, Germany. The reagents were used as received. All solutions were prepared with Milli-Q deionized water (18 MΩ, Millipore, Bedford, MA, U.S.A.).

The RNA aptamer for dopamine (Dopa_RNA aptamer)[50] and a 5′-amino-C_6-modified RNA aptamer were synthesized by RiboTask, Denmark, and Metabion, Germany. For redox labeling, amine-terminated RNA aptamer was modified with an MB redox label by coupling between the amine terminus of RNA and MB-NHS by following the published procedure.[56] More specifically, 0.5 mg of MB-NHS was dissolved in 28 μL of DMSO; to this solution were added 15 μL of 1 M MOPS buffer (pH 8.5) and 157 μL of RNA (100 μM). This mixture was left to shake for 6 h at 900 rpm and 21 °C. After lyophilization, the pellet was dissolved in water and purified by reversed-phase HPLC (triethylammonium acetate, pH 7.0). Arbitrary DNA sequence (5′-AAA AAA CAA CTA GGA CCT AA-3′) was synthesized by the DNA Technology, Risskov, Denmark. For control experiments, a mutant version of the RNA aptamer has been constructed, carrying five point mutations designed to render the aptamer nonfunctional. To do so, a DNA template for in vitro transcription was prepared by a few rounds of polymerase chain reaction (PCR) with two partially overlapping primers (*Eco*RI_T7_DopaMut_Apt_F and DopaMut_Apt__R). DNA templates were gel-purified using GeneJet (Fermentas) as described by the manufacturer. In vitro transcription was carried out using the MegaScript kit (Ambion). Transcripts of correct size were gel-extracted, purified by phenol/chloroform extraction, and precipitated with ethanol. The integrity of the RNA was verified by gel electrophoresis. DNA sequences and the produced RNA are listed in Table S1, Supporting Information.

Electrode Modification. Prior to modification, gold disk electrodes (CH Instruments, U.S.A., 0.2 cm in diameter) were mechanically polished to a mirror luster in 1 μm diamond and 0.1 μm alumina slurries (both from Struers, Denmark) on microcloth (Buehler, Germany), ultrasonicated in an ethanol−water bath for 5 min, and further electrochemically polished by cycling in 1 M H_2SO_4 and 0.5 M H_2SO_4/10 mM KCl as described elsewhere.[52] The electrode surface area was determined from the gold surface oxide reduction peaks[59] during the final scan in 0.1 M H_2SO_4 and was typically 0.068 ± 0.014 cm^2. The electrodes were further washed in water and kept in absolute ethanol for 30 min. Self-assembled monolayers of alkanethiols were formed in the course of 2 h of adsorption of alkanethiols onto the electrodes immersed in a 20 mM alkanethiol aqueous solution (16 h in the case of C_{11}−NH_2 solution in ethanol). After that, the SAM-modified electrodes were washed with 20 mM phosphate buffer solution, 0.15 M NaCl, pH 7.4 (PBS). Then, 10 μL of a 10 μM DNA or RNA solution in PBS (RNase-free water) was placed on the top of the SAM-modified electrode and left for 2 h at room temperature (rt) under the lid. Subsequently, the DNA- or RNA-modified electrodes were carefully washed in PBS to remove weakly bound nucleic acid (NA). To cut off the negatively charged species, the Nafion-coated cellulose acetate membrane (prepared by casting 1 μL of 5 wt % Nafion solution onto the membrane, 3 kDa cutoff, and drying it for 10 min under ambient conditions) was placed onto a homemade Teflon cap tightly fitted with a rubber O-ring. The Teflon cap placed onto the electrode formed a well with a wall height of 1.6 mm and its bottom representing the gold disk electrode surface.[60] The RNA aptamer surface coverage as determined by integration of the MB reduction/oxidation peaks in the CVs, recorded for the MB-labeled RNA aptamer adsorbed onto the SAM-modified electrodes, was 6.6 ± 0.3 pmol cm^{-2}.

Instrumentation and Procedure. Cyclic voltammetry (CV) experiments were performed in a standard three-electrode cell connected to the potentiostat AUTOLAB PGSTAT 30 (Eco Chemie B.V., Utrecht, The Netherlands) equipped with a GPES 4.9.006 software. A Ag/AgCl (4 M KCl) electrode (Metrohm, Denmark) was the reference, and a Pt wire was the auxiliary electrode. All experiments were performed in PBS, pH 7, prepared with RNase-free water. Stock solutions of dopamine and other NT were freshly prepared and diluted to the required concentrations in PBS. Chronoamperometry (CA) was performed at +0.185 V. The reproducibility of the results was verified by measurements with at least five equivalently prepared electrodes.

RESULTS AND DISCUSSION

Electrochemistry of Selected Neurotransmitters at Gold Electrodes. As can be seen from representative CVs in

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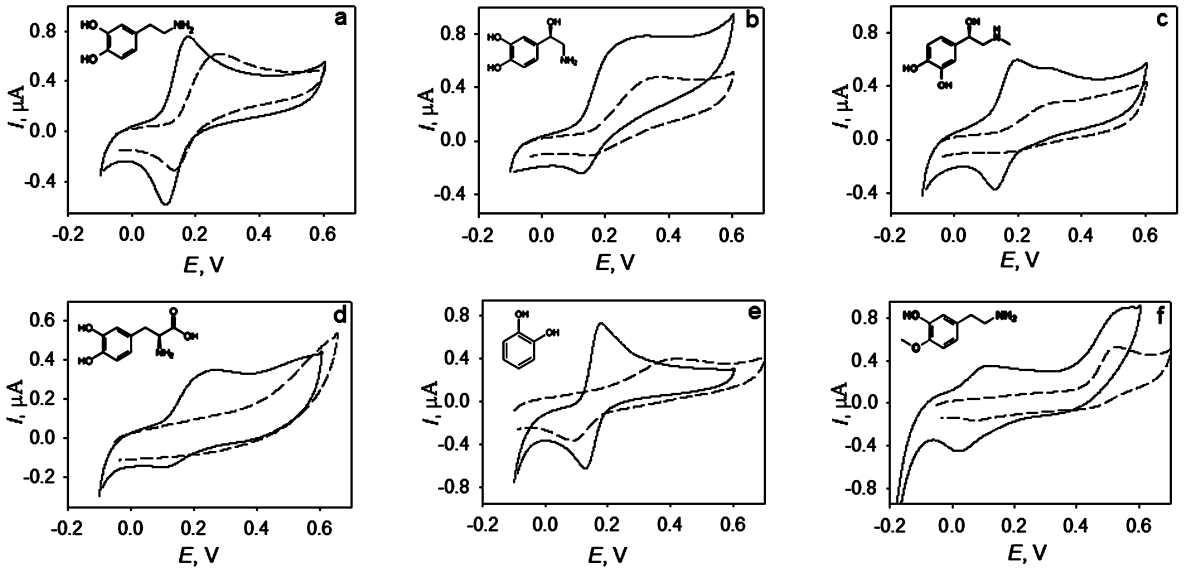

Figure 2. Representative CVs recorded with (solid lines) the bare gold electrodes and (dashed lines) the RNA aptamer/C2−NH_2/Au electrodes in solutions of 30 μM (a) dopamine, (b) norepinephrine, (c) epinephrine, (d) L-DOPA, (e) catechol, and (f) HMP. Potential scan rate is 100 mV s^{-1}.

Figure 2 (solid line curves), such NTs as dopamine, epinephrine, and norepinephrine, to which dopamine is a precursor, L-DOPA, which is a precursor of dopamine, and catechol display a very similar electrochemistry, characterized by a couple of redox peaks centered at ~0.15 V and correlating with a $2e^-/2H^+$ oxidation/reduction the catechol group of these compounds.[61−64] Other NT, structurally related to dopamine, and some methoxylated derivatives are also electrochemically active within the same potential window (Figure 2f and Figure S1 in the Supporting Information).

Overlapping electrochemical signals from the studied NT at unmodified electrodes do not allow selective sensing of dopamine in complex, physiologically relevant mixtures of NT. Aside from the lack of selectivity, the detection limit for dopamine (1 μM, Figures S2 and S3, Supporting Information) was higher than the analytically important submicromolar concentration range observed during the dopamine release.[5,31] To achieve the required specificity of the dopamine analysis, we employed a biosensing strategy based on biorecognition and binding of dopamine to the 57 nts long RNA aptamer sequence, which has already been shown to specifically recognize and selectively bind dopamine in solution.[50]

Electrochemistry of Dopamine at Aptamer-Modified Electrodes. Since the preliminary experiments showed that structural reorganization of the existing RNA aptamer[50] upon dopamine binding was insufficient for the development of the electrochemical aptasensor exploiting the conformational beacon approach, conveniently used in aptamer beacon sensors for electroanalysis of small-molecule analytes,[46,49,55,57,65] we used an alternative biosensor strategy that involved immobilization of the negatively charged RNA aptamer on the positively charged SAM of alkanethiols. Electrostatic interactions between the positively charged SAMs and negatively charged aptamer were expected to provide both stable immobilization of the aptamer on and sufficient conformational freedom to bind dopamine. This was confirmed by measuring the inherent electrochemical activity of dopamine. Several alkanethiol SAMs formed by cysteamine (C2−NH_2) and by longer amine-terminated alkanethiols (C6−NH_2 and C11−NH_2) have been studied. Representative CVs of the RNA aptamer/C2−NH_2-modified electrode in dopamine-containing solutions are shown in Figure 2a (dashed line curve) and Figure S4a (Supporting Information). As can be seen, the signal from dopamine oxidation could be followed starting from 0.1 μM, which was within the physiologically relevant concentration range and is below the 2.8 μM limit shown with the aptamer in solution.[50] However, no signal from dopamine was observed in the presence of 1 nM dopamine, which is the concentration corresponding to the background level of dopamine in the brain. From a practical point of view, such sensitivity seems to be sufficient for analysis of bursts of dopamine-related activity.

In control experiments, mutated aptamer sequence Dopa_RNA Mut_aptamer, carrying five point mutations rendering the aptamer nonfunctional (Table S1, Supporting Information), and DNA of arbitrary composition (Experimental and Methods Section) were immobilized on the C2−NH_2-modified electrodes, and electrochemistry of dopamine at these electrodes was studied. As seen in Figure S4b, Supporting Information, electrooxidation of dopamine at the mutated aptamer-modified electrode was much less efficient than at the RNA aptamer-modified electrode, with a signal from dopamine recorded only in the presence of 2 μM dopamine (similar data were obtained with the electrodes, modified with the arbitrary DNA sequence, Figure S5, Supporting Information). This concentration is 20 times higher than 0.1 μM providing the dopamine signal at the RNA aptamer-modified electrodes. Notably, the mutated aptamer sequence was designed in a way that the dopamine binding domains were positioned within the stem region of the sequence. This made the structure thermodynamically much more stable against the conformational changes required for dopamine binding and thus preventing it.

The specificity and sensitivity of dopamine detection at different RNA aptamer- and DNA-functionalized C2−NH_2/Au electrodes were evaluated by measuring dopamine CV oxidation currents in response to dopamine concentrations (Figure 3). As expected, dopamine analysis is feasible both with unmodified and RNA- or DNA-modified Au electrodes; however, the intensity of dopamine oxidation and the detectable concentration range differ. It can be seen that submicromolar electroanalysis of dopamine, which will be the

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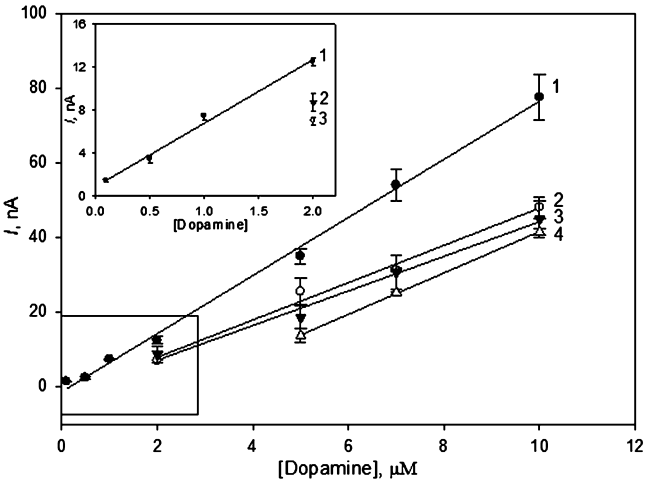

Figure 3. Concentration dependence of the CV peak currents corresponding to dopamine oxidation at cysteamine-modified Au electrodes additionally modified with (1) Dopa_RNA aptamer, (2) Mut_aptamer, (3) arbitrary DNA sequence, and (4) with no NA. Inset: larger scale for the calibration curves at the lowest concentrations of dopamine.

relevant concentration regime for dopamine binding by the aptamer, is feasible only at the RNA aptamer-modified electrode. Nonspecific analysis of dopamine, correlating with a solution electrochemistry of dopamine (a square-root dependence of the peak currents on the potential scan rate[66]) could be followed at all electrodes starting from 2 μM (NA-modified electrodes) or 5 μM (C2−NH_2-modified electrodes) dopamine levels. Along with this, the RNA aptamer-modified electrode enabled selective detection of dopamine in 0.1 μM dopamine solutions, where no other NA-modified electrode responded to dopamine.

We assumed that at low concentrations of dopamine the aptamer−dopamine binding should follow the Langmuir behavior and therefore correlate with the surface electrochemistry of dopamine oxidation. Hence, at a concentration of dopamine higher than 2 μM a strong contribution to the observed oxidation signal from oxidation of dopamine diffusing from the bulk solution to the electrode surface would be expected. Under assumption of the direct proportionality between the number of RNA aptamers and RNA-bound dopamine molecules, integration of the CV peaks related to dopamine oxidation in its bound state (a $2e^-$ process) allows estimations of the assumed surface coverage of the electrode with the aptamer. It was found to be 1.86 ± 0.12 pmol cm^{-2} as estimated from the dopamine-binding saturation level calculated by fitting the data to the Langmuir isotherm equation (Figure S6, Supporting Information). These results are consistent with a moderate NA surface coverage that should not restrict the conformation freedom of the immobilized NA[56] and retain its ability to recognize and bind the dopamine ligand. An aptamer surface coverage determined by the analysis of the voltammetric signals from the redox-labeled aptamer (when the cysteamine SAMs were modified by the MB-labeled aptamer analogue, Experimental and Methods Section and Figure S7, Supporting Information) was 6.6 pmol cm^{-2}, which designated that not all immobilized aptamer molecules were capable of dopamine binding as a result of possible conformational restrictions.

The effect of the length of SAM-forming amino-terminated alkanethiols on the ligand-binding ability of the aptamer immobilized onto those SAMs was studied (Figure 4). The

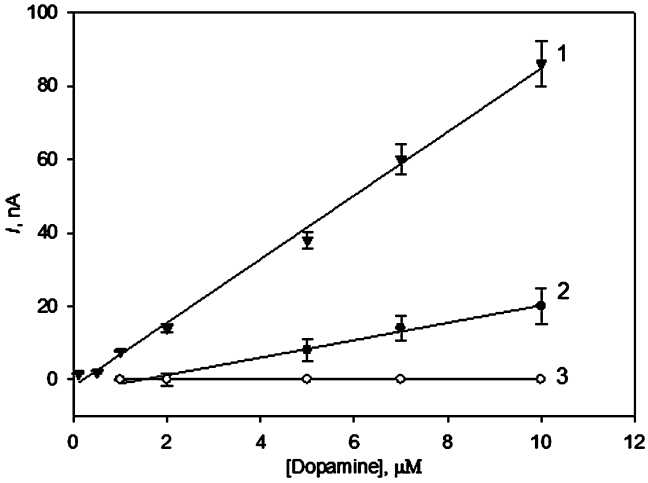

Figure 4. Effect of the alkanethiol length on the dopamine signal measured at (1) RNA aptamer/C2−NH_2/Au, (2) RNA aptamer/C6−NH_2/Au, and (3) RNA aptamer/C11−NH_2/Au electrodes. Dopamine oxidation signals were obtained from the corresponding CVs recorded at 50 mV s^{-1}.

dopamine oxidation signal significantly decreased with the increasing length of the alkanethiol chain (from C2−NH_2 to C6−NH_2) and becomes totally depressed in the presence of 11 methylene groups. It is known that short alkanethiols form disordered SAMs with a number of pinholes, while SAMs formed by alkanethiols with more than six methylene groups are well-ordered, with no pinholes, thus providing a perfect media for studies of ET reactions.[67,68] Though we cannot exclude contribution from the ET blocking ability of long SAMs to the dopamine signal depression, the short cysteamine SAM seems to provide less dense positive charge at the electrode surface and surface properties more appropriate for the aptamer immobilization. A similar phenomenon was earlier observed with the complex membrane enzyme theophylline oxidase, for which immobilization onto a short alkanethiol SAM "cushion" was compatible with biological function of the enzyme and enabled its bioelectrocatalytic function not achievable with longer alkanethiols.[40]

With longer alkanethiols, the immobilization state of the aptamer seems to be similar to the one reported for DNA immobilizations on cationic surfaces, amine-silanized glass,[58] or polycationic polymer-modified electrodes.[69,70] Though immobilized DNA retained its ability for specific base pairing, a highly asymmetrical and unwound nonhelical duplex was suggested as a product of hybridization, the conclusion supported by analysis of other reports.[71] A very special DNA duplex structure resulted from the conformational changes in DNA sequences stretched over the cationic surfaces in a comblike fashion, with bases exposed to the solution and sugar backbones electrostatically interacting with the positively charged surface. In contrast to such conformational restrictions still providing DNA the ability to hybridize,[58] ligand binding requires the conformational freedom of the aptamer sequence. This is not achieved when the aptamer is immobilized on the well-ordered positively charged alkanethiol SAMs with no pinholes. In addition, long-chain amine-terminated alkanethiols

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generally provide better blocking of the surface against any interactions between the electrode and positively charged dopamine molecules. That can be followed from the decrease of the dopamine oxidation signal at dopamine concentrations corresponding to the conditions of diffusional electrochemistry of dopamine (Figure 4). In our further studies, only cysteamine SAMs were used for the electrode modification.

Electroanalysis of Dopamine in the Presence of Other Neurotransmitters. Specificity of electroanalysis is crucial for electrochemical in vivo studies of NT release and metabolism in the brain.[21] Thus, the possibility of specific detection of dopamine in the presence of structurally (and electrochemically) related NTs was studied (Figure 2, dashed line curves). On comparison of the electrochemical responses from NTs at unmodified and aptamer-modified electrodes, certain changes in NT electrochemistry could be followed (Figure 2). For most of the studied NTs the essential change in the reversibility of their electrochemical transformations was observed, consistent with the increased peak separation and shift of the oxidation peak to higher potentials; for L-DOPA the signals actually disappeared. The least changes were observed with dopamine, though there was a 50 mV redox potential shift relative to the dopamine redox potentials at the bare electrodes.

The observed variations in electrochemical properties of NTs at the unmodified and aptamer-modified electrodes enabled us to perform a specific electroanalysis of dopamine at the RNA aptamer-modified electrodes in presence of other NTs. Chronoamperometry was performed at the half-wave potential of dopamine oxidation (0.185 V), which is sufficiently low to avoid oxidation of other NTs while still high enough for observation of the pronounced signal from the dopamine oxidation. As can be seen from the background-corrected CA responses in Figure 5, the response of the aptamer-modified

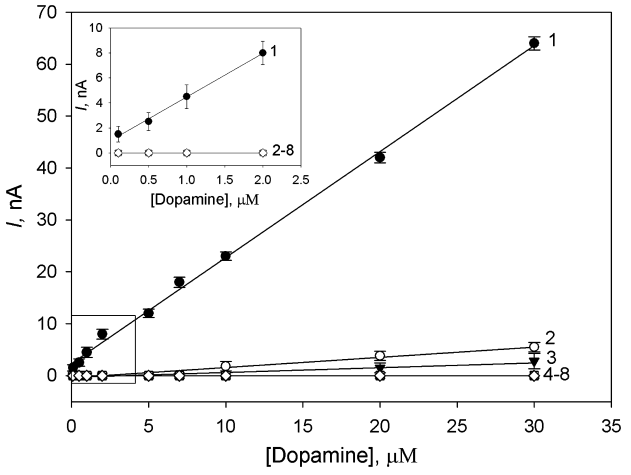

Figure 5. Dependences of the CA response of the Au/Cys/Dopa_RNA aptamer-modified electrode on the concentration of the added NT for (1) dopamine, (2) norepinephrine, (3) epinephrine, (4–8) L-DOPA, DOPAC, catechol, tyramine, and HMP, respectively. Inset: data for concentrations of NT below 2 μM.

electrodes to dopamine is much more pronounced than to other NT and is exclusively derived from dopamine at concentrations lower than 2–5 μM. The response time of the aptamer electrode toward the changes in the dopamine concentration was quite fast, 0.8 s, based on the analysis of the time required to achieve 90% of the maximum response signal, and the sensitivity of analysis was 62 ± 4 nA μM^{-1} cm^{-2}. Hence, this allows for selective electroanalysis of dopamine without disturbance from other NTs as long as their concentrations remain as low as they are expected to be in vivo.[5,30]

Effect of Ascorbic and Uric Acids on the Aptasensors Response. Under physiological conditions, in addition to NT, AA and UA also interfere with the dopamine redox chemistry: both species are electrochemically active and present in relatively high concentrations (0.2–0.5 mM) compared to dopamine, usually present within the submicromolar to nanomolar concentration range. At bare gold electrodes oxidation of dopamine and AA proceeds almost at the same potentials, which complicates signal separation. At the RNA aptamer-modified electrode the oxidation peak of AA shifts toward more positive potentials, though still insufficiently for separation of the dopamine and AA voltammetric response (Figure S8, Supporting Information). Upon addition of 0.3 mM AA the signal from 5 μM dopamine is almost totally masked by the AA oxidation wave, which does not allow quantitative analysis of dopamine. To overcome this problem, the surface of the RNA aptamer-modified electrode was covered with a negatively charged Nafion-coated cellulose membrane. Under physiological pHs, the Nafion-coated membrane strongly repulses such anionic interfering agents as AA and UA, while dopamine is attracted to the electrode surface via electrostatic interactions.[72,73] The signal from oxidation of 0.3 mM AA at the aptamer-modified electrode essentially disappeared in the presence of the Nafion membrane, which resulted in the improved selectivity of dopamine analysis in the presence of a higher concentration of AA (Figure S8A, Supporting Information). With UA, the voltammetric signals from dopamine and UA oxidation are well-separated at the aptamer-modified electrodes and allow quantitative electroanalysis of dopamine in the presence of UA (Figure S8B, Supporting Information). Nevertheless, application of the Nafion-coated membrane cuts off UA from the electrode surface, which results in concomitant disappearance of the UA oxidation signal.

Perspectives of Neurotransmitter Analysis with Aptamers. The main challenge of dopamine analysis is its real-time in vivo detection in the presence of other NTs.[21] To our best knowledge, hitherto there were no reports on modified electrode systems enabling such a highly specific analysis. The existing methods for dopamine discrimination over other NTs currently involve PET, which is noninvasive, sensitive, and selective, but along with that expensive, moderately time-resolved (<10 s), and not suited for small behaving animals technique.[4] Another widely used approach is microdialysis, in which NTs are extracted through the dialysis membrane, placed in the brain region of interest, into the flow of the perfusion fluid, which is further analyzed by EC HPLC.[3] The approach is a good alternative to PET; however, the dopamine levels determined tend to depend on the mathematical models applied for analysis, and temporal resolution is restricted to sampling intervals of 10 min, during which an amount of dialysate sufficient for analysis can be collected.[3] That time is profitable for analysis of the effects of pharmacological agents but does not allow real-time analysis of the NT release and metabolism.[21] Interpretation of microdialysis results may be also complicated by the tissue damage.[3] Microelectrode sensors produce less damage, allow improving time resolution to subsecond levels, and are sensitive within the nanomolar dopamine range, important for in vivo analysis.[15,21,74] However,

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they do not allow discrimination between catecholamines and are applicable solely in the dopamine-rich areas of the brain.

As follows from our data, dopamine can be selectively detected at the RNA aptamer-modified electrode at its physiologically relevant levels and in the presence of other electrochemically active catecholamine NTs, its own precursors, and metabolites. The specificity of the electroanalysis is much improved compared to what has been demonstrated in solution,[50] among other factors due to screening of the negative charge of the aptamer by the cysteamine SAM, thus decreasing nonspecific electrostatic interactions between the aptamer and positively charged amine group of NT. The developed immobilization protocol provides the conformational freedom of the aptamer sufficient for specific dopamine binding and its further analysis, while further chemical modifications of RNA enable its stabilization toward RNase digestion,[53] which is essential for in vivo applications. The time scale of dopamine binding and sensing with the aptamer-modified electrodes is less than 1 s, which is expected to be further improved within the microelectrode formats, eliminating diffusion limitations characteristic of macroelectrodes. Thus, the demonstrated aptasensor design can be considered as promising for possible in vivo applications, which require selective analysis of dopamine in multiplexed NT samples within the subsecond time scale.

Along with that, several issues should be definitely addressed. In comparison with a fair reproducibility of the assay (Figure S9A, Supporting Information), the reusability of the aptamer electrodes important for any in vivo application is insufficient, due to the removal of the electrostatically bound aptamer from the electrode surface (Figure S9B, Supporting Information). While regeneration itself may be performed in vivo by using the microdialysis setups,[3] the stability and mode of the aptamer binding to electrodes become crucial: any variations in the aptamer immobilization at the electrodes may result in the dramatic change of the aptasensor response, and further work should be focused on optimization of the aptamer−electrode binding strategies.

CONCLUSIONS

Here, we investigated the ability of electrochemical label-free and real-time monitoring of dopamine in the presence of other NTs using an RNA aptamer-modified electrode. The developed strategy for immobilization of the RNA aptamer onto the cysteamine SAM-modified gold electrode through electrostatic interactions allowed retaining the conformational freedom of the aptamer and its ability to selectively bind dopamine. Electrochemical oxidation of the bound dopamine enabled sensitive and selective measurements of its concentration in the presence of other NTs. The proposed biosensor for dopamine provided fast response time (<1 s) and sensitivity sufficient for electroanalysis of dopamine within the physiologically relevant 100 nM to 10 μM concentration range. Specificity of dopamine binding by the aptamer allowed selective analysis of dopamine within the 100 nM to 5 μM range in the presence of the competitive concentrations of other NTs. Since the proposed design of the aptasensor was shown not to restrict the conformational freedom and binding properties of the aptamer, it may be used with other aptamers that do not undergo structural rearrangements upon their ligands binding. In the ideal case, if an aptamer capable of conformational switching upon dopamine binding could be selected, then electroanalysis of the binding event may be moved to a potential window completely free from interference from other NTs and such interfering compounds as ascorbic and uric acids. The current work is focused on selection of such conformationally switchable aptamer, with a higher affinity and selectivity of dopamine binding over other NTs and related compounds.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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