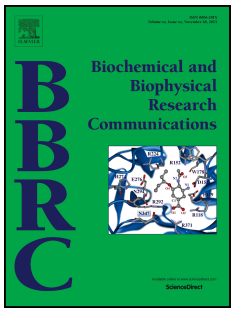

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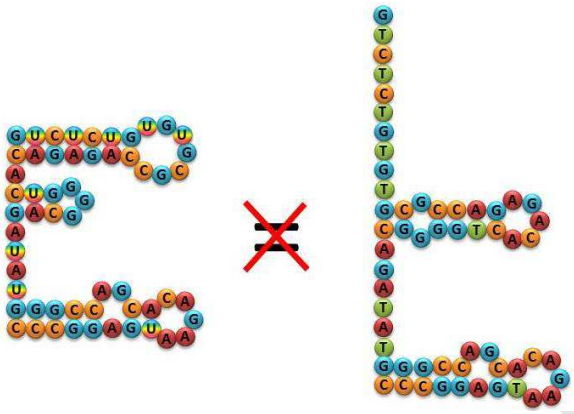
TOC figure



A DNA Sequence Obtained by Replacement of the Dopamine RNA Aptamer Bases Is Not an Aptamer

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Abstract

A unique specificity of the aptamer-ligand biorecognition and binding facilitates bioanalysis and biosensor development, contributing to discrimination of structurally related molecules, such as dopamine and other catecholamine neurotransmitters. The aptamer sequence capable of specific binding of dopamine is a 57 nucleotides long RNA sequence reported in 1997 (*Biochemistry*, 1997, **36**, 9726). Later, it was suggested that the DNA homologue of the RNA aptamer retains the specificity of dopamine binding (*Biochem. Biophys. Res. Commun.*, 2009, **388**, 732). Here, we show that the DNA sequence obtained by the replacement of the RNA aptamer bases for their DNA analogues is not able of specific biorecognition of dopamine, in contrast to the original RNA aptamer sequence. This DNA sequence binds dopamine and structurally related catecholamine neurotransmitters non-specifically, as any DNA sequence, and, thus, is not an aptamer and cannot be used neither for *in vivo* nor *in situ* analysis of dopamine in the presence of structurally related neurotransmitters.

Keywords: Aptamer, RNA, DNA, dopamine, electrochemistry, analysis

1. Introduction

Psychiatric and neurodegenerative diseases associated with malfunctions in neurotransmitter (NT) metabolism in the brain put a burden on the society shoulders, with only dementia global costs approaching 1% of world's GDP [1]. Disturbance in dopaminergic neurotransmission contributes to such disorders as Parkinson's and Alzheimer's diseases, schizophrenia, recreational drug addiction, and psychosis, all of them being related to abnormal levels of dopamine, a small molecular catecholamine NT secreted by the brain neurons [2]. Therefore, there is no surprise that dopamine occupies one of the central places in the current neuroscience research aimed at understanding of mechanisms of the disease development and their treatment.

Similarly to other NTs, the dopamine action depends both on its basal and transient levels in the extracellular space of the brain, and real-time, selective and sensitive tools for *in vivo* monitoring of dopamine are most important for studies of biochemical pathways that affect dopamine release and uptake, mechanisms of its interactions with dopaminergic receptors, and the role dopamine plays in specific neurological disorders and human behavior [3].

Clinical monitoring of NTs can be performed by microdialysis [4], photoelectron transmission scanning [5], and electrochemically, with microelectrodes inserted in the brain [6], the latter methodology being best suited for sensitive real-time *in vivo* analysis of dopamine levels, not the least due to its high temporal resolution [3]. However, the electrochemical approach suffers from insufficient selectivity: dopamine and structurally-related catecholamine NTs have similar redox potentials. Such NTs as catechol, dopamine, products of its metabolic transformation epinephrine and norepinephrine, and its precursor L-dopa can be electrochemically oxidized and reduced in the $2e^-/2H^+$ electrode reaction at around 0.15 V vs. Ag/AgCl (pH 7.4) [7]. The overlapping potentials of their electrochemical activity do not allow specific electrochemical monitoring of dopamine in the

presence of other NTs [8], and that complicates their identification in complex matrix and impedes specific analysis of their metabolism in the brain.

Selectivity of biological recognition reactions allowed avoiding interference from other catecholamines [7, 9, 10]. Among those, aptamer electrodes offer fast and simple, and still sensitive and selective tools for real-time dopamine analysis [7, 11, 12]. The only one currently available sequence capable of specific binding of dopamine is an RNA aptamer sequence [13]. The most useful for dopamine binding sequence (among several selected), in its most stable conformation, forms a three stem-loop region structure (T_m of 75.4 ^oC) (Figure 1A), with two conserved loop regions, U8-C12 and G42-A46, involved in dopamine binding, as shown by nuclease mapping and RNA sequence mutation, with a K_d approaching 1.6 μM (2.8 μM for the original sequence clone) [13].

This sequence was selected by the SELEX procedure (a systematic evolution of ligands by exponential enrichment) [14, 15] using the dopamine molecules attached to a solid support through their amine functionalities, and some affinity towards other catecholamines was also demonstrated [13]. Though, compared to dopamine, binding of other NTs to the aptamer was weaker: norepinephrine eluted the RNA aptamer bound to the dopamine-agarose gel with a 58% efficiency and L-dopa - with 30%, while for dopamine it was 100% [13]. Other catecholamines demonstrated even lower elution ability. Later studies showed that electrostatic interactions between the sugar-phosphate backbone of RNA and the amine group of catecholamines were involved in NT-RNA binding, and partial screening of the negative charge of RNA by the positively charged electrode surface (Figure 1B) allowed optimization of the RNA aptamer binding properties and absolute discrimination of dopamine biorecognition over other NTs [7, 12].

Here: Figure 1

Though the RNA aptamer immobilized on cysteamine-modified electrodes (Figure 1) demonstrated an improved stability (also in serum) [12, 16, 17], general issues of chemical instability of RNA against hydrolytic digestion in solution triggered the search of new, DNA-based aptamer sequences for dopamine. The DNA sequence obtained by the replacement of the RNA aptamer bases for their DNA analogues and exhibiting folding different from RNA, particularly, in the suggested dopamine-binding T8-C12 region (Figure 1B), was reported to be "*able to bind dopamine with improved affinity and similar specificity relative to the RNA aptamer*" [18]. Here, we show that dopamine binding by this DNA sequence is different from that shown with the RNA aptamer, and that this DNA sequence is not able of specific biorecognition of dopamine, in contrast to the original RNA aptamer sequence.

2. Materials and methods

2.1. Chemicals and reagents

Sodium hydroxide, sulfuric acid (99.99 %), ethanol (≥99.88 %), sodium chloride, components of buffer solutions, Tris-(2-carboxyethyl)phosphine hydrochloride (TCEP), cysteamine, dopamine, norepinephrine, catechol, and levodopa (L-dopa) were from Sigma-Aldrich (Germany). All chemicals were used as received without further purification. Ultrapure water from Milli-Q® water purification system (18 MΩ.cm, Millipore, Bedford, MA, USA) was used throughout the work. Stock solutions of each NT in a 20 mM phosphate buffer solution containing 0.15 M NaCl (PBS), pH 7.4, were freshly prepared before measurements and protected from light.

All RNA and DNA sequences were synthesized, purified through HPLC and mass checked by MetaBion (Germany) and used as received. They were dissolved in PBS to a final concentration of 100 µM and kept at -4 °C until use. The 5' end of each was modified with a C_6-alkanethiol linker to facilitate self-assembly to the gold electrode. The sequences used in this work were: a 57-mer RNA

aptamer specific for dopamine [13] HO-C_6-S-S-C_6-5'-GUCUCUG**<u>UGUGC</u>**GCCAGAGACAGUGGGGCAGAUAUGGGCCA**<u>GCACA</u>**GAAUGAGGCCC-3'). Dopamine binding site bases are underlined and bolded; a 57-mer DNA homolog [18] HO-C_6-S-S-C_6-5'-GTCTCTGTGTGCGCCAGAGACACTGGGGCAGAT ATGGGCCAGCACAGAATGAGGCCC-3'; a 59-mer mutated DNA: HO-C_6-S-S-C_6-5'-**g**GTCTCTGTGTGC**aa**CAGAGA**a**CACTGGGGCAGATATGGGCC**c**GCACAGAAT**cc**GGCCC-3'), mutations are highlighted with low letters.

2.2. Preparation of the aptamer modified electrodes

The polycrystalline 2 mm gold electrodes (CH Instruments, Austin, Texas) were cleaned by potential cycling in 0.5 M NaOH, polished with 1 µm diamond and 0.1 µm alumina slurries (Struers, Denmark), sonicated for 20 min in a mixture ethanol:water (1:1 v/v) and electrochemically cleaned by series of oxidation and reduction cycles in 1 M H_2SO_4 and 0.5 M H_2SO_4/10 mM KCl. Their electrochemical surface area was 0.066±0.004 cm^2 as determined from the gold oxide reduction peaks. The electrodes were kept in ethanol for at least 30 min prior modification. Clean electrodes were incubated in freshly prepared 20 mM cysteamine solution overnight (rt). Rinsed with Milli-Q water, they were afterwards placed for 30 min in 1 µM solution of either DNA or RNA, in which disulfide bonds were reduced in a 1 h reaction with 0.5 mM TCEP. To ensure the stable sensor signal, the modified electrodes were kept in PBS for at least 30 min prior to use. When not in use modified electrodes were kept in 20 mM PBS, pH 7.4, at 4˚C.

2.3. Electrochemical measurements

All measurements were performed using a three-electrode setup connected to an AUTOLAB PGSTAT 30 potentiostat (Eco Chemie B.V., Utrecht, the Netherlands) equipped with a NOVA 1.10 software. Gold disk electrodes, an Ag/AgCl filled with 3 M KCl solution (Metrohm, Denmark) and

a Pt wire were used as working, reference and counter electrodes, respectively. To enhance the NT diffusion to electrodes, NT solutions were stirred at 300 rpm for 2 min prior recording cyclic voltammograms (CVs).

3. Results and discussion

Specificity of catecholamine binding by the RNA aptamer and by the homologue DNA sequence was evaluated voltammetrically, by direct oxidation of catecholamines after their binding to the RNA- and DNA-modified electrode surface (Figure 1B). Analysis was performed under conditions not restricting binding ability of RNA [11] and improving the specificity of biorecognition of dopamine [12]. More specifically, thiolated nucleic acid (NA) sequences were co-immobilized with cysteamine on gold electrodes through their thiolated ends (Figure 1). This allowed a partial screening of the NA acid negative charges and prevented, to a certain extent, strong non-specific adsorption of catecholamines on NA-modified electrode surfaces, which is otherwise promoted by electrostatic interactions between positively charged catecholamines and negatively charged NA [7, 12].

Both at the RNA-modified and DNA-modified electrodes pronounced voltammetric peaks due to oxidation of NA-bound dopamine could be followed in the presence of dopamine, already at its 0.1 μM levels (Figure 2). These concentrations are below the K_d values reported for the RNA aptamer sequence (1.6 and 2.8 μM [13]) and the DNA homologue (1.6 μM [18]), as a consequence of non-equilibrium conditions of measurements. Electrochemical analysis under the equilibrium conditions gives K_d of 1.03±0.09 μM [12] consistent with the values reported in solution.

Here: Figure 2

The RNA aptamer could discriminate between dopamine and other catecholamine NTs, such as norepinephrine, L-dopa and catechol, which oxidation waves occurred at more positive potentials,

their half-wave potentials being shifted ca. 40-70 mV more positive versus that of dopamine oxidation (Figure 2A), consistent with previous reports [7, 11, 12]. In contrast to that, the homologous DNA sequence showed essentially similar responses for dopamine and other NTs at potentials around 180-210 mV (the half-wave potentials, Figure 2B). Those potentials are similar to those at which norepinephrine, L-dopa and catechol, non-specifically bound to the RNA-aptamer, are oxidized at the RNA-aptamer modified electrodes (Figure 2A) and are more positive than the 140 mV half-wave potential of oxidation of dopamine specifically bound to the RNA aptamer. It is important to stress that within the 0.1 - 2 μM range electrochemical oxidation of dopamine at NA-modified electrodes (and that of other NTs) followed the regularities of a surface-confined electron transfer (ET) process (a square root dependence of the peak currents on the potential scan rate) [19], consistent with previous reports [7, 11]. Such mechanism implies NT binding and further electrochemical oxidation in the bound state. At those concentrations, the distinctions in NT binding and oxidation can be followed. At NTs concentrations higher than 2 μM, the ET reaction starts to be limited by the diffusion of NTs to the electrode surface and their subsequent oxidation in the unbound state [7, 11]. Discrimination between dopamine and other NTs then becomes more challenging as a result of diminished contribution of the RNA aptamer-dopamine biorecognition reaction to the overall electrode reaction rate and, thus, is not considered here.

The DNA sequence with mutations that destabilized the dopamine-binding region of the original RNA aptamer sequence and disabled its specific dopamine-binding properties [7] showed the same catecholamine-binding pattern as the DNA homologue sequence itself (Figure 3) – and actually, similar to that of any arbitrary DNA sequence that can be used for non-specific dopamine binding studies [12]. No discrimination between dopamine and other catecholamines could be followed both with the DNA homologue and mutated DNA homologue sequences. Similar mutations introduced

in the dopamine-binding region of the RNA aptamer sequence totally depressed its dopamine binding ability [7].

Here: Figure 3

Ability of negatively charged DNA of any composition to nonspecifically interact with dopamine and related species is currently routinely used for dopamine electroanalysis: negatively charged DNA promotes electrostatic adsorption of positively charged dopamine (pK_a=8.9) on the DNA-modified surface [20, 21]. However, other catecholamine NTs such as epinephrine and norepinephrine can be also detected with the same electrodes, at the same potentials [22, 23]. Catecholamine-binding properties of the DNA homologue sequence were used for studies of oxidation of dopamine and L- and D-dopa enantiomers by the hemin/G-quadruplex-DNA complex, in a catalytic peroxidase-mimicking reaction with H_2O_2 [24]. Electrostatic compatibility between DNA and positively charged biogenic amines thus does not automatically imply any specificity of biorecognition. Our data show that a similar situation occurs with the homologue DNA sequence, and no preferential binding of dopamine over other NTs can be observed. Thus, this homologous DNA sequence is obviously not an aptamer.

This observation somehow conflicts with the statements of Walsh and deRosa that the DNA sequence produced by the replacement of the RNA aptamer bases for their DNA analogues possesses "*similar specificity relative to the RNA aptamer*" [18]. It is not. This statement misleads and actually conflicts with their own experimental evidence that dopamine and norepinephrine have similar affinities (norepinephrine displaying even higher affinity/the lower K_d value) for the DNA homologue [18]. Our observations are consistent with the experimental data shown by these authors. Replacement of the RNA bases for their DNA analogues detrimentally affects both

structural (Figure 1B) and binding properties (Figure 2B) of the original RNA aptamer sequence, none of those being found in the produced DNA homologue.

RNA aptamers represent the majority of hitherto selected aptamer sequences due to the unique ability of RNA to form highly ordered 3D architectures particularly useful for high-affinity binding of a variety of ligands [25]. This ability results from the additional 2'-hydroxyl group in the ribose sugar; this hydroxyl group forces RNA to adopt the A-form rather than the B-form helix most typical for DNA. Exactly this group also acts as an intramolecular nucleophile in ribonuclease and base-catalyzed hydrolysis, making RNA most susceptible to enzymatic digestion and chemical cleavage [26]. With no doubt, availability of DNA aptamer sequences solves the stability problems, particularly important for the aptamer applications in solutions, and it is attractive to consider that replacement of RNA bases for DNA bases may conserve the ligand binding properties of the RNA aptamers. Our data show that it is not the case, and currently it is difficult to find examples in literature to support this idea.

To summarise, here we showed that the DNA sequence obtained by the replacement of the RNA bases in the dopamine-specific RNA aptamer sequence for their DNA analogues [18] did not show any specificity of biorecognition and binding of dopamine. Such structurally related catecholamine NTs such as norepinephrine, catechol and L-dopa were able to bind to this sequence and be oxidized in the same way as dopamine itself. Thus, this DNA sequence is not an aptamer and not suitable for specific binding and analysis of dopamine, and cannot be used neither for *in vivo* nor *in situ* analysis of dopamine in the presence of other NTs.

Acknowledgement

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Figure Captions

Figure 1. Upper panel: structures of (A) the RNA aptamer for dopamine [13] and (B) the DNA sequence produced by replacing the RNA aptamer bases for DNA bases [18], their folding was evaluated by the DINAMelt [27, 28] for the buffer solution conditions used in the current work. Bases that form the dopamine binding site are marked with red ovals. (C) Electrochemical set-up used in this work for screening catecholamines' binding ability and schematic representation of the working electrode (WE) modification with cysteamine and thiolated RNA or DNA sequences. After either affinity or electrostatic binding of dopamine or norepinephrine to NA, bound catecholamines are electrochemically oxidized at the NA-modified WE.

Figure 2. Representative CVs recorded with the (A) RNA and (B) DNA aptamer/cysteamine modified gold electrodes in (1) solutions of 1 µM dopamine (DA, brown line), norepinephrine (NE, orange line), levodopa (L-DOPA, blue line), and catechol (CA, green line), and (2) without NTs (black lines). Potential scan rate is 10 mV s^{-1}. Insets show the response of those electrodes to increasing concentrations of dopamine and norepinephrine at +0.13 V.

Figure 3. Representative CVs recorded with the (A) RNA aptamer/cysteamine, (B) DNA homologue/cysteamine– and (C) mutated DNA homologue/cysteamine modified gold electrodes in the presence of 1 µM (1) dopamine and (2) norepinephrine. Potential scan rate 10 mV s^{-1}.

Figures

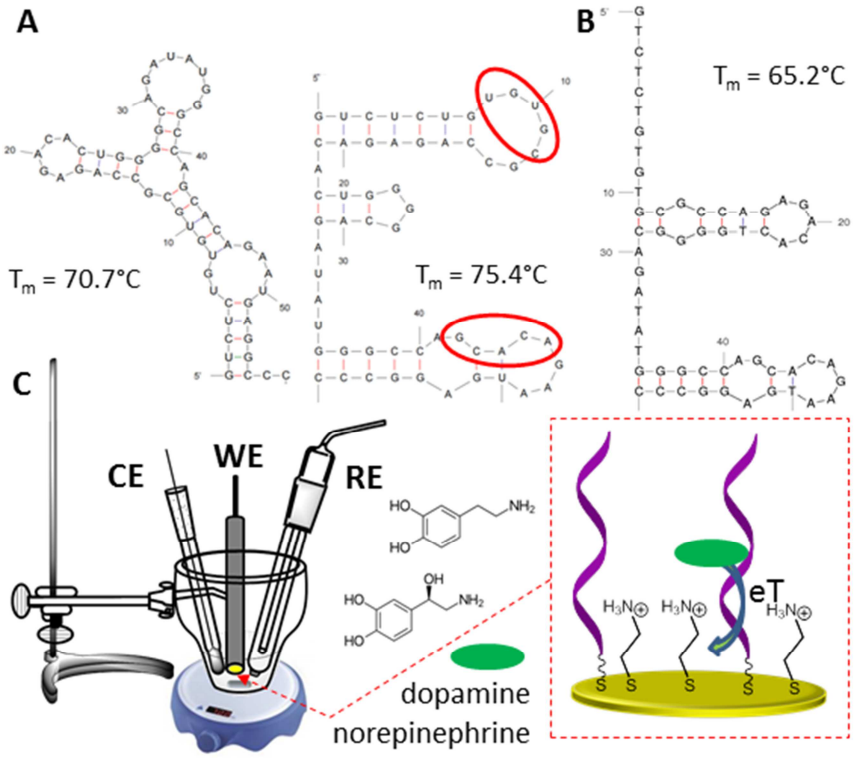


Figure 1

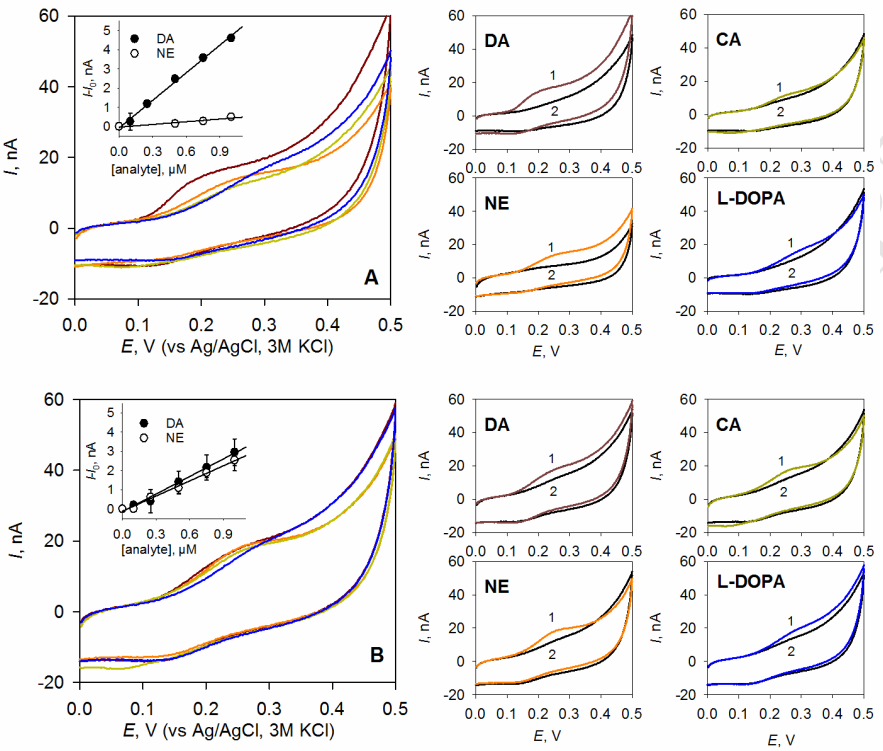


Figure 2

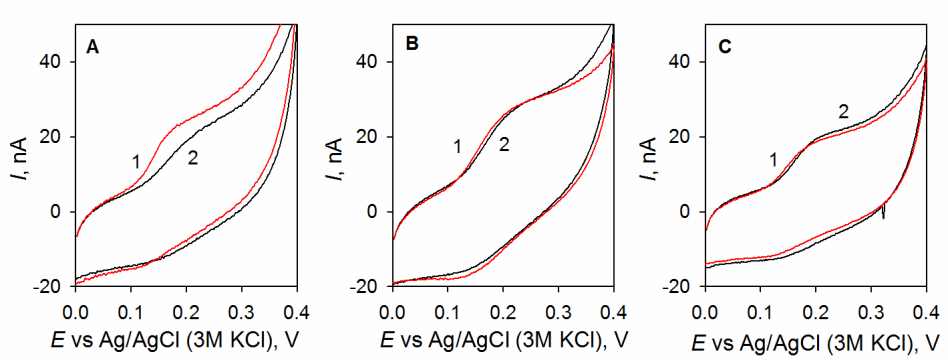
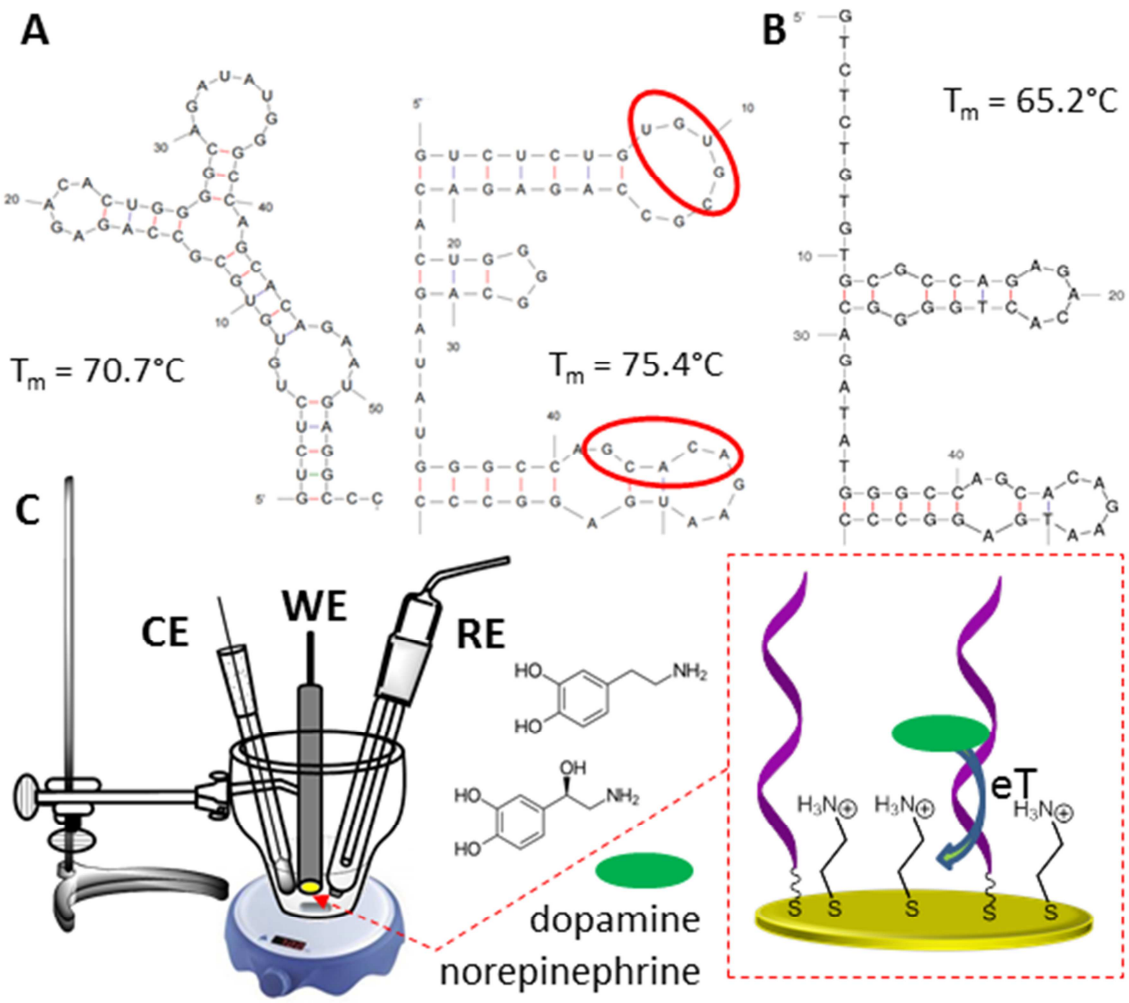
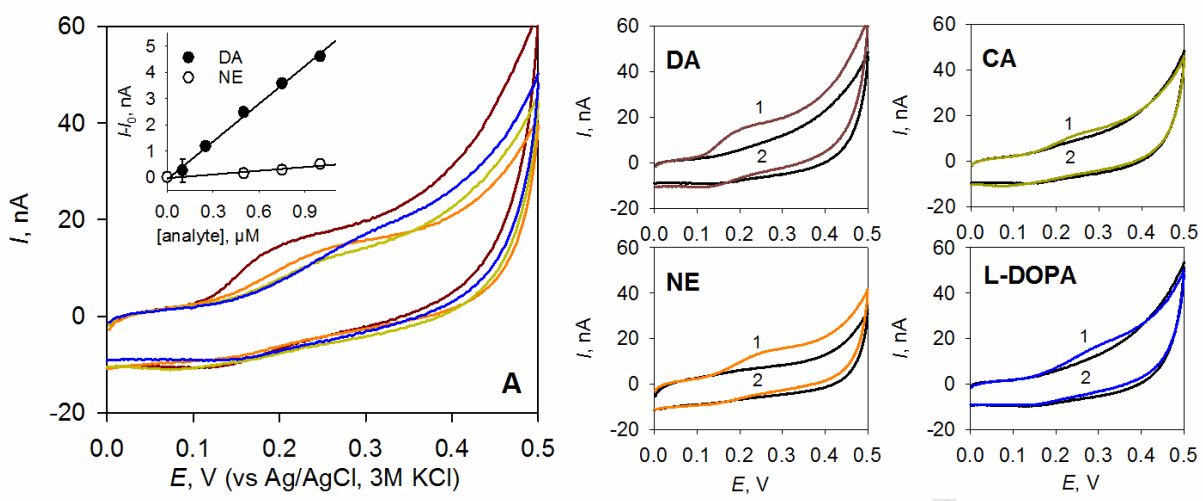
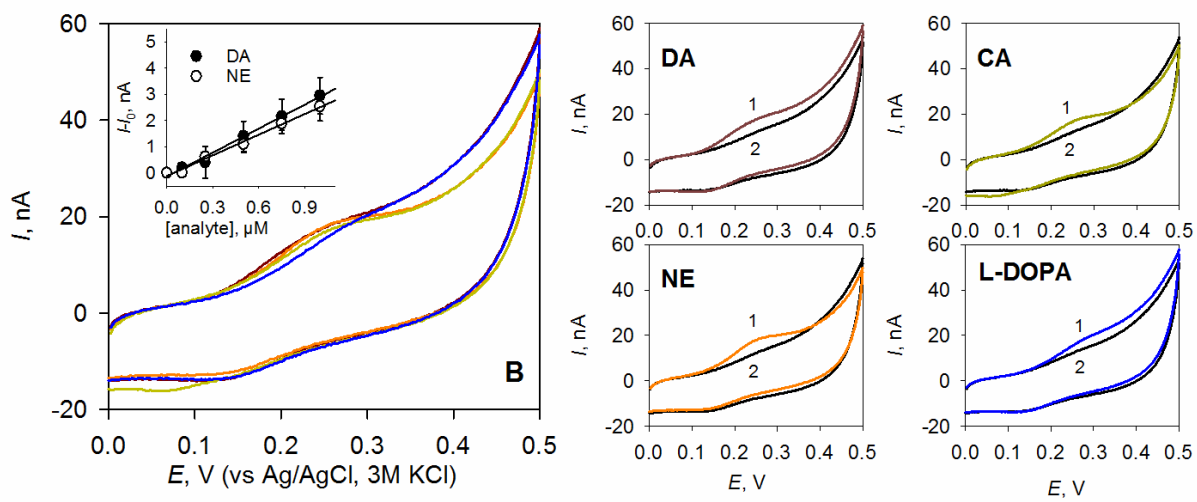
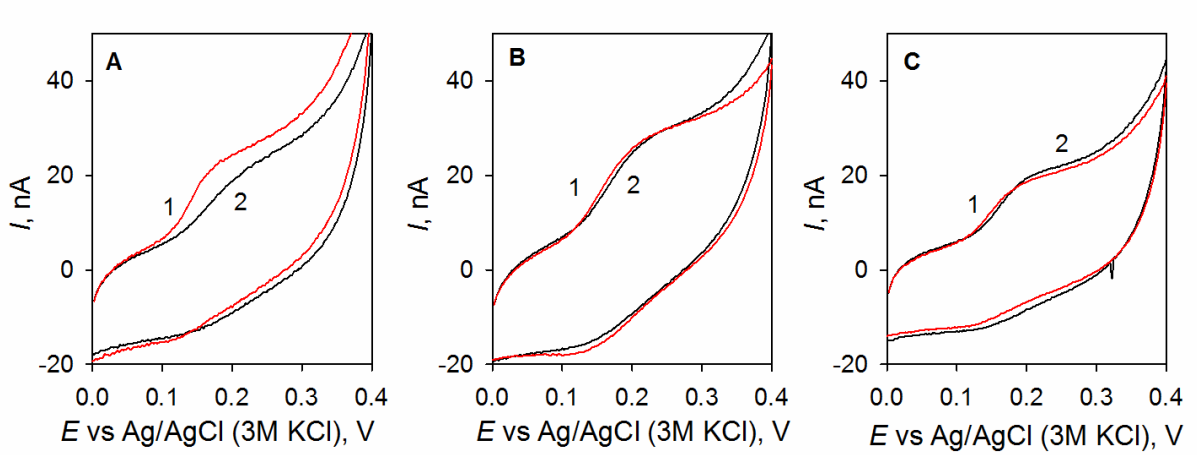


Figure 3









Highlights

- Available RNA aptamer sequence specifically binds dopamine over other catecholamines
- Specific RNA aptamer-dopamine binding allows selective detection of dopamine
- DNA sequence obtained by RNA bases replacement binds dopamine non-specifically
- Any DNA sequence can non-specifically bind dopamine and other catecholamines
- DNA–dopamine binding cannot be used for selective detection of dopamine