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Supporting Information

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Enzymatic synthesis of Redox-Labeled RNA and Dual-Potential Detection at DNA-Modified Electrodes

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Experimental Section

Abbreviations

Aq1-UTP, 5-(3-anthraquinonecarboxamidopropenyl-1)-uridine 5'-triphosphate; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; Fc1-UTP, 5-(3-ferrocenecarboxamidopropenyl-1)-uridine 5'-triphosphate; HATU, O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; HBTU, O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate; MCH, 6-mercapto-1-hexanol; RP-HPLC, reverse phase high pressure liquid chromatography; TEAB, triethylammonium bicarbonate.

Materials

Pfu DNA polymerase, DNase I, Rnasin, deoxyribonucleotides and unmodified ribonucleotides were purchased from Promega. T7 and SP6 RNA polymerases were purchased from MBI Fermentas. Bio-Spin P6 gel filtration columns were purchased from Bio-Rad. 6-Mercapto-1-hexanol (MCH) was purchased from Aldrich Chemicals. Unless otherwise stated, starting materials for chemical syntheses were obtained from Sigma-Aldrich or Bio-Rad and were used without further purification. 5-(3-aminopropenyl-1)-uridine 5'-triphosphate was synthesized according to procedure of Langer et al. (Proc. Natl. Acad. Sci. USA **1981**, *78*, 6633-6637). Other general chemicals and solvents were purchased from Ajax Chemicals. All reagents were used without further purification unless otherwise stated. Solutions were prepared with Milli-Q deionized water and the pH adjusted by addition of HCl or NaOH where necessary. All common buffers were prepared according to standard recipes.

Oligonucleotides

Oligonucleotides were purchased from Proligo or Sigma-Genosys and stored in Milli-Q water. Thiolated primers contained a C6 linker group and were purified on Bio-Spin P6 gel filtration columns following reduction to the monothiol form with dithiothreitol.

PAI-FP(SP6)
5'- CTATTTAGGTGACACTATAGCCTGGGTCAAGACTCAAACC -3'

PAI-RP(T7)
5'- CTAATACGACTCACTATAGGGCATCTGTACAGGTGTGCGCTG -3'

PAI-C2
5'- HS-GTCCTGGTGAATGCTGTCTACTT -3'

2A
5'- GGGGCAGTGCCTCACAACCT-SH -3'

EP
5'- HS-CAACGTCCGCCGAGCAGTACA -3'

Electrotide synthesis

For all synthetic protocols, ^{1}H spectra were recorded on a Bruker DPX-300 spectrometer, with chemical shifts reported in parts per million (δ) relative to an external standard. HPLC separation and analyses were performed with an Äkta Purifier system (Pharmacia Biotech). A reverse phase C18 column (Zorbax ODS, 250 x 9.4 mm) was utilized for preparative separations.

Ferrocene-UTP (Fc1-UTP) was synthesized as follows: 20 μmol of 5-(*trans*-3-aminopropenyl-1)-uridine 5'-triphosphate (triethylammonium salt) was evaporated twice from absolute ethanol to remove traces of water and dissolved in 0.5 mL of anhydrous DMF/DMSO (1:1). A mixture of 23 mg (0.1 mmol) of ferrocenecarboxylic acid, 38 mg (0.1 mmol) of HATU, and 17.4 μL (0.1 mmol) of N,N-diisopropylethylamine in 1 mL of anhydrous DMF was added to the solution of nucleotide. The reaction mixture was incubated overnight at room temperature, diluted with 20 mL of 5 mM β-mercaptoethanol in water, and the yellow precipitate of ferrocenecarboxylic acid filtered off with a 0.45 μm polypropylene membrane filter (Gelman

Sciences). The filtrate was applied to a DEAE-cellulose column (1 x 15 cm) equilibrated with 5 mM β-mercaptoethanol in water, and separated in the linear gradient of TEAB (0 – 0.5 M in 5 mM β-mercaptoethanol, 0.5 L). Product eluted as a large peak at the end of the gradient. Product-containing fractions were pooled, evaporated, and purified by RP-HPLC with a linear gradient of 0 – 50% acetonitrile in 0.05 M $LiClO_4$. The solvent was removed by rotary evaporation, the residue dissolved in 0.5 mL of water and the product precipitated by addition of 5 mL 2% $LiClO_4$ in acetone. The precipitate was washed with acetone and air-dried. Yield was 4 μmol (20%). UV (H_2O) λ_{max} = 439 nm (ε = 300 $M^{-1}cm^{-1}$). ^1H NMR (D_2O) δ 3.95 (d, J = 4.5 Hz, H9, 2H), 4.0- 4.45 (m, H2', H3', H4', H5', 5H, broad peaks), 4.2 (s, C_5H_5 of Fc, 5H), 4.53 (s, H2'', 2H), 4.78 (s, H1'', 2H), 5.7 (d, J = 6 Hz, H1', 1H), 6.3 (s, H7, 1H), 6.4 (t, J = 4.5 Hz, H8, 1H), 7.9 (s, H6, 1H).

Anthraquinone-UTP (Aq1-UTP) was synthesized as follows: The procedure was similar to the synthesis of Fc1-UTP except that anthraquinone carboxylic acid (25 mg, 0.1 mmole, 5 eq.) was used instead of ferrocenecarboxylic acid. Yield was 6 μmol (30%). λ_{max} = 260 nm (ε = 30,800 $M^{-1}cm^{-1}$). ^1H NMR (D_2O) δ 3.9 (d, J = 4.5 Hz, H9, 2H), 4 – 4.4 (m, H2', H3', H4', H5', 5H, broad peaks), 5.72 (d, J = 6 Hz, H1', 1H), 6.12 (s, H7, 1H), 6.34 (t, J = 4.5 Hz, H8, 1H), 7.62 (m, H of anthraquinone ring, 7H), 7.85 (s, H6, 1H).

Transcription template

The human plasminogen activator inhibitor-2 (PAI-2) coding sequence cloned into a pQE-30 vector (Qiagen) was used for the production of a transcription template. A 232-basepair fragment of the PAI-2 gene was PCR amplified using two chimeric primers (PAI-FP(SP6) and PAI-RP(T7)), each of which introduced a promoter at either end of the amplified fragment for SP6 and T7 polymerases, respectively. The 30 μL reaction volumes contained 200 μM dNTPs, 30 pmol of each primer, 1 μg of plasmid DNA and 3 units of *Pfu* DNA polymerase. PCR conditions were as follows: 1 cycle of 95 ˚C for 1 min, followed by 30 cycles of 95 ˚C for 45 s, 46 ˚C for 45 s then 70 ˚C for 3 min, completed with 1 cycle at 70 ˚C for 2 min.

Electrochemical RNA production

In general, for a 20 μL transcription reaction, 300 ng of template DNA, 20 units of RNasin (RNase inhibitor) and 50 units of RNA polymerase were combined with ATP, GTP and CTP each at 400 μM, unmodified UTP at 250 μM and the electrotide at concentrations ranging from 0-500 μM. Following a 37 ˚C incubation for 4 h, 20 units of DNase I was added and incubated for a further 30 min to digest template DNA. Enzymes were inactivated by heating to 75 ˚C for 15 min and the RNA purified by passing the solution sequentially through two Bio-Spin 30 columns (40 kDa cutoff). Transcript production was monitored by denaturing polyacrylamide gel electrophoresis and subsequent Sybr Green II staining and UV detection. RNA transcript concentrations were estimated by comparison with standards following denaturing polyacrylamide gel electrophoresis. Additional confirmation was achieved by spectrophotometry at 260 nm (Cary Bio 100) assuming 1 A_{260} = 40 μg/mL RNA.

Electrode construction

Polycrystalline gold wire (>99,99% gold, Aldrich) was sealed with epoxy in 4mm-diameter glass tubes to serve for mounting the working electrode. The bulk gold wire electrodes with a nominal geometrical area of approximately 0.02 cm^2 and a measured area of 0.0087 cm^2 were polished with 1.0, 0.3 and 0.05 μM alumina slurries on microcloth pads (Buehler, Lake Bluff, Il). Trace alumina was removed from the electrode surface by rinsing with Milli-Q water. Further processing included brief cleaning in an ultrasonic bath and electrochemical etching by cycling the electrode potential between -0.2 and +1.5 V in 0.05 M H_2SO_4 at a scan rate of 100 mVs^{-1} until a reproducible voltammogram was obtained.

SAM construction, hybridization and washing

Polished and cleaned electrodes were immersed in a solution of thiolated DNA oligonucleotide at concentrations of 300-1000 nM in 1 x PBS buffer for a period of 12 h at room temperature. Following adsorption of DNA onto the gold surface, electrodes were thoroughly rinsed in additional PBS buffer and incubated in 150 μM MCH in ethanol for 2.5 h. Further rinsing and incubation in PBS for at least 5 h was performed to ensure a well-ordered monolayer. Generally, CV scans of the constructed SAMs were undertaken in order to obtain background readings and minimize any scan-related changes to the monolayer. Electrode oligonucleotide density measured by the ruthenium hexammine method was 9 x 10^{12} molecules/cm^2 [Wong, E.L. and Gooding, J.J., 2003. *Anal. Chem*. 75, 3845-3852].

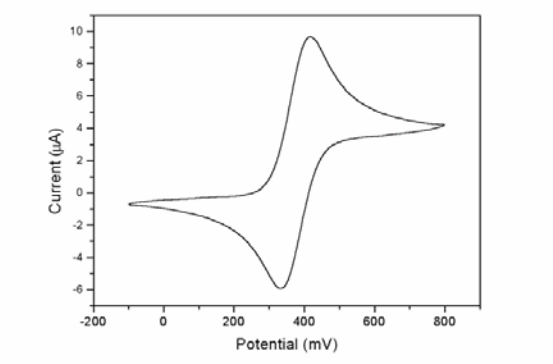
Prepared electrodes bearing the DNA probe SAMs were incubated in solutions containing labeled RNA in hybridisation buffer (1 x SSC, 1 x PBS) for at least 6 h at room temperature prior to measurement, followed by three 1 ml washes of 1 x PBS prior to electrochemical measurements.

Electrochemical measurements

Electrochemical measurements were performed at room temperature using a BAS 100B electrochemical analyser (Bioanalytical System Inc.). A conventional three-electrode system consisting of the DNA-modified working electrode, platinum flag auxiliary electrode, and a Ag/AgCl reference electrode (E_{ref} = 206 mV vs SHE) (Bioanalytical System Inc.) was used for cyclic voltammetry (CV) and Osteryoung square wave voltammetry (OSWV). Cyclic voltammetry (CV) was performed at a sweep rate of 100 mV s^{-1}. The following parameters were employed for OSWV: pulse amplitude = 25 mV, step = 4 mV, and frequency = 10 Hz. CV of nucleotides was performed in a solution of 20 mM Tris-acetate, 100 mM KCl, 1 mM $MgCl_2$ (pH 7.4). CV and OSWV of DNA SAMs were performed in hybridisation buffer. Solutions were degassed with argon for approximately 20 min prior to data acquisition.

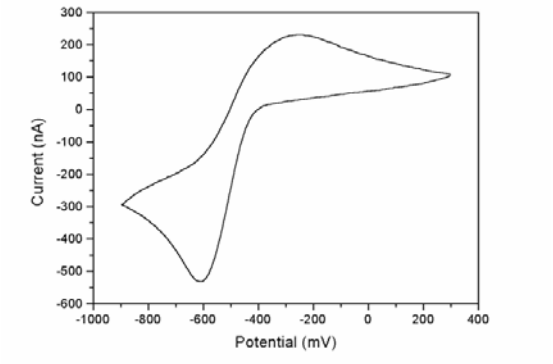
Cyclic Voltammograms

Fc1-UTP



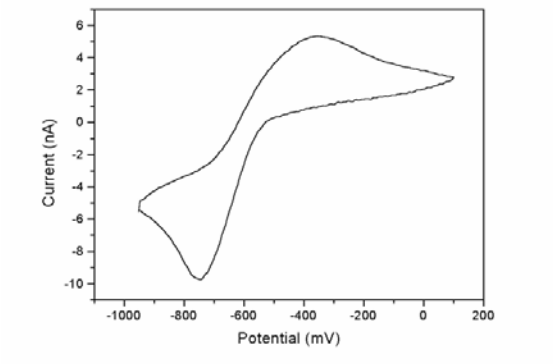
Cathodic peak potential = +409 mV
Anodic peak potential = +334 mV

Anthraquinone carboxylate



Cathodic peak potential = -311 mV
Anodic peak potential = -608 mV

Aq1-UTP



Cathodic peak potential = -399 mV
Anodic peak potential = -741 mV