Electronic Supplementary Information for

Electrochemical direct detection of DNA deamination catalyzed by APOBEC3G

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Materials and Methods

Preparation of A3G protein and nucleotides

The A3G CTD, spanning residues 193-384, was expressed in BL21 (DE3) (Agilent Technologies) and purified as described previously.[s1] Additionally, the protein was purified by gel chromatography using a Superdex 75 column (GE Healthcare) with a running buffer (50 mM sodium phosphate, pH 7.3, 100 mM NaCl, 0.005% Tween 20, 1 mM DTT). The purity of A3G CTD protein was confirmed by SDS-PAGE, and the protein concentration was estimated according to the absorbance at 280 nm and the theoretical value of molar extinction coefficient (ε = 40,450). Single-strand DNAs, 15-mer nucleotides 15-CCC (5'-GGATTCCCAATTGAG-3'), 15-CCU (5'-GGATTCCdUAATTGAG-3'), and 15-CUU (5'-GGATTCdUdUAATTGAG-3') were synthesized and purified by HPLC (Integrated DNA technologies).

Preparation of DNA probes and immobilization of the DNA probes onto gold electrodes

DNA probes Fc-GGG (5'-Fc-CTCAATTGGGAATCC-$(CH_2)_3$-SH-3'), Fc-GGA (5'-Fc-CTCAATTAGGAATCC-$(CH_2)_3$-SH-3'), Fc-GAA (5'-Fc-CTCAATTAAGAATCC-$(CH_2)_3$-SH-3'), Fc-GAG (5'-Fc-CTCAATTGAGAATCC-$(CH_2)_3$-SH-3'), Fc-AGG (5'-Fc-CTCAATTGGAAATCC-$(CH_2)_3$-SH-3'), Fc-AAA (5'-Fc-CTCAATTAAAAATCC-$(CH_2)_3$-SH-3'), and Fc-AGA (5'-Fc-CTCAATTAGAAATCC-$(CH_2)_3$-SH-3') were synthesized according to a procedure previously reported.[s2,s3] Commercially available gold electrodes (Tanaka Kikinzoku, Tokyo, Japan) were cleaned as a reported procedure[s2,s3] and dried under argon stream before use. For immobilization of DNA probes, 1 μL of a probe DNA (100 μM) in a buffer solution (10 mM phosphate buffer that contained 1 M $NaClO_4$, pH 7.0) was placed on the gold electrode and kept in a closed container under high humidity for 90 min at room temperature. After having been rinsed with the buffer solution (300 μL), the probe DNA-modified gold electrode was soaked in a solution of 1 mM

6-mercaptohexan-1-ol in the buffer solution contained 1% Tween 20 (300 μL) for 90 min at room temperature. Then, it was thoroughly washed with Milli-Q water and the buffer solution successively. For hybridization of target DNAs, 5 μL of a target DNA (10 μM) in the buffer solution was placed on the probe-modified gold electrode and kept in a closed container under high humidity for 90 min at room temperature, then it was rinsed with the buffer solution (300 μL).

Deamination reaction

The deamination reaction was performed in a buffer (50mM sodium phosphate, pH7.3, 100mM NaCl, 0.005% Tween 20, 1mM DTT) at 20˚C with 10 μM A3G CTD and 10 μM substrate nucleotide, 15-CCC. After incubation for 30 min to 24 hours, 60 μl of the reaction mixture was mixed with 6 μl of phenol/chloroform/isoamyl alcohol (25:24:1) to terminate the reaction, and then centrifuged at 18,000 x g for 2 min. 50 μl of the supernatant was applied to a centri-spin 10 column (Princeton separations) to remove buffer salts. The elute was lyophilized and stored at -30˚C for further analysis.

Electrochemical measurements

Square-wave voltammetry (SWV) measurements was carried out as previously reported.[s2] The buffer solution (10 mM phosphate buffer that contained 1 M $NaClO_4$, pH 7.0) was used as the electrolyte solution for all electrochemical studies. SWV measurement was carried out at 25 °C on the probe-modified electrodes by means of a normal three-electrode configuration consisting of the gold working electrode, a saturated Ag/AgCl reference electrode, and a platinum wire auxiliary electrode. The working compartment of the electrochemical cell was separated from the reference compartment by a glass frit.

Table S1. Optimized frequency for each voltammetry measurement

Probe	Optimized frequency (Hz)	Complementary hybrid	Mismatched hybrid	Normalized Peak Current Ratio [mismatched] / [complementary]
Fc-GGG	**40**	CCC	CCU	0.10 ± 0.07
Fc-GGA	**90**	CCU	CCC	0.23 ± 0.19
Fc-GAA	**100**	CUU	CCU	0.05 ± 0.02
Fc-AGG	**60**	UCC	CCC	0.13 ± 0.04
Fc-GAG	**140**	CUC	CCC	0.32 ± 0.06
Fc-AAA	**110**	UUU	CUU	0.04 ± 0.01
Fc-AGA	**120**	UCU	CCU	0.20 ± 0.15

References for ESI

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