Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Modulation of the current signature of DNA abasic site adducts in the α-hemolysin ion channel

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Methods

Materials

KCl, EDTA, Tris-HCl, taurine (Trn), Arg-His (RH), D-(+)-glucosamine (GlcN), Gly-Pro-Arg-Pro amide (GPRP), streptomycin (STM), $NaBH_3CN$, wild-type α-HL, phospholipid 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC), 2-aminomethyl-15-crown-5 (15c5), 2-aminomethyl-18-crown-6 (18c6), streptavidin and uracil-DNA glycosylase (UDG) were purchased from commercial suppliers and used without further purification.

Preparation and characterization of DNA AP site adducts

The oligodeoxynucleotides (ODN) were synthesized from commercially available phosphoramidites (Glen Research, Sterling, VA) by the DNA-Peptide Core Facility at the University of Utah. After synthesis, each ODN was cleaved from the synthetic column

and deprotected according to the manufacturer's protocols, followed by purification using a semi-preparative ion-exchange HPLC column with a linear gradient of 25% to 100% B over 35 min while monitoring absorbance at 260 nm (A = 20 mM Tris, 1 M NaCl pH 7 in 10% CH_3CN/90% ddH_2O, B = 10% CH_3CN/90% ddH_2O, flow rate = 3 mL/min). Uridine-containing oligomers (10 μM, 1 nmole) and 1 unit UDG were thermally equilibrated in UDG buffer (pH 8.0) at 37 ^oC for 30 min, followed by dialysis against ddH_2O for 12 h. The resulting AP-containing ODNs (10 μM, 1 nmole) were dried down and resuspended in MOPS buffer (pH 6.5), followed by the addition of the appropriate amine (20 mM, 2 μmoles) and $NaBH_3CN$ (100 mM, 10 μmoles); then the reactions were kept at 37 ^oC for 24 h. Unreacted AP sites-containing ODNs were cleaved with 0.1 M NaOH. After dialysis against ddH_2O for 12 h, all products were purified by analytical ion-exchange HPLC running a linear gradient of 25% to 100% B over 35 min while monitoring absorbance at 260 nm (A = 20 mM Tris, 1 M NaCl pH 7 in 10% CH_3CN/90% ddH_2O, B = 10% CH_3CN/90% ddH_2O, flow rate = 1 mL/min) Analysis of the crude-reaction products indicated yields of approximately 85-90%. The identities of the 3'-biotinylated ODNs were determined by negative ion electron spray (ESI^-) mass spectrometry on a Micromass Quattro II mass spectrometer equipped with Zspray API source in the mass spectrometry laboratory at the Department of Chemistry, University of Utah (Table S1).

Ion channel recordings

Ultrapure water (>18 MΩ•cm) was prepared by a Barnstead E-pure water purifier and used to make buffered electrolyte solution (1.0 M KCl, 1.0 mM EDTA, 25 mM Tris, pH=7.9) that was used for the single ion channel current recordings. The electrolyte was filtered with a sterile 0.22 mm Millipore vacuum filter before the measurement. The protein α-HL was diluted to a 0.5 mg/mL solution in ultra pure water and the lipid DPhPC was dissolved in decane to a concentration of 10 mg/mL, both of which were stored in a -20 ^oC freezer. The glass nanopore membrane (GNM) (radius = 600 nm) was fabricated as previously established,[1] and was silanized in 2% (v:v) 3-cyanopropyldimethylchlorosilane in CH_3CN for 6 h. Ag/AgCl electrodes were prepared by soaking silver wires (diameter = 0.25 mm) in bleach.[2] In the immobilization studies,

the 3'-biotinyliated ODNs (160 pmol) were mixed with streptavidin (40 pmol) and kept at 23 ^{o}C for 20 min before the measurements, while in the translocation studies, the 87-mer ODNs were used directly after purification and dialysis.

A custom built high-impedance, low noise amplifier and data acquisition system, donated by Electronic Bio Sciences, San Diego, CA, was used for the current-time (*i*-*t*) recordings. The GNM was rinsed with CH_3CN, ethanol and ultrapure water, and then filled with the electrolyte described above. A pipette holder with a pressure gauge and a 10 mL gas-tight syringe was used to locate the GNM to the DC system. Two Ag/AgCl electrodes were positioned inside and outside of the GNM to apply an electrical potential.

The lipid DPhPC solution (1 μL) was painted on the GNM surface using a plastic pipette tip (flat gel-loading tips, 1-200 μL) to form a suspended bilayer, which was confirmed by the resistance of approximately 100 GΩ, a dramatic decrease from that of an open GNM orifice (10 MΩ). After the addition of α-HL (0.2 μL), a pressure was applied to assist the insertion of the ion channel protein, which had a resistance of around 1 GΩ under these conditions.

In the immobilization studies, Strep-Btn DNA (40 pmol, 200 nM) was added in the cell and more than 200 capture/release events were collected under -120 mV bias with a 10 kHz low pass filter, and 50 kHz data acquisition rate. Then the same amount of Strep-Btn C_{40} was added as an internal standard, and ~200 events were collected for each strand under the same conditions.

Notes and references

1. B. Zhang, J. Galusha, P. G. Shiozawa, G. Wang, A. J. Bergren, R. M. Jones, R. J. White, E. N. Ervin, C. C. Cauley and H. S. White, *Anal. Chem.*, 2007, 79, 4778-4787.
2. R. J. White, E. N. Ervin, T. Yang, X. Chen, S. Daniel, P. S. Cremer and H. S. White, *J. Am. Chem. Soc.*, 2007, 129, 11766-11775.

$C_{39}X_{\omega 14}$ (X =)	Calculated Mass	Experimental Mass
C	12075.2	12075.3
U	12076.2	12076.0
Trn	12091.2	12091.9
GlcN	12145.3	12144.8
RH	12276.4	12276.8
GPRP	12390.4	12390.1
STM	12547.5	12546.9
15c5	12215.4	12215.2

Table S1. Mass spectrometry (ESI) characterization of the oligonucleotides. The ODNs presented here were biotinylated at the 3' terminus, and position 14, relative to the 3'-biotin tag, was substituted with various amines (X) in a poly-dC background.

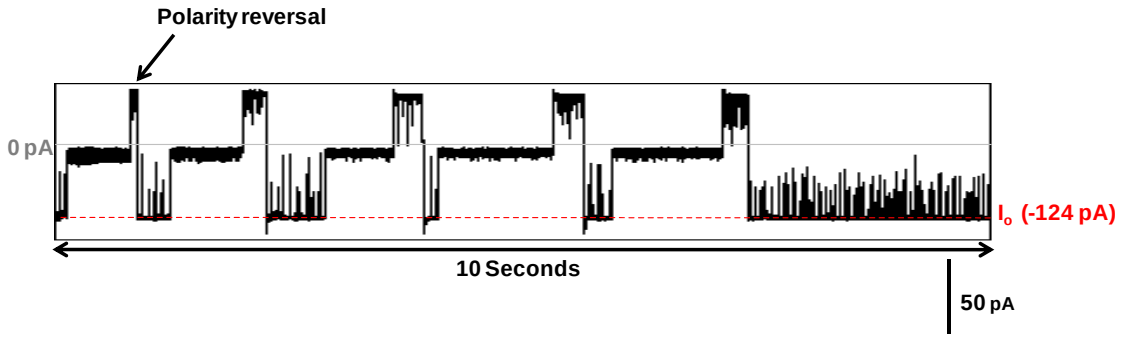

Fig. S1 Example of *i*-*t* trace for $C_{43}STMC_{43}$ at 120 mV (*trans* vs. *cis*).