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

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Abstract

Electrical current signatures of DNA adducts were investigated during immobilization of strands inside the membrane-bound α -hemolysin ion channel. The current blockages produced by these adducts were found to depend on both size and shape, providing insights into the DNA-protein interactions and the size limitation of bulky adducts to be translocated.

The α -hemolysin (α -HL) protein ion channel has been under intensive investigation as a DNA analysis platform.^{1, 2} Most studies are focused on rapid, low-cost sequencing technique development via monitoring the different current blockage levels and the durations of native DNA bases as an electric field drives single-stranded DNA (ssDNA) through the channel.^{3–8} One of the biggest challenges of this technology arises from the low current amplitude resolution between the nucleobases while ssDNA translocates through the protein channel. Along with the high speed of the ssDNA electrophoretic movement, the available electronics cannot provide a high enough signal-to-noise ratio to achieve single-nucleotide identification.¹ Approaches have been developed to overcome these technical hurdles: DNA polymerases have been utilized to ratchet the DNA molecule one nucleotide at a time to control the translocation speed, consequently increasing the accuracy.^{7, 8} Simultaneously, site-directed mutagenesis was applied to engineer the protein ion channel, producing narrower recognitions and higher capture rates.^{9, 10} Experimental conditions were explored to assist the unraveling of DNA secondary structures, and to slow down the translocation speed.^{11–13} Additionally, small molecules have been synthesized to bind the α -HL ion channel as adapters for the ssDNA translocation.^{14, 15} The porin MspA of *Mycobacterium smegmatis* has also been explored as another transmembrane protein ion channel with promising properties.⁷

Recently, an 18-crown-6 motif was selectively attached to the DNA abasic (AP) sites, which yielded unique current signatures and provided the detection of individual AP sites along the same strand at a single-molecule level.¹⁶ In order to gain insights into the correlation between the molecular structure and the electrical currents produced in the α -HL ion channel, we now report an investigation of various AP site adducts with different sizes, shapes and rigidities synthesized through reductive amination in the presence of a primary amine (Fig. 1; see ESI for experimental procedures). ssDNA was immobilized inside the nanopore by capturing 3'-biotinylated strands in the presence of streptavidin, which is too large to enter the protein cavity.^{17, 18} Each strand contained the modification located at the ω 14 position, the site that presented the best current discriminations between native bases.¹⁷

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An electrical potential (120 mV) drove the complex to enter the *cis* side of the protein channel, reducing the open channel current I_0 to a lower level I , both of which were recorded, and $\%I/I_0$ is presented here as the percentage of residual current (Fig. 2).

The amines attached to AP sites included taurine (Trn), Arg-His (RH), glucosamine (GlcN), Gly-Pro-Arg-Pro amide (GPRP), 15-crown-5 (15c5), 18-crown-6 (18c6) and streptomycin (STM) (Fig. 1). These compounds represent readily available water-soluble amines that would explore the relationship of size, shape and rigidity to changes in electrical current levels. In a poly-dC background sequence, the $\%I/I_0$ histograms of the adducted DNA ($C_{39}X_{\omega 14}$) were compared to those of the non-substituted C_{40} strand, which was set to 0 (Fig. 3A). For comparison, we also plotted the histograms of $C_{39}AP_{\omega 14}$ and $C_{39}18c6_{\omega 14}$, which were taken from published results.¹⁶ Except for STM and 18c6, all adducts exhibited single distributions of residual current levels. $C_{39}Trn_{\omega 14}$ had a broader but similar $\%I/I_0$ compared to C_{40} , not surprisingly as the molecular size of this adduct is fairly close to an unmodified C_{40} . For all other adducts, the disturbance of the poly-dC secondary structure caused different interactions of the ssDNA with the interior of the protein channel, resulting in broader $\%I/I_0$ distributions. Additionally, the bulkier adducts $C_{39}RH_{\omega 14}$ and $C_{39}GlcN_{\omega 14}$ blocked the open channel current ~2% and ~3% more than an AP site ($\Delta\%I/I_0 = \sim -1.1\%$), respectively. Even though the RH dipeptide is larger than GlcN, $C_{39}GlcN_{\omega 14}$ had a larger $\Delta\%I/I_0$ than $C_{39}RH_{\omega 14}$ did, and this could be explained by the different shapes these two amines display; RH is more linear than GlcN, suggesting that there are more factors than merely size affecting the electrical signatures of the DNA AP adducts. The tetrapeptide GPRP produced an even deeper blockage, and the $\Delta\%I/I_0 = \sim 8\%$ compared to AP sites presented an absolute residual current difference of $\Delta I \sim 10$ pA in 1 M KCl under 120 mV (*trans* vs. *cis*), as expected for an oligopeptide adduct.¹⁹

The two flexible crown ethers can adopt rather different conformations upon complexation with the K^+ ion present in the electrolyte. While the 18c6- K^+ complex has a single quasiplanar symmetric D_{3d} conformer, due to the ideal sizing matching between the cation and the polyether cavity,²⁰ the K^+ ion is too big to fit entirely into the cavity of 15c5. Thus, 15c5 is forced to distort its backbone to achieve the greatest interaction with the cation lying above it, producing a smaller and more dynamic conformation.²¹ As a result, $C_{39}15c5_{\omega 14}$ presented a single current amplitude peak, indicating the successful positioning of the adduct in the narrow β barrel of α -HL, but the large, rigid $C_{39}18c6_{\omega 14}$ adduct had difficulty passing the constriction of the ion channel.¹⁶ Similarly to $C_{39}18c6_{\omega 14}$, $C_{39}STM_{\omega 14}$ yielded multiple peaks in the $\%I/I_0$ distribution, which featured a ~9% more blocking $\%I/I_0$ relative to AP sites in addition to another peak in the histogram lining up with C_{40} (Fig. 3A). Given the large size of STM, this is likely indicating that $C_{39}STM_{\omega 14}$ could not immediately enter the β barrel of α -HL, and thus it hesitated at the constriction, causing the electrical current to reflect the positioning of only the poly-dC portion of the strand. Apparently, STM could be forced into the constriction, but not in a reproducible manner, generating the broader and deeper blockage current distribution. To confirm this hypothesis, a translocation study of the STM adduct was performed in the absence of streptavidin, and this led to an 'immobilization-like' current-time trace. This indicates trapping of the adducted strand within α -HL without passing to the *trans* side of the channel (Fig. S1).

By merely examining the molecular weights of all these adducts, we see that the current blockage levels of Trn, GlcN, RH, GPRP and STM followed such a general trend, with STM as the size limitation of not being translocated by the α -HL ion channel. However, shape and rigidity have a greater effect on the electrical signatures of the crown ether adducts compared to molecular weight alone. The molecular weight of 18c6 is much lower than GPRP; yet, it cannot pass the constriction, due to the rigid and planar shape of its K^+ complex. The less rigid and smaller 15c5- K^+ complex had less difficulty doing so.

In order to investigate the sequence context effect on the current blockage level, U, AP and the GPRP adduct were incorporated at the $\omega 14$ position in a 40-mer oligonucleotide containing a portion of the *K-ras* gene sequence (Fig. 3B). This particular site at which U, AP and the GPRP adduct were positioned is highly prone to a G→A transition mutation, predominantly in colon, breast, lung and brain cancers.²² As one major source of the G→A transition mutation, deamination of C to U could be specifically recognized and removed by UDG, a base excision repair glycosylase, generating an AP site that could be further functionalized to form a GPRP adduct. Fig. 3B presents the $\%I/I_o$ histograms for *K-ras*, *K-rasU* $_{\omega 14}$, *K-rasAP* $_{\omega 14}$ and *K-rasGPRP* $_{\omega 14}$ compared to *C* $_{40}$, and they all showed single residual current distributions. *K-ras* and *K-rasU* $_{\omega 14}$ both had similar but sharper $\%I/I_o$ histograms compared to *C* $_{40}$, while *K-rasAP* $_{\omega 14}$ blocked electrical current ~0.5% less than *K-ras* ($\Delta \%I/I_o = \sim 1\%$ for *C* $_{39}AP$ $_{\omega 14}$ and *C* $_{40}$), indicating that sequence context has a slight effect on the electrical signatures of AP sites and native bases. Recently, it has been reported that the sequence context could change the blocking orders of DNA damaged bases and using an engineered α -HL nanopore could also alter the percentage residual current of native bases.^{17, 23} In the *K-ras* gene sequence, the GPRP adduct reduced the open channel current ~8% more than *K-rasAP* $_{\omega 14}$, which is significant enough to be distinguished from native bases irrespective of the sequence context, even with wild-type α -HL.

In conclusion, the investigation of the electrical signatures of amine adducts to the DNA AP site in both homopolymer and heterosequences demonstrates the power of chemical modification to shift the residual current of AP sites in a wide range. In particular, the presence of U compared to C in the *K-ras* gene sequence could be clearly identified by enzymatic conversion to an AP site followed by chemical ligation of a tetrapeptide GPRP and analysis with the α -HL ion channel. The behaviours of the various adducts depend on their size, shape and rigidity, a finding that provides insights into the design of chemical labels and contrast markers for both lesion detection and sequencing efforts with single molecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. DNA-AP adducts synthetic scheme. Taurine (Trn), glucosamine (GlcN), Arg-His carboxamide (RH), Gly-Pro-Arg-Pro carboxamide (GPRP) and streptomycin (STM), 2-aminomethyl-15-crown-5 (15c5) and 2-aminomethyl-18-crown-6 (18c6) were attached to DNA AP to produce adducts (AP-NR) via reductive amination.

Fig. 2.

Illustration of the immobilization events. Four biotinylated ssDNA strands (red) were bound to the streptavidin tetramer (black) to form a complex. When electrically driven into the α -HL ion channel (blue), the strand remained captured, causing a reduction of the open channel current I_o to the blockage level I . The polarities of electrodes were reversed to release the DNA strand prior to capture of another molecule. Histograms of changes in current blockage levels I/I_o are presented in subsequent figures.

Fig. 3.

(A): $\Delta\%II_o$ histograms of U, AP sites and AP adducts at $\omega 14$ position relative to C in a poly-d C_{40} sequence. (B): $\Delta\%II_o$ histograms of U, AP sites and GPRP adduct at $\omega 14$ position relative to C in the *K-ras* sequence. Every histogram has more than 200 events with a bin increment of 0.1%, and $\%II_o$ of C_{40} was set to 0.