Published in final edited form as:

Chem Commun (Camb). 2012 February 1; 48(10): 1520–1522. doi:10.1039/c1cc16124e.

Nucleobase recognition at alkaline pH and apparent pK_a of single DNA bases immobilised within a biological nanopore

Lorenzo Franceschini^a, Ellina Mikhailova^b, Hagan Bayley^b, and Giovanni Maglia^a

Giovanni Maglia: giovanni.maglia@chem.kuleuven.be

^aDepartment of Chemistry, University of Leuven, 3001, Heverlee, Leuven, Belgium

^bDepartment of Chemistry, University of Oxford, Oxford, OX1 3TA, United Kingdom

Abstract

Ionic currents through DNA filled biological nanopores can be used to identify the four DNA bases at high alkaline pH and to measure the apparent pK_a values of single nucleobases.

Keywords

alpha-hemolysin; DNA sequencing; nanopore sequencing; DNA stretching; pH dependence; nucleobase ionisation

The identification of single nucleobases during electrophoretic translocation of single stranded DNA molecules (ssDNA) through nanopores is under intense scrutiny in the effort to develop a low-cost and high-speed third-generation DNA sequencing technology.¹ One of the challenges in nanopore sequencing is that ssDNA samples must be both prepared from genomic dsDNA and their local structure disrupted in order to allow translocation through the nanopores.² Direct nanopore sequencing at alkaline pH would be a valuable solution because it would promote the denaturation of genomic dsDNA samples into ssDNA^{3–5} and, at the same time, disrupt the intramolecular base-paired interactions in the ssDNA molecules. Previously, we have shown that although the α HL nanopore displays stable open pore current at alkaline pH up to 13, DNA can translocate through the pore only at high ionic strength (e.g. 3 M KCl) and when using a modified version of the α HL nanopore in which an arginine residue substitutes for Met113 at the constriction (Fig. 1).⁶ In this work, we investigated whether such modifications also allow the identification of the four DNA bases at pH values up to 11.7, at which dsDNA denatures into ssDNA.^{3–5} Because the additional positive charges required for DNA translocation at high pH reduce the ionic current employed for nucleobase recognition,^{7, 8} for this task, we used an α HL nanopore (E111N/K147N/M113R or NN-M113R) engineered to have a wider and neutral central constriction (Glu111 $\rightarrow$ Asn and Lys147 $\rightarrow$ Asn) and the Met113 $\rightarrow$ Arg substitution at the central constriction. Since the electrophoretic translocation of DNA through the pore is too quick for direct base identification, we used a biotinylated ssDNA molecule and halted translocation through the pore by the interaction with streptavidin (Figure 1).^{9, 10} By using the streptavidin approach, we have shown before that the α HL nanopore contains three

recognition point for nucleobases, one of which is at the central constriction of the pore and recognizes bases at positions 8 to 11 in an immobilised DNA strand with a peak at position 9.¹⁰ We also showed that at pH 8.0, in 1 M KCl and under an applied potential of +160 mV, the NN-M113R nanopore can recognize the four DNA bases at position 9.¹¹

By using the same immobilisation technique with the NN-M113R pore, we investigated four ssDNA oligonucleotides containing a single nucleobase difference at position 9 (Fig. 1b) from pH 7.0 to pH 11.7, at +120 mV and in 3 M KCl. At pH 11.7, although the blocked pore current was lower than at pH 8.0 (Fig. 2a and Table S1), the differences between the blocked current signals (ΔI_B) among all four nucleotides were comparable to the ΔI_B values recorded at pH 8.0 (Fig. 2A), indicating that α HL nanopores are able to discriminate between the four DNA bases in DNA strands immobilized within the pore under the alkaline conditions that denature dsDNA. Since the sampling of the recognition point by the Streptavidin conjugated DNA molecules requires a well defined folded structure of the nanopore, these results also suggest that the α HL retains its native-like structure at pH up to 11.7. Although the four nucleotides evoked four separate I_B levels over the pH range investigated, the discrimination between the nucleobases varied with pH. As a measurement of discrimination, we used δ , which is the product of the three sequential differences between each of the four I_B levels.¹¹ The best discrimination between the four DNA bases was observed at pH 8.0, while the worst discrimination was at pH 10.4 (Fig. 2B). Interestingly, the change in δ was not due to a change in the open pore current (I_O) of the NN-M113R pore, which was constant from pH 7.0 to pH 11.0 and only slightly decreased at higher pHs (~5% at pH 11.7), but was mainly due to changes in the blocked pore current.

Experimental^{10–12} and theoretical^{12–14} works have shown that the ionic current through a nanopore is strongly affected by the fixed-charge distribution within the lumen of the pore and by the ionic strength of bulk solution. Further, ionic currents can be used to determine the pK_a of residues within the lumen of a nanopore.^{6,15,16} For example, we have shown that in the WT- α HL nanopore the rectification ratio (expressed as the current at +100mV divided by the current at –100mV) is likely related to the protonation state of the seven Lys147 at the constriction.⁶ Interestingly, their apparent cumulative pK_a value (the seven lysine residues on each monomer are ~ 5nm apart) seem not significantly shifted from the expected value of single lysine residues in solution.⁶ In a DNA-filled NN-M113R pore, I_B is most likely influenced by the negative charges on the DNA (~12 nucleobases are expected to fill the β barrel under the experimental conditions)^{10,17–19} and by the 7 positive charges of the arginine residues at the constriction. It should be noted, however, that from pH 7.0 to pH 11.0 both the DNA and the M113R residues are expected to retain their respective charges (the pK_a of the arginine side-chain is 12.5). On the contrary, over the same pH range G and T nucleobases in the immobilised DNA strand should deprotonate²⁰ (Fig. 3A). In an effort to observe the deprotonation of a single nucleobase, we compared the residual current signals ($I_{RES} = I_B/I_O \times 100$) of immobilised oligonucleotides containing nucleobases at position 9 that deprotonate in the pH range 7.0–11.0 (T and G) with the I_{RES} values of the oligonucleotides containing nucleobases that do not deprotonate in the same pH range (C and A). Interestingly, we observed that the residual current differences between the oligonucleotides containing C and A bases at position 9 ($\Delta I_{RES}^{C-A} = I_{RES}^C - I_{RES}^A$) was independent of pH (Fig. 3B). On the other hand, the ΔI_{RES} between poly dC (*i.e.* C at position 9) and the oligonucleotides containing either G or T at position 9 (ΔI_{RES}^{C-G} or ΔI_{RES}^{C-T}) decreased with the pH in a sigmoidal fashion (Fig. 3c and 3d), showing transition mid-points at pH 9.2 ± 0.2 and pH 9.5 ± 0.1 , respectively. These values correspond reasonably well to the pK_a values of G and T nucleosides (9.2 and 9.7, respectively,²⁰ Fig. 3A). Almost identical transition mid-point values were observed for ΔI_{RES}^{A-G} and ΔI_{RES}^{A-T} (Fig. S2). It should be noted, however, that in ssDNA or RNA, due to interactions with the phosphate group and with the other DNA bases, G and T generally show more basic pK_a than their

respective nucleosides (0.2–1.0 pK_a unit increase depending on the DNA sequence).^{21, 22} Therefore, although we cannot exclude a local effect of the guanidine groups of the Arg113 residues, it is tempting to suggest that when DNA is immobilised within the nanopore and stretched by the applied potential, the phosphate group of the DNA backbone (or other nearby DNA bases) no longer influences the pK_as of the nucleobases, which become more similar to the pK_a values of the corresponding nucleosides. These findings are important because, other than showing a novel strategy to measure the pK_a of individual nucleobases within a DNA strand, they suggest that the chemical properties of nucleic acids might be influenced by physical factors such as the stretching of the nucleic acid itself.

In conclusion, we have shown that individual nucleobases in oligonucleotides immobilised within the α HL nanopore can be identified at pH values up to 11.7. Since the production and manipulation of long ssDNA molecules is not trivial, these conditions might be used to denature unamplified genomic dsDNA into individual ssDNA strands, which, if further aspects of nanopore sequencing can be addressed, could then be directly sequenced during translocation through a α HL nanopore. Finally, we have also shown that ionic current through nanopores are amenable to measure the protonation state of single DNA base immobilised within the nanopore.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Branton D, Deamer DW, Marziali A, Bayley H, Benner SA, Butler T, Di Ventra M, Garaj S, Hibbs A, Huang X, Jovanovich SB, Krstic PS, Lindsay S, Ling XS, Mastrangelo CH, Meller A, Oliver JS, Pershin YV, Ramsey JM, Riehn R, Soni GV, Tabard-Cossa V, Wanunu M, Wiggins M, Schloss JA. *Nature Biotechnology*. 2008; 26:1146–1153.
2. Japrun D, Henricus M, Li QH, Maglia G, Bayley H. *Biophysical Journal*. 2010; 98:1856–1863. [PubMed: 20441749]
3. Zimmer C. *Biochim Biophys Acta*. 1968; 161:584–586. [PubMed: 5667300]
4. Ageno M, Dore E, Frontali C. *Biophys J*. 1969; 9:1281–1311. [PubMed: 4982056]
5. Chapman RE Jr, Sturtevant JM. *Biopolymers*. 1970; 9:445–447. [PubMed: 5436171]
6. Maglia M, Henricus M, Wyss R, Li Q, Cheley S, Bayley H. *Nano Letters*. 2009; 9:3831–3836. [PubMed: 19645477]
7. Maglia G, Rincon Restrepo M, Mikhailova E, Bayley H. *Proc Natl Acad Sci U S A*. 2008; 105:19720–19725. [PubMed: 19060213]
8. Rincon-Restrepo M, Mikhailova E, Bayley H, Maglia G. *Nano Lett*. 2011; 11:746–750. [PubMed: 21222450]
9. Purnell RF, Mehta KK, Schmidt JJ. *Nano Lett*. 2008; 8:3029–3034. [PubMed: 18698831]
10. Stoddart D, Heron A, Mikhailova E, Maglia G, Bayley H. *Proc Natl Acad Sci USA*. 2009; 106:7702–7707. [PubMed: 19380741]
11. Stoddart D, Heron AJ, Klingelhoefer J, Mikhailova E, Maglia G, Bayley H. *Nano Lett*. 2010; 10:3633–3637. [PubMed: 20704324]
12. Eisenberg RS. *Journal of Membrane Biology*. 1999; 171:1–24. [PubMed: 10485990]
13. Aguilera VM, Pellicer J, Aguilera-Arzo M. *Langmuir*. 1999; 15:6156–6162.
14. Daiguji H, Oka Y, Shirono K. *Nano Letters*. 2005; 5:2274–2280. [PubMed: 16277467]
15. Bayley, H.; Luchian, T.; Shin, S-H.; Steffensen, MB. *Single Molecules and Nanotechnology*. Rigler, R.; Vogel, H., editors. Springer; Heidelberg: 2008. p. 251-277.
16. Bezrukov SM, Kasianowicz JJ. *Physical Review Letters*. 1993; 70:2352–2355. [PubMed: 10053539]
17. Meller A, Nivon L, Branton D. *Phys Rev Lett*. 2001; 86:3435–3438. [PubMed: 11327989]

18. Sauer-Budge AF, Nyamwanda JA, Lubensky DK, Branton D. *Phys Rev Lett*. 2003; 90:238101-238101-238101-238104.
19. Vercoutere W, Winters-Hilt S, Olsen H, Deamer D, Haussler D, Akeson M. *Nat Biotechnol*. 2001; 19:248–252. [PubMed: 11231558]
20. Bloomfield, VA.; Crothers, DM.; Tinoco, I. *Nucleic acids: structures, properties, and functions*. University Science Books; Sausalito, CA: 2000.
21. Acharya P, Cheruku P, Chatterjee S, Acharya S, Chattopadhyaya J. *Journal of the American Chemical Society*. 2004; 126:2862–2869. [PubMed: 14995203]
22. Acharya S, Barman J, Cheruku P, Chatterjee S, Acharya P, Isaksson J, Chattopadhyaya J. *Journal of the American Chemical Society*. 2004; 126:8674–8681. [PubMed: 15250719]

Fig. 1.

Oligonucleotide Immobilisation within an α HL nanopore. a) Cartoon representation of a homopolymeric DNA strand with a single purine base substituted at position 9 (red) from the 3' biotin-TEG¹⁰ (yellow) immobilised within a α HL pore (grey) by complexation with streptavidin (red). Notice that for the sake of clarity, the DNA is not to scale and not all the nucleobases are included in the drawing. The introduced arginine residue at position 113 in the constriction site is coloured blue. b) The sequences of the four poly(dC) oligonucleotides with single nucleobase substitutions at position 9 used in this work.

Fig. 2. Nucleobase recognition at different pH. a) I_B values at pH 8.0 and 11.7 for the M113R-NN pore when blocked by a poly-dC strand containing a C, T A and G nucleobase at position 9 from the biotinylated TAG. b) pH dependence of the product of the three successive differences between the four current levels in a histogram of residual currents (δ).¹¹ The buffer was 10 mM Tris.HCl, 10 mM CAPS, 15 mM K phosphate, 3.0 M KCl, 100 μ M EDTA and the applied potential +120mV. Errors are shown as standard errors.

Fig. 3.

Apparent pK_a of a single nucleobases immobilised in the M113R-NN nanopore. a) Acid association constants for the T and G nucleosides. b) pH dependence of the ΔI_{RES} differences between a poly dC oligonucleotide containing a single A at position 9 from the 3' biotin-TEG and an a poly dC oligonucleotide containing a single T in the same position. c and d) pH dependences of ΔI_{RES} differences between poly dC and an oligonucleotide containing a single T and G substitution at position 9 from the 3' biotin-TEG, respectively. The midpoints of the transitions were 9.5 ± 0.1 and 9.2 ± 0.2 , respectively. Errors are shown as standard errors.