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Silver(I) ions modulate the stability of DNA duplexes containing cytosine, methylcytosine and hydroxymethylcytosine at different salt concentrations†

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Abstract

Silver(I) ions can stabilize cytosine–cytosine, cytosine (C)–methylcytosine (5mC) and cytosine–hydroxymethylcytosine (5hmC) mismatched-base pairs. While cytosine modifications regulate DNA stability to regulate cellular functions, silver ions can modulate the stability of C–C, C–5mC and C–5hmC containing DNA duplexes in a salt concentration dependent manner.

Natural A–T and C–G Watson–Crick base pairs¹ in double-stranded DNA (dsDNA) are essential for the replication and expression of genetic information. Cytosine modifications such as 5mC and 5hmC have been studied as gene regulators,² through their effect on DNA secondary structures such as i-motifs (iMs) in C-rich DNA³ and DNA–protein interactions.^{2b,4} Studies have found that 5mC increases dsDNA stability⁵ while 5hmC decreases DNA stability.⁶ DNA stability is critical in maintaining normal gene function.⁷ 5mC and 5hmC detection and discrimination also have attracted a great deal of recent interest.⁸

In DNA duplexes, silver(I) ions specifically interact with C–C mismatches,⁹ while mercury ions specifically interact with T–T mismatches.¹⁰ These interactions strongly stabilize DNA duplexes and have been extensively studied.¹¹ Since 5mC and 5hmC are important epigenetic markers associated with gene expression and tumorigenesis,¹² we were motivated to explore the interactions of Ag(I) with a DNA duplex containing single C–C, 5mC–C or 5hmC–C mismatch to observe how the interactions affect DNA stability. Primarily, Ag(I) ions had no notable effects on the shape of the thermal transition profile for dsDNA containing A–T and C–G pairs.^{9b} Only silver ions exhibited specific interactions with C–C mismatch, but not Hg(II), Cu(II), Ni(II), Pd(II), Co(II), Mn(II), Zn(II), Pb(II), Cd(II), Mg(II), Ca(II), Fe(II), Fe(III), and Ru(III) metal ions.^{9b} We designed a dsDNA containing a single C–C, 5mC–C or 5hmC–C mismatch to study the effect of Ag(I) ions on the stability of these DNA duplexes. The sequence is: 5′-AATAAAATAXTATAAA-3′/5′-TTTATACTATTTTATT-3′, where X = C, 5mC or 5hmC. In the following text, C–C, 5mC–C and 5hmC–C stands for the dsDNA contains C–C, 5mC–C and 5hmC–C mismatch, respectively.

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In 100 mM KNO₃ about physiological condition, we found that Ag(I) ions can stabilize C–C, 5mC–C and 5hmC–C (Fig. 1). Without Ag(I) ions, the melting temperatures (T_m) obtained from UV melting curves are 20.0 ± 0.0 °C (C–C), 21.7 ± 0.4 °C (5mC–C) and 18.0 ± 0.5 °C (5hmC–C), respectively. These results suggest that 5mC increases dsDNA stability but 5hmC decreases DNA stability, which are consistent with previous reports.^{5,6} With the addition of Ag(I) ions, T_m are 35.0 ± 0.0 °C (C–Ag–C), 34.3 ± 0.3 °C (5mC–Ag–C) and 33.2 ± 0.5 °C (5hmC–Ag–C), respectively. Therefore, Ag(I) ions changed the stability of the DNA duplexes, from 5mC–C > C–C > 5hmC–C to C–Ag–C > 5mC–Ag–C > 5hmC–Ag–C (Fig. 1a–c). Additionally, Ag(I) ions increased the stability of C–C ($T_m = 15.0 \pm 0.0$ °C) and 5hmC–C ($T_m = 15.2 \pm 0.7$ °C) similarly, but less of 5mC–C ($T_m = 12.6 \pm 0.5$ °C) (Fig. 1d). Standard deviations for these changes were calculated by error propagation equation. A recent study found that a distinct exception for 5hmC contacts UHRF2 protein by binding to the narrow pocket adjacent to the positively charged area of the protein to form multiple interactions.¹³ These results suggest that positively charged molecules may be able to interact with 5hmC and modulate the stability of the complex.

In 1 M KNO₃, we also found that Ag(I) ions can stabilize C–C, 5mC–C and 5hmC–C containing dsDNAs (Fig. 2). Without Ag(I) ions, T_m are 28.5 ± 0.6 °C (C–C), 29.1 ± 0.8 °C (5mC–C) and 27.6 ± 0.3 °C (5hmC–C), respectively. With the addition of Ag(I) ions, T_m are 43.5 ± 0.6 °C (C–Ag–C), 42.4 ± 1.0 °C (5mC–Ag–C) and 39.9 ± 1.8 °C (5hmC–Ag–C), respectively. Similarly, Ag(I) ions changed the stability of the DNA duplexes, from 5mC–C > C–C > 5hmC–C to C–Ag–C > 5mC–Ag–C > 5hmC–Ag–C (Fig. 2a–c). But interestingly, duplex stability is most increased for C–C ($T_m = 15.0 \pm 0.8$ °C) with Ag(I) ions, followed by 5mC–C ($T_m = 13.3 \pm 1.3$ °C) and then 5hmC–C ($T_m = 12.3 \pm 1.8$ °C) (Fig. 2d). If we compare these changes with 100 mM salt condition (Fig. 1d and 2d), we can notice that 5hmC has the most notable difference (see Table S1† for summary). These results suggest that salt concentration not only regulates the stability of DNA duplex itself, but also plays a very important role in regulating the stabilizing effect of Ag(I) ions on C–C, 5mC–C and 5hmC–C.

In 10 mM KNO₃, there is no measurable T_m for C–C (Table S1†). The T_m values were evaluated to be 0.6 °C, 18.6 °C and 31.1 °C for C–C in 10 mM, 100 mM and 1 M NaCl (<http://biophysics.idtdna.com>), which are close to our experimental results in KNO₃. Our result is also consistent with our previous nanopore measurements. Nanopore is a single molecule detection platform.¹⁴ In 1 M KNO₃, without Ag⁺, the duration of duplexes dissociation follows mC–C > C–C > hmC–C (in nano-pore measurement, longer duration would indicate a higher binding energy between two ssDNAs). With Ag⁺, the duration of duplexes dissociation C–C > mC–C > hmC–C.^{14c} In our current T_m measures, without Ag⁺, T_m follows mC–C > C–C > hmC–C. With Ag⁺, T_m follows C–C > mC–C > hmC–C. The same trend was also found in 100 mM KNO₃.

The T_m measurements for C–C mismatch with and without Ag⁺ were well studied. For example, for a 11bp DNA (containing 6 CG pairs) in 1 M NaClO₄, T_m for C–C and C–Ag–C were 31.7 °C and 36.5 °C, respectively.¹⁵ Another study reported that a 21bp DNA (containing no CG pairs) in 100 mM NaNO₃, T_m for C–C and C–Ag–C were 31 °C and 39 °C, respectively.^{9b} Our result using the 16bp DNA duplex also demonstrated that the T_m

is higher for C–Ag–C than C–C in both 100 mM and 1 M salt concentrations. X-Ray diffraction results suggest that each of the methylcytosine residues doubly cross-linked by two Ag(I) ions at the base binding sites N₃ and O₂.¹⁶ Previous studies by isothermal titration calorimetry (ITC) and electrospray ionization mass spectrometry measurement, the binding of silver(I) to a DNA duplex containing a single C–C mismatch was identified at a 1 : 1 molar ratio.^{9d,17}

Thermodynamic properties of C–Ag–C complexes were studied by isothermal titration calorimetry (ITC) and circular dichroism (CD) and the results suggest that the specific binding between the Ag(I) ion and the single C–C mismatch was mainly driven by the positive dehydration entropy change of Ag(I) ion and the negative binding enthalpy change from the bond formation between the Ag(I) ion and the N₃ positions of the two cytosine bases^{9d,17} (Fig. 3a). Previous Molecular dynamics (MD) simulations found that H₂O molecules have the highest affinity for 5hmC when compared to C and 5mC, which increases the rotation probability.^{5b} Atomic force microscopy (AFM) studies have found that the persistent length follows the trend 5mC > C > 5hmC,^{5b} suggesting that 5hmC-containing DNA has the largest flexibility and least structural stability. Finally, the –OH group in 5hmC can chelate with the phosphate group¹⁸ which may prevent a stable 5hmC–C complex formation. These results suggest a mechanism behind the lower stability of the base-pairing in 5hmC–C mismatches (Scheme in Fig. 3a–c). Our recent MD simulations indicate that hydrogen bonds are alternatively formed between N4_A and N3_B atoms and between N3_A and N4_B atoms. This type of pairing results in the formation of a binding site for a cation^{14c} (Fig. 3d). Under any circumstances, specific interactions between Ag(I) ion and C–C, 5mC–C or 5hmC–C can regulate the effects of cytosine modifications on the DNA duplexes stability (Fig. 3), and this regulation could be very complex depending on the salt concentration.

Conclusions

In conclusion, for the first time, our results demonstrated that Ag(I) can modulate the stability of dsDNA which contains cytosine and cytosine modifications in a salt concentration dependent manner. When close to physiological salt concentration (100 mM), Ag(I) has the most notable effect on 5hmC–C duplex ($T_m = 15.2 \pm 0.7$ °C), followed by C–C and then 5mC–C duplexes. At high salt concentration (1 M), duplex stability is most increased for C–C ($T_m = 15.0 \pm 0.8$ °C) with Ag(I) ions, followed by 5mC–C and then 5hmC–C. Salt concentration not only regulates the stability of DNA duplex itself, but also plays a very important role in regulating the stabilizing effect of Ag(I) ions on C–C, 5mC–C and 5hmC–C. By utilizing the chemical interactions with metal ions that regulate DNA stability, this phenomenon might be extended to study other cytosine modifications, such as 5-formylcytosine and 5-carboxylcytosine, or applied in epigenetic detections.^{5b,19} It also can be applied to investigate metallo-pair interactions^{10a,20} with modified base pairs, metal ion sensing, metal-coated DNA nanostructures.²¹

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Notes and references

1. Watson JD and Crick FH, *Nature*, 1953, 171, 737. [PubMed: 13054692]
2. (a) Kraus TF, Globisch D, Wagner M, Eigenbrod S, Widmann D, Münzel M, Müller M, Pfaffeneder T, Hackner B and Feiden W, *Int. J. Cancer*, 2012, 131, 1577; [PubMed: 22234893] (b) Yildirim O, Li R, Hung J-H, Chen PB, Dong X, Ee L-S, Weng Z, Rando OJ and Fazio TG, *Cell*, 2011, 147, 1498. [PubMed: 22196727]
3. Bhavsar-Jog YP, Van Dornshuld E, Brooks TA, Tschumper GS and Wadkins RM, *Biochemistry*, 2014, 53, 1586. [PubMed: 24564458]
4. (a) Iurlaro M, Ficiz G, Oxley D, Raiber E-A, Bachman M, Booth MJ, Andrews S, Balasubramanian S and Reik W, *Genome Biol*, 2013, 14, R119; [PubMed: 24156278] (b) Spruijt CG, Gnerlich F, Smits AH, Pfaffeneder T, Jansen PW, Bauer C, Münzel M, Wagner M, Müller M and Khan F, *Cell*, 2013, 152, 1146; [PubMed: 23434322] (c) Mellén M, Ayata P, Dewell S, Kriaucionis S and Heintz N, *Cell*, 2012, 151, 1417. [PubMed: 23260135]
5. (a) Rodríguez López CM, Guzmán Asenjo B, Lloyd AJ and Wilkinson MJ, *Anal. Chem*, 2010, 82, 9100; [PubMed: 20945868] (b) Wanunu M, Cohen-Karni D, Johnson RR, Fields L, Benner J, Peterman N, Zheng Y, Klein ML and Drndic M, *J. Am. Chem. Soc*, 2011, 133, 486. [PubMed: 21155562]
6. Thalhammer A, Hansen AS, El-Sagheer AH, Brown T and Schofield CJ, *Chem. Commun*, 2011, 47, 5325.
7. Branco MR, Ficiz G and Reik W, *Nat. Rev. Genet*, 2012, 13, 7.
8. (a) Yu M, Hon GC, Szulwach KE, Song CX, Zhang L, Kim A, Li X, Dai Q, Shen Y, Park B, Min JH, Jin P, Ren B and He C, *Cell*, 2012, 149, 1368; [PubMed: 22608086] (b) Yu M, Hon GC, Szulwach KE, Song CX, Jin P, Ren B and He C, *Nat. Protoc*, 2012, 7, 2159; [PubMed: 23196972] (c) Booth MJ, Branco MR, Ficiz G, Oxley D, Krueger F, Reik W and Balasubramanian S, *Science*, 2012, 336, 934. [PubMed: 22539555]
9. (a) Lin YH and Tseng WL, *Chem. Commun*, 2009, 6619; (b) Ono A, Cao S, Togashi H, Tashiro M, Fujimoto T, Machinami T, Oda S, Miyake Y, Okamoto I and Tanaka Y, *Chem. Commun*, 2008, 4825; (c) Wen Y, Xing F, He S, Song S, Wang L, Long Y, Li D and Fan C, *Chem. Commun*, 2010, 46, 2596; (d) Torigoe H, Okamoto I, Dairaku T, Tanaka Y, Ono A and Kozasa T, *Biochimie*, 2012, 94, 2431. [PubMed: 22766014]
10. (a) Miyake Y, Togashi H, Tashiro M, Yamaguchi H, Oda S, Kudo M, Tanaka Y, Kondo Y, Sawa R, Fujimoto T, Machinami T and Ono A, *J. Am. Chem. Soc*, 2006, 128, 2172; [PubMed: 16478145] (b) Tanaka Y, Oda S, Yamaguchi H, Kondo Y, Kojima C and Ono A, *J. Am. Chem. Soc*, 2007, 129, 244; [PubMed: 17212382] (c) Torigoe H, Ono A and Kozasa T, *Chemistry*, 2010, 16, 13218; [PubMed: 20886468] (d) Wen S, Zeng T, Liu L, Zhao K, Zhao Y, Liu X and Wu HC, *J. Am. Chem. Soc*, 2011, 133, 18312. [PubMed: 21995430]
11. Ono A, Torigoe H, Tanaka Y and Okamoto I, *Chem. Soc. Rev*, 2011, 40, 5855. [PubMed: 21826352]
12. (a) Brena RM, Huang TH and Plass C, *J. Mol. Med*, 2006, 84, 365; [PubMed: 16416310] (b) Kriaucionis S and Heintz N, *Science*, 2009, 324, 929; [PubMed: 19372393] (c) Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo QM, Edsall L, Antosiewicz-Bourget J, Stewart R, Ruotti V, Millar AH, Thomson JA, Ren B and Ecker JR, *Nature*, 2009, 462, 315. [PubMed: 19829295]
13. Zhou T, Xiong J, Wang M, Yang N, Wong J, Zhu B and Xu R-M, *Mol. Cell*, 2014, 54, 879. [PubMed: 24813944]
14. (a) Wang Y, Zheng D, Tan Q, Wang MX and Gu LQ, *Nat. Nanotechnol*, 2011, 6, 668; [PubMed: 21892163] (b) Wang Y, Tian K, Hunter LL, Ritzo B and Gu LQ, *Nanoscale*, 2014, 6, 11372; [PubMed: 25144935] (c) Wang Y, Luan BQ, Yang Z, Zhang X, Ritzo B, Gates K and Gu LQ, *Sci.*

- Rep, 2014, 4, 5883; [PubMed: 25103463] (d)Luan B, Zhou B, Huynh T and Zhou R, Nanoscale, 2014, 6, 11479. [PubMed: 25154639]
15. Urata H, Yamaguchi E, Nakamura Y and Wada S, Chem. Commun, 2011, 47, 941.
16. Kistenmacher TJ, Rossi M and Marzilli LG, Inorg. Chem, 1979, 18, 240.
17. Torigoe H, Miyakawa Y, Ono A and Kozasa T, Nucleosides, Nucleotides Nucleic Acids, 2011, 30, 149. [PubMed: 21360412]
18. Gunther EL, Nathan BA, James BJ, Patricia C, Joseph RM, Yong SA and Edward T, in Bioinorganic Chemistry, American Chemical Society, 1971, pp. 135–154.
19. (a)Hu J, Chen Y, Xu X, Wu F, Xing X, Xu Z, Xu J, Weng X and Zhou X, Bioorg. Med. Chem. Lett, 2014, 24, 294; [PubMed: 24295785] (b)Li WW, Gong L and Bayley H, Angew. Chem, 2013, 52, 4350; [PubMed: 23559386] (c)Shim J, Humphreys GI, Venkatesan BM, Munz JM, Zou X, Sathe C, Schulten K, Kosari F, Nardulli AM, Vasmatazis G and Bashir R, Sci. Rep, 2013, 3, 1389; [PubMed: 23474808] (d)Kang I, Wang Y, Reagan C, Fu Y, Wang MX and Gu L-Q, Sci. Rep, 2013, 3, 2381. [PubMed: 24135881]
20. (a)Atwell S, Meggers E, Spraggon G and Schultz PG, J. Am. Chem. Soc, 2001, 123, 12364; [PubMed: 11734038] (b)Okamoto I, Iwamoto K, Watanabe Y, Miyake Y and Ono A, Angew. Chem, 2009, 48, 1648. [PubMed: 19156796]
21. (a)Berti L, Alessandrini A and Facci P, J. Am. Chem. Soc, 2005, 127, 11216; [PubMed: 16089430] (b)Burley GA, Gierlich J, Mofid MR, Nir H, Tal S, Eichen Y and Carell T, J. Am. Chem. Soc, 2006, 128, 1398; [PubMed: 16448080] (c)Petty JT, Zheng J, Hud NV and Dickson RM, J. Am. Chem. Soc, 2004, 126, 5207; [PubMed: 15099104] (d)Feldkamp U and Niemeyer CM, Angew. Chem., Int. Ed, 2006, 45, 1856.

Fig. 1.

UV melting curves of C-C (a), 5mC-C (b) and 5hmC-C (c) dsDNA duplexes. Samples contained 1 mM duplex, 100 mM KNO₃ and 10 mM Mops, pH = 7.1. Blue line: melting curves without Ag(I) and red line: melting curves with Ag(I). (d) The changes of T_m for duplexes with and without Ag(I).

Fig. 2. UV melting curves of C-C (a), 5mC-C (b) and 5hmC-C (c) dsDNA duplexes. Samples contained 1 μ M duplex, 1 M KNO₃ and 10 mM Mops, pH = 7.1. Blue line: melting curves without Ag(I) and red line: melting curves with Ag(I). (d) The changes of T_m for duplexes with and without Ag(I).

Fig. 3. Schemes of possible configurations for C-C, 5mC-C and 5hmC-C mismatches according to the references. As well as the possible binding site for Ag(I). See the detailed discussion in the main text.