Supporting Information

Effect of LNA nucleobases as an enhancer for the binding of amiloride to an abasic site in DNA/DNA and DNA/RNA duplexes

Yusuke Sato, Tetsushi Sato, Takaya Sato, Seiichi Nishizawa, and Norio Teramae*

Department of Chemistry, Graduate School of Science, Tohoku University, Aoba-ku, Sendai 980-8578, Japan.

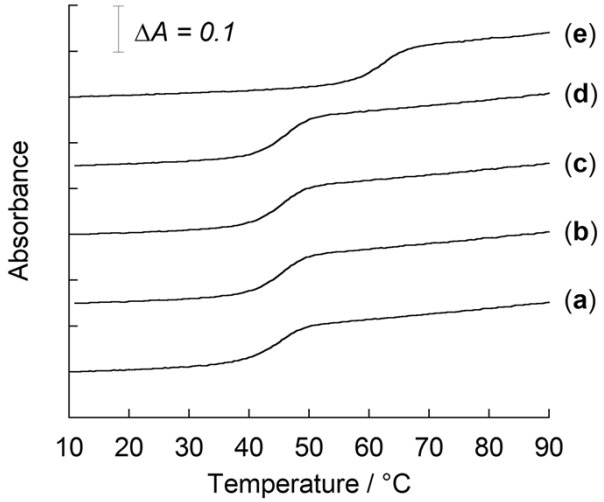
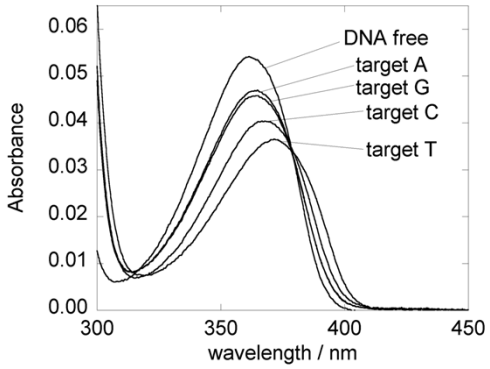


Fig. S1. Thermal denaturation profiles of (a) DNA/DNA, (b) **LD1**/DNA, (c) **LD2**/DNA, (d) **LD3**/DNA, (e) **LD4**/DNA duplexes (1.0 μM) containing the target T. Other solution conditions were the same as those shown in Fig. 2 in the main text. Absorbance of each duplex was measured at 260 nm as a function of temperature, which ranged from 2°C to 92°C with a heating rate of 1.0°C/min. Optical path length = 10 mm.



	Absorption maximum wavelength (nm)	Absorbance at maximum wavelength
DNA free	361.6	0.0542
G	364.8	0.0458
A	364.8	0.0470
C	366.6	0.0404
T	373.0	0.0364

Fig. S2. Absorption response of amiloride (3.0 μM)upon binding to **LD1**/DNA duplex (3.0 μM). Other solution conditions were the same as those shown in Fig. 2 in the main text. Temperature, 20°C.
We observed the decrease in the absorbance and absorption maximum wavelength of amiloride upon binding to duplexesand these responses are also shown.

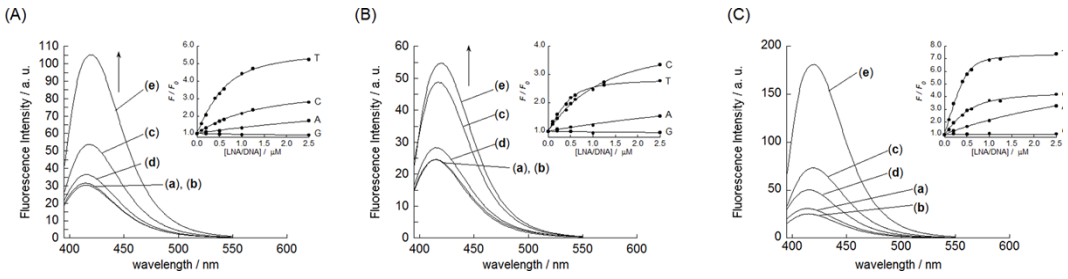


Fig. S3. Fluorescence response of amiloride (0.5 μM) to the target nucleobase in (A) **LD2**/DNA (0.5 μM), (B) **LD3**/DNA (0.5 μM), (C) **LD4**/DNA duplexes (0.5 μM): (a) duplex free, target nucleobase = (b) G, (c) C, (d) A, or (e) T. Other solution conditions were the same as those shown in Fig. 2 in the main text. Excitation, 380.5 nm. Temperature, 20°C. Inset: Nonlinear regression analysis of the changes in the fluorescence intensity ratio at 415 nm based on a 1:1 binding isotherm model. F and F_0 denote the fluorescence intensities of amiloride (0.5 μM) in the presence and absence of duplexes, respectively.

Table S1. The effect of the kind of LNA flanking nucleobases[a] on the dissociation constants (K_d) for amiloride binding for the target T

K_d / nM (n = 3)	GXG/CTC[b]	CXC/GTG[b]	AXA/TTT[c]
DNA	107 ± 17	240 ± 52	384 ± 12
LNA	46 ± 7.3	28.8 ± 4.9	31.6 ± 2.4

[a] Sequence: 5'-GGG GAA GGA wXw' GAA GGA AAA-3'/3'-TTT TCC TTC zTz' TCC TTC CCC-5', w, w' = LNA or DNA nucleobase; G, C, or A, X = AP site (Spacer C3). [b] K_d values were determined by the fluorescence titration experiments as described in the main text. [c] K_d values were determined by UV-vis titration experiments since the fluorescence responses were not well analyzed by the 1:1 binding isotherm, where the changes in the absorbance at 361.5 nm of amiloride with increasing concentration of the duplexes were analyzed for the K_d determination. Sample solutions were buffered to pH 7.0 (10 mM sodium cacodylate) with 100 mM NaCl and 1.0 mM EDTA. The errors are the standard deviations obtained from the three independent experiments.

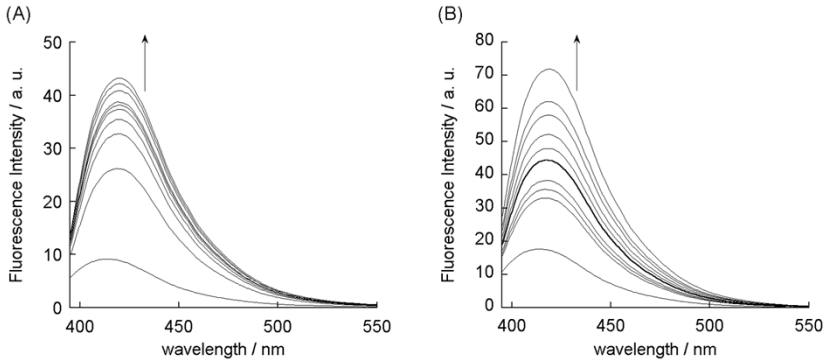


Fig. S4 Fluorescence response of amiloride to target T in (A) **LD1**/DNA and (B) DNA duplexes. All solution conditions are the same as those given in Fig. 3 in the main text. Excitation, 380.2 nm. Temperature, 20°C.

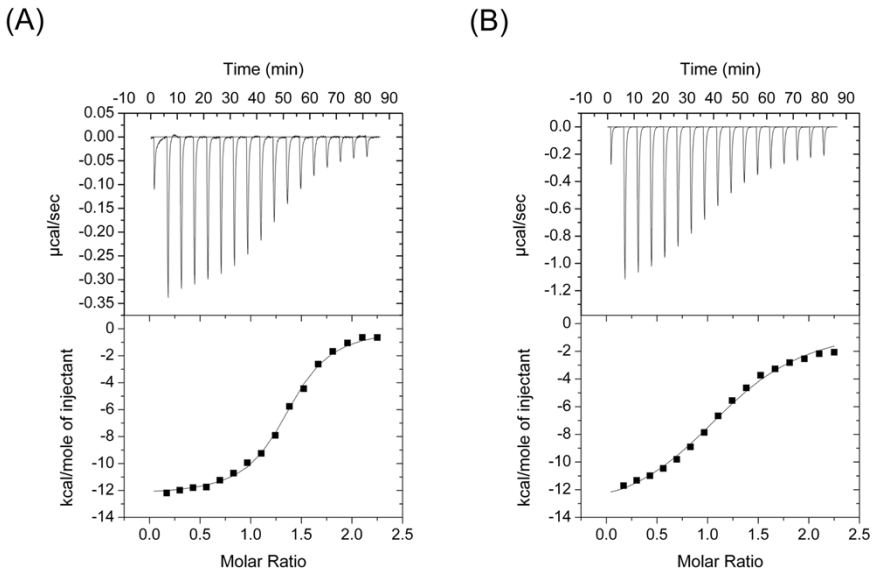


Fig. S5. ITC data for the addition of amiloride aliquots into the solution containing (A) **LD4**/DNA and (B) DNA duplex containing the target T. (A) [amiloride] = 50 μM, [duplex] = 4.0 μM, (B) [amiloride] = 120 μM, [duplex] = 10 μM. Other solution conditions are the same as those given in Fig. 3 in the main text. Temperature, 20°C. The data were best-fitted to a model that assumes a single set of identical binding sites. The analysis gives the binding enthalpy (ΔH_{obs}) and the binding stoichiometry (n).

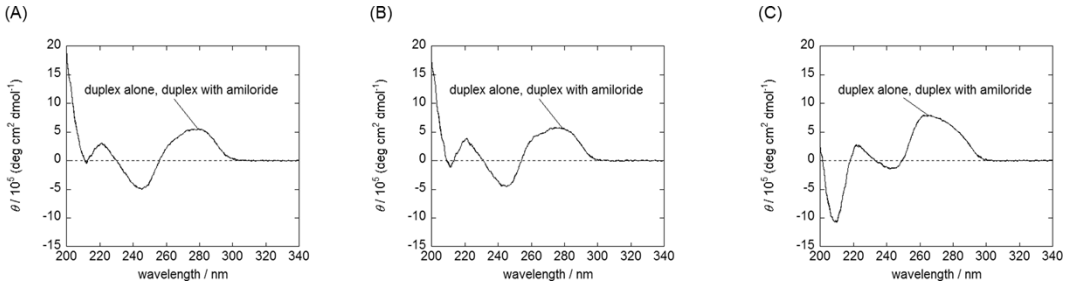


Fig. S6. Changes in the CD spectra of (A) **LD1**/DNA, (B) **LD4**/DNA and (C) DNA duplexes (10 μM) containing the target T upon binding of amiloride (10 μM). Other solution conditions were the same as those given in Fig. 2 in the main text.

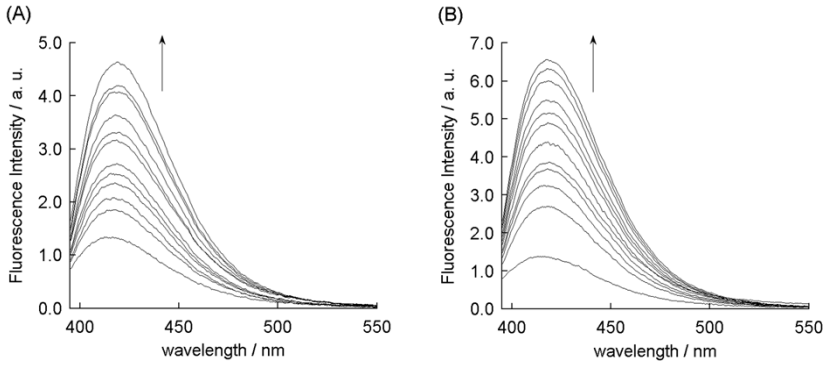


Fig. S7 Fluorescence response of amiloride to target U in (A) **LD1**/RNA and (B) **LD4**/RNA. All solution conditions are the same as those given in Fig. 5 in the main text. Excitation, 380.2 nm. Temperature, 20°C.

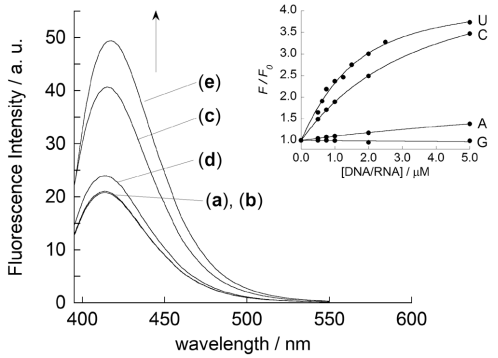


Fig. S8. Fluorescence response of amiloride to the target nucleobase in DNA/RNA hybrid duplexes: (a) duplex free, target nucleobase = (b) G, (c) C, (d) A, or (e) U. [amiloride], [duplex] = 1.0 μM. Other solution conditions were the same as those given in Fig. 2 in the main text. Excitation, 380.5 nm. Temperature, 20°C. Inset: Nonlinear regression analysis of the changes in the fluorescence intensity ratio at 415 nm based on a 1:1 binding isotherm model. F and F_0 denote the fluorescence intensities of amiloride in the presence and absence of duplexes, respectively.