Supplementary Material (ESI) for Chemical Communications

Supporting information for

Strong and selective binding of amiloride to thymine base opposite AP sites in DNA duplexes: simultaneous binding to DNA phosphate backbone

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The following items have been included as supplementary material:

Table S1. Binding constants of amiloride or DCPC to thymine base opposite the AP site in DNA duplex (5'-TCC AGX GCA AC-3'/3'-AGG TCT CGT TG-5', X = AP site) under different salt concentrations.

Figure S1. Salt dependence of binding constants for the ligand-DNA interaction.

Figure S2. Calorimetric isothermal titration for the binding of amiloride or DCPC with DNA duplex (5'-TCC AGX GCA AC-3'/3'-AGG TCT CGT TG-5', X = AP site, T = target thymine).

Figure S3. Fluorescence detection of T-related mutation of PCR products (*K-ras* gene, codon 12).

Table S1. Binding constants of amiloride or DCPC to thymine base opposite the AP site in DNA duplex (5'-TCC AGX GCA AC-3'/3'-AGG TCT CGT TG-5', X = AP site) under different salt concentrations.[a)]

$[Na^+]$ /mM	$K_{(5\,°C)}$ (M^{-1})	
	Amiloride	DCPC
50	1.5×10^7	3.9×10^5
100	6.7×10^6	2.9×10^5
120	5.2×10^6	—
150	4.1×10^6	—
250	—	2.5×10^5
500	—	2.2×10^5

[a)]The binding constants were obtained by fluorescence titration experiments in solutions buffered to pH 7.0 with 10 mM sodium cacodylate containing 1.0 mM EDTA at 5 ºC (cf. Figure 2). The concentration of NaCl ranged from 50 mM to 500 mM. For amiloride titration: [amiloride], 1.0 μM; [DNA duplex], 0-5.0 μM; excitation wavelength, 380.5 nm; analysis, 415 nm. For DCPC titration: [DCPC], 20 μM; [DNA duplex], 0-96 μM; excitation wavelength, 371.5 nm; analysis, 424 nm.

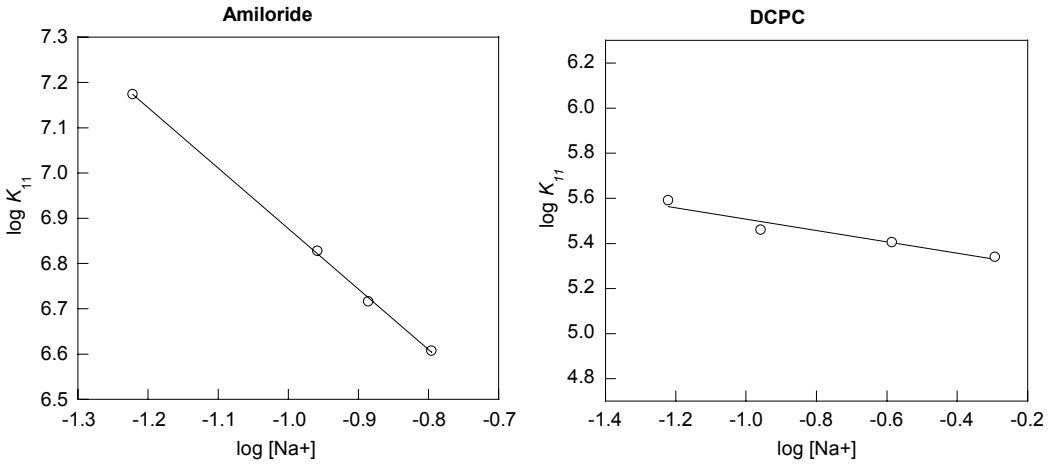

Figure S1. Salt dependence of binding constants for the ligand-DNA interaction. The data are given in Table S2. For amiloride binding: the linear least squares fit to the data yielded a slope of –1.34, from this value, an apparent charge of amiloride was obtained (Z = +1.52). For DCPC binding: the linear least squares fit to the data yielded a slope of –0.26, giving the apparent charge Z of +0.29.

The effect of different NaCl concentrations upon the binding constant of ligands was calculated and used to determine the ionic strength dependence of the equilibrium binding constants according to the polyelectrolyte theory of Record et al.. The observed linear dependence is described by the relationship:

$$\delta(\log K)/\delta\log[Na^+] = -Z\psi \qquad (1)$$

where ψ is the fraction of sodium counterion associated per DNA phosphate (ψ = 0.88 for B-DNA) and Z is the apparent charge of the ligand.

Moreover, the Zψ value was used to evaluate the polyelectrolyte contribution to the free energy of binding to DNA using the relationship:

$$\Delta G_{pe} = Z\psi R T \ln[Na^+] \qquad (2)$$

and the ΔG_{pe} obtained was used to calculate ΔG_t, the non-polyelectrolyte contribution to binding, using the following equation:

$$\Delta G_{obs} = \Delta G_t + \Delta G_{pe}. \qquad (3)$$

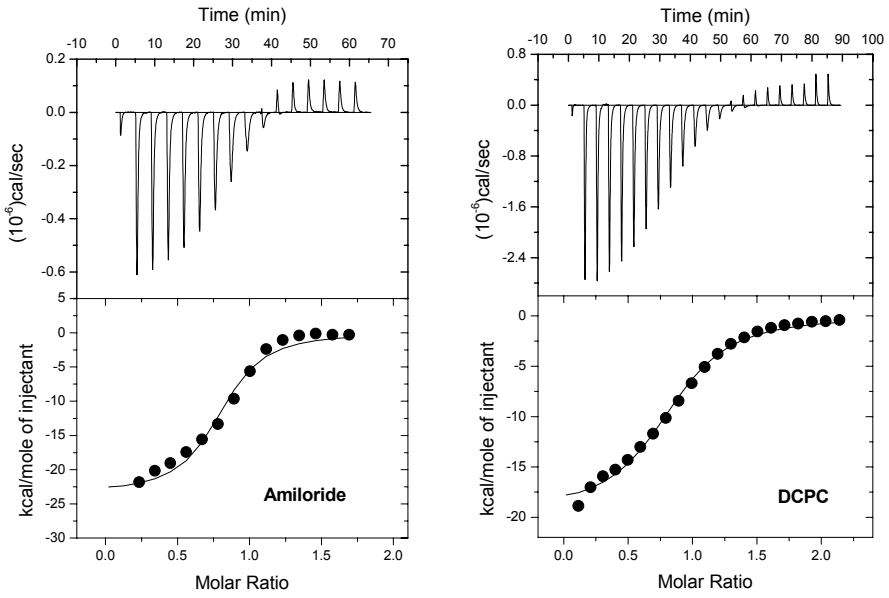

Figure S2. Calorimetric isothermal titration for the binding of amiloride or DCPC with DNA duplex (5'-TCC AGX GCA AC-3'/3'-AGG TCT CGT TG-5', X = AP site, T = target thymine). Measurements were performed using VP-ITC from MicroCal Inc., and a concentrated solution of DNA duplex was added into the ligand solution. [Amiloride], 10 μM; [DCPC], 60 μM; [DNA duplex], 150 μM for amiloride and 800 μM for DCPC; [NaCl], 100 mM; [EDTA], 1.0 mM; [sodium cacodylate], 10 mM; pH, 7.0; temperature, 10 ºC.

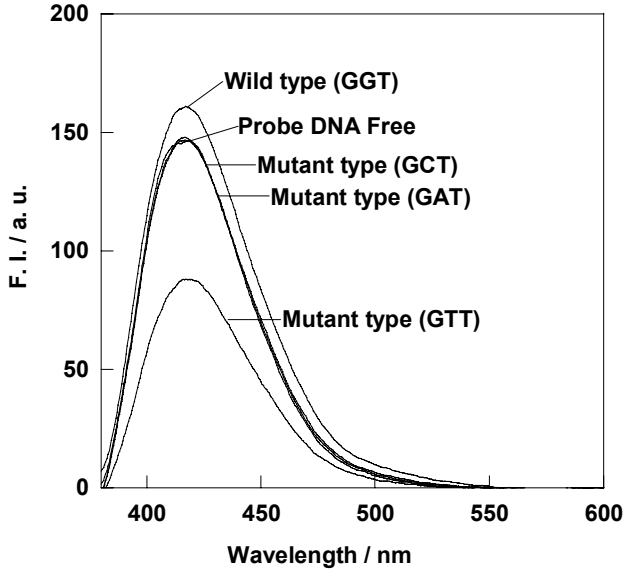

Figure S3. Fluorescence detection of T-related mutation of PCR products (*K-ras* gene,[1)] codon 12). After PCR reactions, the reaction solutions were buffered to pH 7.0 with 100 mM sodium cacodylate containing 1.6 mM EDTA, 0.1 μM amiloride and 5.0 μM AP site-containing probe DNA. PCR product: 5'-GACTGAATAT AAACTTGTGG TAGTTGGAGC TGYTGGCGTA GGCAAGAGTG CCTTGACGAT ACAGCTAATT CAGAATCATT TTGTGGACGA ATATGATCCA ACAATAG-3' (wild type, Y = G; mutant type, Y = T, C, A); probe DNA: 5'-CCTACGCCAXXCAGCTCCAAC-3' (X = AP site). Excitation wavelength, 361.5 nm. Temperature, 5 ºC.

1) Bos, J. L. *Mutat Res.* **1988**, *195(3)*, 255-271.